The Role of Norepinephrine in the Pharmacology of 3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy')

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The research in this thesis is presented in the form of scientific papers that have either been published or are in preparation. Reference lists for each paper are presented at the end the relevant section. A reference list covering the general introduction and discussion is at the end of the thesis.

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Summary

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a widely used recreational drug, which produces strong psychological effects such as increased empathy and sociability. MDMA inhibits the uptake of and releases serotonin, dopamine, and norepinephrine via an interaction with the respective monoamine transporter. While the role of serotonin in the human pharmacology of MDMA has been well described, the role of norepinephrine in the mediation of the effect of MDMA is mostly unexplored. Preclinical data indicate that norepinephrine may play a crucial role in the effects of MDMA. This project focused on the role of norepinephrine in the pharmacology of MDMA in humans. We performed five experimental clinical studies and analyzed data from a previously performed study investigating the role of norepinephrine in the mechanism of action of MDMA. All studies were pharmacological interaction studies with selective medications used as research tools to inhibit the effects of MDMA in healthy subjects. All clinical studies used placebo-controlled, double-bind cross-over designs and were each performed in 16 healthy subjects in the University Hospital of Basel. In the first study, we showed that the selective norepinephrine transporter inhibitor reboxetine reduced the MDMA-induced increases in circulating plasma norepinephrine, psychostimulant, and cardiovascular effects in healthy volunteers. Moreover, we also showed that the observed pharmacodynamic interaction in this study was not attributed to a pharmacokinetic interaction between reboxetine and MDMA, because reboxetine decreased the pharmacodynamics effects of MDMA although it increased MDMA plasma levels. The results demonstrate a critical role for transporter-mediated norephinephrine release in the cardiovascular and the subjective stimulant-like effects of MDMA in humans. Norephinephrine transporter inhibitors could therefore be useful in the clinical treatment of stimulant addiction. In the second study, we then demonstrated that the α_2 agonist clonidine, an inhibitor of the vesicular norepinephrine release, did not inhibit the response to MDMA in healthy subjects confirming that vesicular release of norepinephrine is not responsible for the effects of MDMA in humans. This study also confirmed indirectly that the monoamine transporter is the primary target of psychostimulants such as MDMA and that physiological impulse-dependent release of monoamines does not appear to be critical for the effects of MDMA in humans. In the ensuing studies we addressed the roles of the postsynaptic adrenergic receptors where the norepinephrine released by MDMA would be expected to act. We analyzed data from a previously performed study and showed that the non-selective β adrenergic receptor antagonist pindolol reduced MDMA-induced increases in heart rate but had no effect on blood pressure or body

temperature elevations produced by MDMA. In the third study, we assessed the effects of the postsynaptic α_1 and β adrenergic receptor antagonist carvedilol on the cardiostimulant, thermogenic, and subjective response to MDMA in healthy subjects. Carvedilol reduced elevations in blood pressure, heart rate, and body temperature but did not affect the subjective effects produced by MDMA. Thus, α_1 and β adrenergic receptors contribute to the cardiostimulant and thermogenic effects of MDMA in humans. The findings suggest that carvedilol would be useful in the treatment of cardiovascular and hyperthermic complications associated with ecstasy use. In the fourth study, we determined the effect of a pretreatment with the selective α_1 adrenergic receptor blocker doxazosin on the response to MDMA. Doxazosin reduced MDMA-induced increases in blood pressure but did not affect heart rate responses to MDMA. Doxazosin also attenuated some of the subjective effects of MDMA indicating that α_1 adrenergic also contribute to the psychotropic effects of MDMA. Taken together, the studies suggest a role for norepinephrine in particular in the cardio- and the psychostimulant aspects of the MDMA effect in humans. However, MDMA is not only a potent releaser of norepinephrine but also serotonin. Previous clinical studies have shown that inhibition of the serotonin transporter reduced positive psychotropic effects produced by MDMA. However, selective serotonin or norepinephrine transporter inhibitors, when used alone, only moderately affected the response to MDMA in humans. In fifth study we therefore tested the effects of the dual serotonin and norepinephrine transporter inhibitor duloxetine on the acute effects of MDMA in humans. We demonstrated that duloxetine almost completely prevented the pharmacodynamic response to MDMA despite an increase in duloxetine-associated elevation in MDMA plasma levels. The serotonin and norepinephrine transporters may therefore both be potential targets for the treatment of psychostimulant dependence.

In an additional analysis of the Duloxetine-MDMA study, we showed that MDMA increased plasma copeptin, a marker for arginine vasopressin (AVP) secretion, in women but not in men. This sex-difference in the MDMA-induced AVP secretion may explain why hyponatremia is typically reported in female ecstasy users. The copeptin response to MDMA is likely mediated via MDMA-induced release of serotonine and/or noreinephrine because it was prevented by duloxetine which blocks the interaction of MDMA with the serotonin and norepinephrine transporter. Finally, we addressed two research questions that require a larger study sample in pooled analyses across several of our clinical studies. To study the effects of MDMA on social cognition, we investigated the effects of MDMA on the ability to infer mental states of others from social cues of the eye region using the

Reading the Mind in the Eyes Task in 48 subjects. We showed that MDMA differently affected mind reading depending on the emotional valence of the stimuli. MDMA enhanced the accuracy of mental state decoding for positive stimuli (e.g., friendly), impaired mind reading for negative stimuli (e.g., hostile), and did not affect mind reading for neutral stimuli (e.g., reflective). MDMA also produced subjective prosocial effects, including feelings of being more open, talkative, and feeling closer to others. The shift in the ability to correctly read socioemotional information toward stimuli associated with positive emotional valence, together with the prosocial feelings elicited by MDMA, may enhance social approach behavior and sociability when MDMA is used recreationally and facilitate therapeutic relationships in MDMA-assisted psychotherapy settings. In a second pooled analysis, we investigated the effect of MDMA on pupillary function using infrared pupillometry data from 80 subjects from all our five clinical studies. We demonstrated that while MDMA-induced mydriasis is lasting and mirrors the plasma concentration-time curve of MDMA, the impairment in the reaction to a light reflex is associated with the subjective and other autonomic effects induced by MDMA and exhibits acute tolerance. Taken together, these experimental clinical studies contributed to the understanding of the mechanism of action of MDMA in humans.

Introduction

1.1 What is MDMA?

The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA) (Fig. 1) is the main compound found in ecstasy tablets. Ecstasy use is often associated with recreational settings such as nightclubs and raves frequented by young people. An ecstasy tablet generally contains between 80 mg and 150 mg of MDMA[1], although this is highly variable and contamination with other drugs such as methamphetamine, caffeine, and aspirin, is common.[2,3] In 2009, the United Nations drug report estimated that up to 28 million people had used ecstasy at least once in this particular year. A number which remained stable over the past few years.[4] In the late 90ies ecstasy was the second most commonly used drug in Europe[5] and a survey in 2007 in the Swiss population revealed that at least 1.8% of the over 15 years old had consumed ecstasy at least once in their life time.[6]

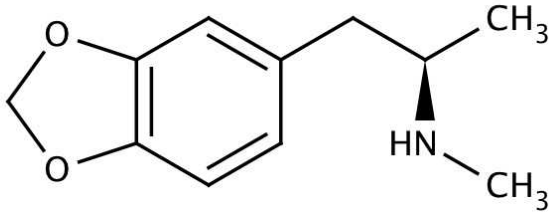

Figure 1 Chemical structure of 3,4-methylenedioxymethamphetamine (MDMA)

As a psychotropic drug, MDMA has remarkable and relatively rare characteristics. When administered to healthy volunteers in a controlled setting, MDMA produces a mental state that is usually enjoyable to almost every subject, almost every time. Most psychotropic substances, like psilocybin or ketamine for example additionally induce feelings of unease, whereas MDMA mostly produces enjoyable drug effects (e.g. “blissful state”) and only minimal negative effects (Fig. 2)[7]. In a great majority of the subjects taking MDMA in a controlled setting, MDMA produces arousal, deep euphoria, closeness to others, and extroversion.[8-10] There are also sex differences in response to MDMA, while women are more sensitive to the drug than men.[9] The onset of the drug takes between 20 and 60 minutes, with peak effects usually occurring 60 to 90 minutes after ingestions. MDMA’s main effects normally last for 3 to 5 hours.[9,11,12] One of the unusual properties of the MDMA effect is that the substance encourages social contacts and breaks down emotional barriers. David Nicholas, co-author of the first publication about the

pharmacology of MDMA, suggested to classify the drug in a novel pharmacological class called "entactogens".[13] Further, MDMA increases oxytocin levels in animals[14] and humans.[15] Oxytocin is a neuro-peptide, which has received abundant attention for its role as a key regulator of emotional and social behavior.[16,17] MDMA produces an increased social behavior in male rats, an effect which can be attenuated by the administration of an oxytocin receptor antagonist prior to MDMA.[14] There might be a connection between the "love drug" and the "love hormone"; but the link between the two has not been fully elucidated.

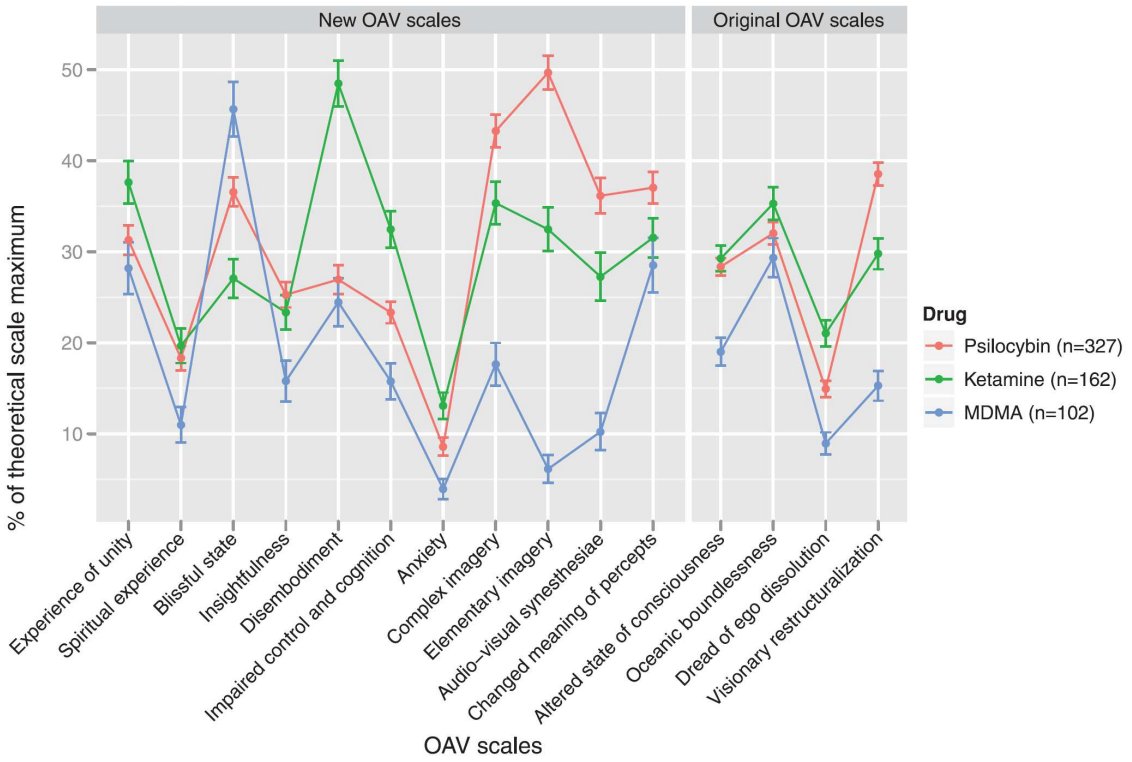

Figure 2 Psychotropic effects produced by psilocybin, ketamine, and MDMA in healthy volunteers. Subjective effects were assessed by the altered state of consciousness (OAV) questionnaire.[7]

MDMA also exerts amphetamine-like stimulant effects including increased alertness and heightened energy level. These effects are very likely linked to the somatic sympathomimetic effects MDMA which include a marked increase in blood pressure, heart rate, pupil size, and slight elevations in body temperature. The sympathomimetic effects of MDMA are responsible for some of the adverse effects of MDMA. Mild adverse effects are common after MDMA ingestion, but-generally short lived. In addition, MDMA might also induce bruxism, trismus, dry mouth, loss of appetite, sweating, or headache.

1.2 Safety and risks of MDMA

Until the late 1980s the use of ecstasy was not controlled by legislation, but since then it has been considered a dangerous narcotic on both sides of the Atlantic and placed in the highest category of harmful drugs, along with heroin and cocaine.[18] The decision of banning MDMA was initiated by the widespread use of ecstasy and partly by the first reports of ecstasy-related deaths among young people.[19 20] Most deaths resulted from a syndrome of persistent hyperthermia, which leads to rhabdomyolysis, with subsequent kidney and other organ-system failure.[21 22] From the 87 ecstasy-associated deaths reported in the literature until 2001, 30 were due to hyperthermia.[22] Also, in a controlled clinical setting, MDMA produced a small increase in body temperature of about 0.5°C.[23-25] This slight increase might not be enough to produce hyperthermia. However, additional factors such as prolonged dancing in a hot environment without rest and a lack of liquid intake increases the risk of hyperthermia. In an attempt to guard against hyperthermia and dehydration, recommendation was given that dancers at rave parties should drink plenty of liquid. Unfortunately, some took this advice too literally and cases of acute hyponatremia occurred among ecstasy users. However, the increased fluid intake seems not to be the only reason for this high prevalence of cases of ecstasy-related hyponatremia. MDMA associated hyponatremia is also possibly due to inappropriate secretion of the hormone arginine vasopressin (AVP) particularly in women.[26-29] A large retrospective series of ecstasy exposures reported to the California poison center found hyponatremia in 73 of 188 cases.[28] Of the 73 cases with hyponatremia, 55 were women and only 18 men. In a small laboratory study MDMA also produced a significant increase in plasma concentrations of AVP.[30,31]

A case series studied 19 366 deaths between January 1997 and July 2000 in New York City. Toxicological post-mortem analysis revealed that only 22 of the cases were considered to be ecstasy related.[32,33] A total of 81 ecstasy use related deaths were reported in the United Kingdom between 1997 and 2000 for which also post-mortem analysis was available.[34] Only six of the total 81 cases appeared to have died after taking ecstasy alone. Most of those who died had been taking other drugs at the same time as ecstasy and more than half had taken heroin or a related opiate.[33] The rare fatalities attributed to ecstasy need to be considered in relation to the very large number of individuals using the drug. In 2007 two groups of experts met to assess the harms of a range of illicit drugs (heroin, cocaine, alcohol, barbiturates, amphetamine, methadone, benzodiazepines, solvents, buprenorphine,

tobacco, steroids, cannabis, LSD, and ecstasy) in an evidence-based fashion.[35,36] The analysis revealed that ecstasy was on the third bottom place for all categories of harm whereas heroin, cocaine, barbiturates, and alcohol were on the top of all.[36]

High production, availability, and consumption of ecstasy[4] render appropriate a contemporary review of the dependence potential of the drug. However, MDMA dependence might be less likely than dependence upon other drugs. Some preclinical studies demonstrated that animals self-administer MDMA,[37,38] whereas other studies failed to document acquisition of MDMA self-administration behavior in animals even after extended training periods.[37] The animal model of self-administration is a widely used test of whether a drug is likely to produce dependence in humans.[39] The mesolibmic-frontocortical system is implicated in the development and maintenance of drug dependence.[39] Acute and chronic use of many drugs of abuse directly or indirectly affect this dopaminergic pathway[40] and MDMA is no exception.[41,42] However, MDMA also releases 5-HT and this effect of MDMA attenuates its reinforcing effects compared with other amphetamines.[41] Co-administration of MDMA also attenuates the reinforcing effects of cocaine[43] and methamphetamine[44,45] in animals, suggesting an interference of MDMA with the DA release actions of these drugs. Together, animal studies have shown that MDMA has weaker effects on the reward system than most other illicit drugs.[46]

There is a large scientific literature on the fact that administration of MDMA to animals results in neurotoxicity, especially to the serotonin system.[47-49] MDMA-induced alterations include serotonin (5-HT) depletion, lower levels of 5-HT metabolites, lower levels of 5-HT transporters, and higher levels of 5-HT_2 receptors.[48,50] These changes mostly represent neuroadaptive changes. Structureal serotonergic neuronal damage including gliosis in is only seen at very high doses of MDMA and in the presence of additional permissive factors such as hyperthermia and inconsistently across species.[47,49,51] A further issue of controversies is that in most animals studies MDMA was administered by injection, where fast drug absorption and high peak plasma levels are reached compared to the slower oral route employed by most human users.[49] Nevertheless, it is clear that high doses of MDMA are neurotoxic to the serotonergic system of animals. The question is, whether the data on the neurotoxicity of MDMA in rodents or primates are relevant for humans. The problem with human neurotoxicity studies are that direct measures of serotonin concentration for example cannot be performed in the human brain. Therefore indirect approaches must be adopted. One approach is to use brain imaging studies. These

experiments assess whether the activity of specific brain regions differs in drug-users, performing a specific task, from the brain activity of non-drug users performing the same task. For example, Bauernfeind and colleagues showed different visual stimuli to MDMA users and matched controls while performing functional magnetic resonance imaging (fMRI). The study revealed that higher lifetime MDMA exposure was associated with increased activation in specific serotonergic areas in the brain.[52] This so called increase in cortical excitability was then interpreted as a loss of serotonergic neurons.[52] Two other neuro-imaging studies, that also included ex-ecstasy users as subjects found that the subjects who had stopped using ecstasy had no reductions in serotonin transporter densities, suggesting that the changes observed in current users are reversible.[53,54]

A further indirect method to assess MDMA's neurotoxicity is to test whether cognitive impairments are consistently found in ecstasy users and whether they persist after drug use. There are various reviews on studies demonstrating that ecstasy users display negative residual effects on various cognitive tasks, especially on lowered verbal memory.[55-57] However, such naturalistic studies always suffer from methodological issues. For example the non-user controls in most studies were not members of the 'rave' subculture. Thus, unlike ecstasy users, they were not exposed to repeated sleep and liquid deprivation from all-night dancing, both factors which can also produce cognitive deficits.[58,59] An additional problem of almost all studies is that the ecstasy users included in the studies reported an extensive life-time history of other drug use, including cocaine, methamphetamines, cannabis, or hallucinogens –which might themselves produce neurotoxic effects. In a recent study Halpern and colleagues have overcome those methodological issues and found that of 15 neuropsychological tasks ecstasy users performed as well as controls.[58] Simply the Revised Strategy Application Test showed a clear indication of poorer performance in heavy ecstasy users, suggesting poorer strategic self-regulation and hence perhaps greater reflection impulsivity.[58] If impulsivity is an effect of ecstasy use or a cause for substance abuse is a matter of considerable debate.

1.3 What do we know about the pharmacology of MDMA?

Like other amphetamines MDMA works indirectly by causing an acute and rapid release of presynaptic monoamine transmitters including serotonin (5-HT), dopamine (DA), and norepinephrine (NE).[60-62] The MDMA-induced release of 5-HT, DA and NE is thought to be due to reverse-transport of these monoamines through the corresponding

uptake transporters, resulting in increased concentrations of the monoamines in the synaptic cleft. As in all phenethylamines of the amphetamine class, MDMA has a chiral center and therefore two enantiomers the (*S*)- and (*R*)-isomer.[63] The (*S*)-MDMA isoform is thought to be more active than the (*R*)-MDMA isoform.[63] MDMA also binds to the classical neurotransmitter receptors such as 5-HT_2, α_2 adrenergic, M_1 muscarinic, and histamine H_1 receptors.[64] While the neurochemical effects of MDMA have been relatively well studied in preclinical models, it is less clear how the neurochemistry translates into the psychotropic and physiological effects in humans. Pretreatment with selective serotonin reuptake transporters (SSRIs), drugs typically used in the treatment of depression or anxiety disorders, decreased the MDMA-induced release of serotonin in the rat brain[62,65] and the behavioral response to MDMA in animals.[66,67] In healthy volunteers, the SSRIs citalopram, fluoxetine, and paroxetine partially reduced the subjective and somatic response to MDMA.[12,25,68,69] These findings confirmed that the interaction of MDMA with the serotonin uptake transporter is one mode of action of MDMA. Additional studies in healthy subjects demonstrated that the moderate hallucinogen-like perceptual changes produced by MDMA were reduced after pretreatment with a postsynaptic 5-HT_2 antagonist[70] while blockade of 5-HT_1 receptors only slightly attenuated positive mood effects of MDMA.[71] MDMA also releases dopamine from cerebral tissue, as has been shown by both in vivo microdialysis[72-75] and by in vitro studies using tissue slices.[76,77] However, the involvement of the dopamine uptake site in MDMA-induced dopamine release is controversial. Whereas some *in vitro* studies demonstrated an inhibition of the MDMA-induced dopamine release by selectively blocking the dopamine transporter,[60,78] others were not able to show this effect.[65] There is also evidence that dopamine release might not be directly involved in the mechanism of action of MDMA but via 5-HT_2 receptor stimulation.[72] This mechanism is supported by the fact that MDMA-induced striatal dopamine release is reduced by pretreatment with fluoxetine.[74]

In a gene knock-out model, mice with a dopamine D_1, D_2, and D_3 receptor deletion did not produced MDMA-induced locomotor effects. In humans, the D_2 receptor antagonist haloperidol attenuated positive mood effects produced by MDMA.[79] However, haloperidol alone produced dysphoric mood effects in healthy subjects compared to placebo, possibly explaining the attenuation on MDMA-induced euphoric mood effects.[79]

The mesolimbic dopamine plays an important role in the mediation of reward and reinforcement but to which extend the dopaminergic system is involved in the mechanism of action of MDMA remains unclear.

MDMA is rapidly absorbed after oral administration.[80-82] There are two main metabolic pathways for MDMA in humans. MDMA is mostly metabolized to 3,4-dihydroxymethamphetamine (HHMA) that is then rapidly methylated to the main metabolite found in plasma and urine; 4-hydroxy-3-methoxymethamphetamine (HMMA).[80,82] The active but minor metabolite 3,4-methylenedioxyamphetamine (MDA) is formed by *N*-demethylation of the mother substance.[51,82]

1.4 Role of norepinephrine in the pharmacology of MDMA

The noradrenergic system can be divided into the central and the peripheral noradrenergic pathways. The main chemical messenger of the noradrenergic system NE and serves as a transmitter to manifold brain functions including arousal, attention, mood, learning, memory and the stress response.[83] The central noradrenergic neurons are localized in brainstem nuclei, such as the locus ceruleus, but noradrenergic nerves project diffusely to almost every part of the brain.[84] In the neurons, NE is synthesized from the amino acid tyrosine, which is supplied by the blood and extracellular fluid.[85] To date, little is known about the role of NE in the mediation of the effects of psychostimulants such as MDMA. To investigate the ability of MDMA and other drugs to bind to the presynaptic transporter and release the corresponding monoamines (Fig 3), nerve endings were isolated from rat brain and studied *in vitro*.[60,62] These studies revealed that MDMA binds to the NE transporter with the highest affinity compared to 5-HT or DA. In addition MDMA also releases NE more potently than it releases 5-HT or DA.[60] This finding was confirmed in *in vitro* models using human cells transfected with human monoamine transporters.[78] Finally, it is also highly likely for NE to play an important role in mediating peripheral effects of MDMA. In animals, MDMA dramatically increased circulating plasma levels of NE, causing a positive chronotropic effect and vasoconstriction, both effects being blocked by the NE transporter inhibitor desipramine.[48] In rats, the postsynaptic α_1 adrenergic receptor antagonist prazosin reversed MDMA-associated locomotor stimulation[86] or vascular effects.[87] In addition, blockade of the effects of NE at postsynaptic α_1 and β adrenergic receptors reversed the hyperthermic response to MDMA in rats or mice.[88,89] In healthy

volunteers, MDMA increases heart rate, blood pressure and body temperature via stimulation of the sympathetic nervous system.[11,25]

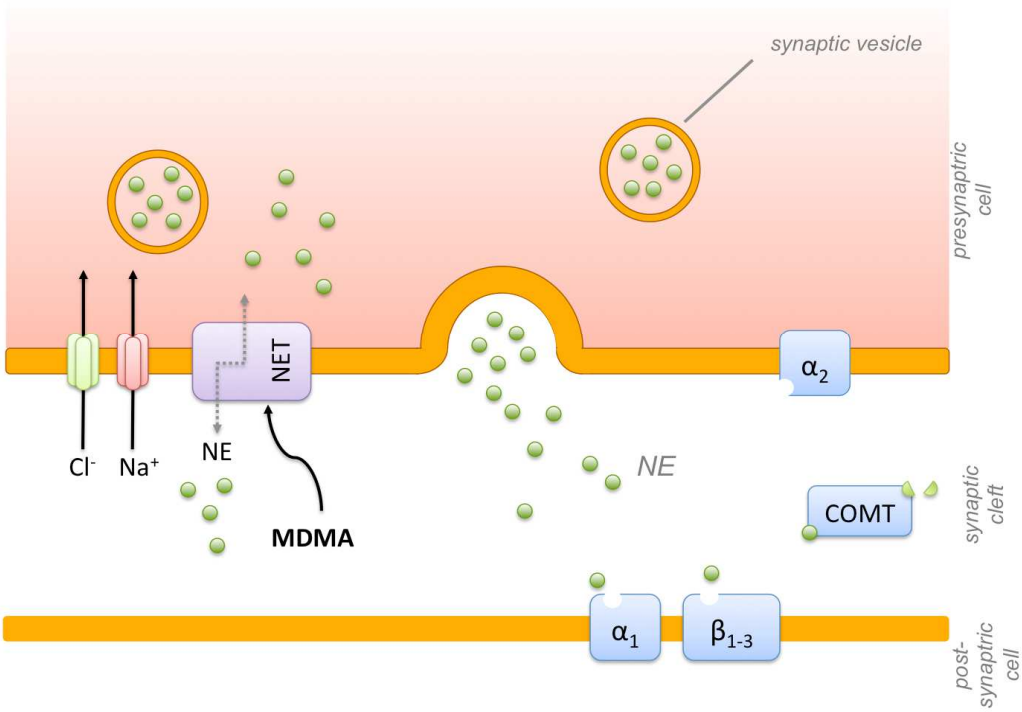

Figure 3 Norepinephrine (NE) is usually released into the synaptic cleft, where it is then inactivated by the catechol-*o*-methyl transferase (COMT) or carried back into the presynaptic cell via the norepinephrine transporter (NET). Preclinical models have shown that MDMA binds to the norepinephrine transporter (NET) and releases norepinephrine (NE).

In rats, the postsynaptic α_1 adrenergic receptor antagonist prazosin reversed MDMA-associated locomotor stimulation[86] or vascular effects.[87] In addition, blockade of the effects of NE at postsynaptic α_1 and β adrenergic receptors reversed the hyperthermic response to MDMA in rats or mice.[88,89] In healthy volunteers, MDMA increases heart rate, blood pressure and body temperature via stimulation of the sympathetic nervous system.[11,25] Serious adverse effects of uncontrolled ecstasy use also include cardiovascular and hyperthermic reactions that are likely to be mediated by a MDMA-induced activation of the adrenergic system.[90] The cardiovascular effects evoked by MDMA are only partly attenuated by serotonin transporter inhibition[12,68] and largely unaffected by postsynaptic serotonergic receptor antagonists.[70,71] Further support for a crucial role of NE in the effects

of psychostimulants derives from the clinical findings that the NE inhibitor atomoxetine attenuated cocaine-induced systolic blood pressure increases[91] and cardiovascular and subjective responses to *D*-amphetamine.[92] Together, these preclinical and clinical findings suggest that NE may critically be involved in the mediation of the psychotropic and particularly the cardiovascular and thermogenic effects of psychostimulants including MDMA in humans.

In addition, the noradrenergic system may play an important role in stimulant addiction.[85] Pre-clinical models show that NE is critically involved in the mediation of the effects of psychostimulants including sensitization, drug discrimination, and reinstatement of drug seeking.[93,94]

1.5 Significance

Consumption of psychostimulants including MDMA is highly prevalent in our society. In Switzerland, more than 1.8% of the over 15 year olds report having used ecstasy at least once in their life.[6] Besides from being abused as an illicit drug MDMA is also used experimentally in clinical research. For example, a recent pilot study with patients suffering from post-traumatic stress disorders (PTSD) has shown that MDMA positively affected the outcome of psychotherapy.[95] MDMA could also be useful to study mood disorders[96,97] and the neuropharmacology of mood in humans.[15,96] Recreational use of MDMA as ecstasy can result in adverse and potentially fatal medical complications.[90,98] Extensive ecstasy consumption has also been associated with neurotoxic effects to serotonergic brain neurons.[48,52] Because MDMA is widely used, a better understanding of its pharmacology and toxicology is warranted. A better understanding of the clinical pharmacology and toxicology of MDMA will also help in the treatment of intoxications with MDMA and other amphetamine-type stimulants. Finally, selective norepinephrine transporter inhibitors or dual norepinephrine and serotonin transporter inhibitors, as suggested to be evaluated in this thesis, may provide treatments for amphetamine dependence.

1.6 Aims & Hypothesis

The main aim of this thesis was to investigate the role of NE in the mediation of the effects of MDMA in humans. As previously discussed (1.4 Role of norepinephrine in the pharmacology of MDMA), preclinical and clinical studies implicate NE in the mediation of

the effects of psychostimulants, including MDMA. Our overall hypothesis was that an inhibition of noradrenergic targets would reduce the acute effects of MDMA. Consequently, we performed a series of experimental clinical studies to assess the effect of different pretreatments acting on noradrenergic binging sites on the pharmacodynamic and pharmacokinetic response to MDMA in humans.

In the first study, we investigated the effects of the NE transporter inhibitor reboxetine on the subjective, somatic, and neuroendocrine response to MDMA and also studied the pharmacokinetics of both drugs. Based on the preclinical evidence we hypothesized that the pretreatment with reboxetine would reduce acute effects of MDMA to the extent that they depend on NE transporter-mediated NE release. To evaluate the role of impulse-dependent monoamine release in the mediation of the effects of MDMA we similarly evaluated the effect of the α_2 agonist clonidine, an inhibitor of the vesicular NE release, on the response to MDMA. We postulated that clonidine would reduce the subjective and cardiovascular effects of MDMA to the extent that the clinical effects of MDMA are mediated by the exocytotic release of NE. In a further study we then tested the effect of the dual 5-HT and NE transporter blocker duloxetine on the response to MDMA using the same outcome measurements. The hypothesis of this study was that duloxetine would block the transporter-mediated release of 5-HT and NE and therefore significantly reduce the acute subjective, cardiovascular, and neuroendocrine effects induced by MDMA. We then similarly evaluated the effect of the α_1 and β adrenergic receptor blocker carvedilol and of the α_1 adrenergic antagonist doxazosin on the response to MDMA. We postulated that carvedilol would reduce the heart rate, blood pressure and subjective response to MDMA. The hypothesis for a pretreatment with the α_1 adrenergic antagonist doxazosin was that doxazosin would lead to a reduction of the blood pressure elevations and the acute emotional effects evoked by MDMA.

All studies used randomized double-blinded cross-over study designs and 16 healthy volunteers were included in each study.

In addition, we investigated the effect of MDMA on emotion recognition or pupillary function using pooled study data.

Paper One: Role of Carrier-Mediated NE Release in the Psychostimulant Effects

The Norepinephrine Transporter Inhibitor Reboxetine Reduces Stimulant Effects of MDMA ("Ecstasy") in Humans

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This study assessed the pharmacodynamic and pharmacokinetic effects of the interaction between the selective norepinephrine (NE) transporter inhibitor reboxetine and 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in 16 healthy subjects. The study used a double-blind, placebo-controlled crossover design. Reboxetine reduced the effects of MDMA including elevations in plasma levels of NE, increases in blood pressure and heart rate, subjective drug high, stimulation, and emotional excitation. These effects were evident despite an increase in the concentrations of MDMA and its active metabolite 3,4-methylenedioxyamphetamine (MDA) in plasma. The results demonstrate that transporter-mediated NE release has a critical role in the cardiovascular and stimulant-like effects of MDMA in humans.

3,4-Methylenedioxymethamphetamine (MDMA, "ecstasy") is widely used as a recreational drug, but it is also being investigated as an adjunct to psychotherapy in patients with post-traumatic stress disorder.[1] In humans, MDMA produces euphoria, happiness, and cardiovascular activation.[2–4] *In vitro*, MDMA induces carrier-mediated release of dopamine (DA), serotonin (5-HT), and norepinephrine (NE) through DA (DAT), 5-HT (SERT), and NE transporter (NET), respectively.[5–9] However, it is not clear how these monoamines contribute to the acute psychostimulant effects of MDMA in humans.[8,10]

The role of DA in the reinforcing effects of psychostimulants is well established in animal models. However, deletions of dopamine D_1, D_2, and D_3 receptor genes in mice had minimal effects on MDMA-induced acute changes in locomotor behavior,[11] and DAT inhibition did not affect acute responses to MDMA in rhesus monkeys.[12] In humans, DA D_2 receptor antagonists reduced amphetamine-induced and MDMA-induced euphoria only at doses that produced dysphoria.[13–15] Therefore, non-DA systems may be principally responsible for the acute effects of MDMA.

SERT inhibitors (SSRIs) decrease MDMA-induced 5-HT release *in vitro*[7] and in animals[16] and also attenuate behavioral effects of MDMA in animals.[17] Consistent with this preclinical evidence for a role of SERT, SSRIs reduced the subjective and cardiovascular response to MDMA in humans,[18–21] indicating that MDMA-induced, SERT-mediated 5-HT release critically contributes to the psychotropic and physical effects of MDMA in humans. However, the blood pressure response to MDMA is only partly attenuated by blockade of 5-HT release[18] and is largely unaffected by postsynaptic 5-HT_1 or 5-HT_2 receptor antagonist pretreatment.[22,23]

The role of the NET in the mechanism of action of MDMA in humans has not yet been explored. As compared to SERT and DAT, MDMA exhibits higher affinity for human NET.[5,6] MDMA releases NE more potently than 5-HT or DA from monoamine-preloaded human embryonic kidney (HEK) cells transfected with the corresponding human monoamine transporter.[6] The NET inhibitor desipramine and the SERT inhibitor citalopram, but not the DAT/NET inhibitor methylphenidate, reversed the acute cognitive effects of MDMA in rhesus monkeys.[12] NE also plays a role in mediating the peripheral effects of MDMA. MDMA increases the levels of circulating NE in rats.[24] The adrenergic α_1 receptor antagonist prazosin reversed MDMA-associated locomotor stimulation[25] and vascular effects[26] in rats. The NET inhibitor nisoxetine abolished contraction of the rat aorta produced by 4-methylthioampethamine,[27] a compound with a pharmacology similar to that of MDMA. Clinically, MDMA increases plasma NE levels[4] and stimulates

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Table 1 Mean ± SEM values and statistics of drug effects

		Placebo–placebo (mean ± SEM)	Reboxetine–placebo (mean ± SEM)	Placebo–MDMA (mean ± SEM)	Reboxetine–MDMA (mean ± SEM)	F(3,45) =	P <
Circulating catecholamines							
Epinephrine (nmol/l)	At 1 h	0.11 ± 0.02	0.08 ± 0.02	0.35 ± 0.08*	0.21 ± 0.03	6.70	0.001
Norepinephrine (nmol/l)	At 1 h	1.29 ± 0.16	1.18 ± 0.15	2.05 ± 0.20**	1.31 ± 0.13††	12.40	0.001
Physiologic effects							
SBP (mm Hg)	E_{max}	11.1 ± 2.1	8.5 ± 1.5	38.7 ± 2.6***	20.7 ± 2.6*,†††	33.50	0.001
DBP (mm Hg)	E_{max}	8.6 ± 1.5	5.2 ± 1.0	20.8 ± 1.7***	15.8 ± 1.9*	17.40	0.001
MAP (mm Hg)	E_{max}	8.0 ± 1.4	5.1 ± 1.0	25.8 ± 1.9***	16.2 ± 1.9**,††	30.80	0.001
Heart rate (beats/min)	E_{max}	11.3 ± 1.9	12.3 ± 2.4	31.7 ± 3.1***	23.4 ± 3.0**,†	17.90	0.001
Body temperature (°C)	E_{max}	0.41 ± 0.06	0.46 ± 0.07	0.74 ± 0.10*	0.51 ± 0.09	3.40	0.05
Visual analog scales (VAS, %max)							
Any drug effect	E_{max}	1.9 ± 1.3	8.0 ± 3.4	85.4 ± 4.8***	67.7 ± 6.2***,††	120.40	0.001
Drug high	E_{max}	4.9 ± 3.7	9.3 ± 4.6	86.31 ± 4.0***	65.3 ± 8.1***,†	61.10	0.001
Stimulated	E_{max}	2.6 ± 2.6	3.4 ± 2.5	71.9 ± 8.0***	51.1 ± 9.4***,†	34.00	0.001
Closeness	E_{max}	0.25 ± 0.19	0.00 ± 0.00	33.9 ± 5.9***	20.8 ± 4.5***,†	22.70	0.001
Good drug effect	E_{max}	0.06 ± 0.06	6.3 ± 3.4	85.6 ± 4.1***	72.6 ± 7.2***	114.60	0.001
Liking	E_{max}	3.1 ± 3.1	15.6 ± 7.0	86.6 ± 4.7***	77.9 ± 5.1**	75.60	0.001
Adjective Mood Rating Scale (AMRS score)							
Activity	E_{max}	1.56 ± 0.52	1.38 ± 0.45	3.81 ± 0.98*	4.19 ± 1.02**	4.80	0.05
Inactivation	E_{max}	1.88 ± 0.82	3.56 ± 1.03	8.19 ± 2.06	6.50 ± 1.73	4.80	0.05
Extroversion	E_{max}	1.06 ± 0.31	1.25 ± 0.57	4.00 ± 078*	4.13 ± 0.83**	10.10	0.001
Introversion	E_{min}	−0.44 ± 0.20	−1.06 ± 0.35	−3.00 ± 0.82***	−1.19 ± 0.39††	6.20	0.001
Well-being	E_{max}	1.75 ± 0.37	2.56 ± 0.83	6.50 ± 1.28**	6.25 ± 1.46*	16.60	0.001
Emotional excitation	E_{max}	0.44 ± 0.27	1.53 ± 0.69	7.50 ± 1.20***	3.94 ± 1.14*,†	14.20	0.001
Anxiety-depression	E_{max}	0.31 ± 0.18	1.25 ± 0.50	2.25 ± 0.71	1.38 ± 0.62	2.90	0.05
Dreaminess	E_{max}	0.69 ± 0.25	1.63 ± 0.46	4.00 ± 0.74***	3.19 ± 0.68**	7.30	0.001
State-Trait Anxiety Inventory	E_{max}	1.44 ± 0.58	2.19 ± 0.98	10.19 ± 2.36**	3.81 ± 1.06†	9.40	0.001
List of complaints (total score)							
Acute adverse effects	At 3 h	−1.00 ± 0.53	−0.19 ± 1.30	10.13 ± 1.90***	1.94 ± 2.80†	7.73	0.001
Subacute adverse effects	At 24 h	−1.19 ± 0.63	0.06 ± 0.92	5.38 ± 1.47**	0.44 ± 1.33†	5.95	0.01
Ex vivo *binding (K_i, %)*							
NET		24.00 ± 1.28	9.09 ± 1.66***	22.17 ± 1.66	7.16 ± 0.83***,†††	48.02	0.001
SERT		>25	>25	>25	>25		
DAT		>25	>25	>25	>25		

Values are mean ± SEM of changes from baseline of 16 subjects.

DAT, dopamine transporter; DBP, diastolic blood pressure; E_{max}, peak effects; E_{min}, minimum effects; K_i, inhibition constant calculated as % of plasma sample dilution with undiluted plasma set as 100%; MAP, mean arterial pressure; MDMA, 3,4-methylenedioxymethamphetamine; NET, norepinephrine transporter; SBP, systolic blood pressure; SERT, serotonin transporter.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to placebo–placebo. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared to placebo–MDMA.

the sympathetic nervous system, as evidenced by increases in heart rate, blood pressure, pupil size, and body temperature.[18] Serious adverse effects of uncontrolled ecstasy use also include hypertensive and hyperthermic reactions that are likely to be mediated by an activation of the adrenergic system by MDMA.[28] The importance of NE in the mechanism of action of amphetamine-type stimulants in general is further supported by the observation that the subjective effects of these stimulants in humans correlate with their potency to release NE and not with their effect on DA.[8] Further support for a role of the NET in the effects of psychostimulants derives from the clinical findings that the NET inhibitor atomoxetine attenuated cocaine-induced systolic blood pressure increases[29] and cardiovascular and subjective responses to D-amphetamine in humans.[30] Together, the preclinical and clinical findings suggest that NE may contribute critically to the psychotropic and, even more importantly, the

cardiovascular effects of MDMA in humans. This study evaluated pharmacokinetic and pharmacodynamic effects of the interaction between the selective NET inhibitor reboxetine and MDMA in healthy subjects. We hypothesized that pretreatment with reboxetine would attenuate the subjective, neuroendocrine, cardiovascular, and adverse effects of MDMA to the extent that they depend on NET-mediated release of NE.

Pharmacokinetic interactions were evaluated to confirm that the effects of reboxetine on the MDMA response could not be explained by the exposure to MDMA or its active metabolites being too low. MDMA is *N*-demethylated to the active, but minor, metabolite 3,4-methylenedioxyamphetamine (MDA) by cytochrome P450 (CYP) 2B6 and 3A4. The major pathway of MDMA degradation includes CYP2D6-mediated O-demethylation to 3,4-dyhydroxymethamphetamine (HHMA), followed by catechol-*O*-methyltransferase-catalyzed methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA).[31]

RESULTS

Pharmacodynamics

MDMA increased the levels of both circulating NE and epinephrine relative to placebo. Reboxetine prevented the MDMA-induced increase in NE, an endocrine correlate of sympathetic activation (Table 1). It also reduced the cardiovascular and psychostimulant effects of MDMA. Reboxetine decreased MDMA-induced elevations in blood pressure and heart rate (Figure 1 and Table 1) and attenuated MDMA-induced visual analog scale (VAS) score increases in "any drug effect," "drug high," "stimulated," and "closeness to others" (Figure 2 and Table 1). In contrast, reboxetine did not affect MDMA-induced VAS score changes with regard to "good drug effect" and "drug liking" (Figure 2 and Table 1). On the 5-Dimensions of Altered States of Consciousness (5D-ASC) Rating Scale, analysis of variance showed significant main effects of the drug in the sum score and in all the dimensions of the scale ($F_{(3,45)}$ = 32.8, 32.9, 8.5, and 17.8 for ASC, oceanic boundlessness (OB), anxious ego dissolution (AED), and visionary restructuralization (VR), respectively; all $P < 0.001$). MDMA robustly increased scores in the OB, AED, and VR dimensions relative to placebo (all $P < 0.001$) (Figure 3). Reboxetine reduced MDMA's effect on the total ASC score ($P < 0.01$) in the OB dimension ($P < 0.01$) and in the VR dimension ($P < 0.05$), including significant reductions in OB item clusters for "experience of unity" ($P < 0.01$) and "blissful state" ($P < 0.1$). With respect to the Adjective Mood Rating Scale (AMRS) scale, reboxetine prevented MDMA-induced increase in emotional excitation and decrease in introversion (Figure 4 and Table 1). MDMA increased a sense of well-being, extroversion, and dreaminess and produced inactivation at 1.25 h and activation at 2 h, which resulted in peak increases in both activity and inactivation relative to placebo. Reboxetine had no effect on these subjective effects associated with MDMA. Finally, reboxetine reduced MDMA-induced elevations in State-Trait Anxiety Inventory (STAI) anxiety scores (Figure 4 and Table 1).

Adverse effects

MDMA increased the total list of complaints adverse effects score at 3 h and again at 24 h after administration, relative to placebo (Table 1). The most frequently reported adverse effects of placebo–MDMA and reboxetine–MDMA included lack of appetite (n = 12 and n = 8, respectively), difficulty in concentrating (n = 12 and n = 12, respectively), tremor (n = 9 and n = 3, respectively), restlessness (n = 8 and n = 4, respectively), and dizziness (n = 6 and n = 4, respectively). Reboxetine decreased the number of MDMA-induced adverse effects (Table 1).

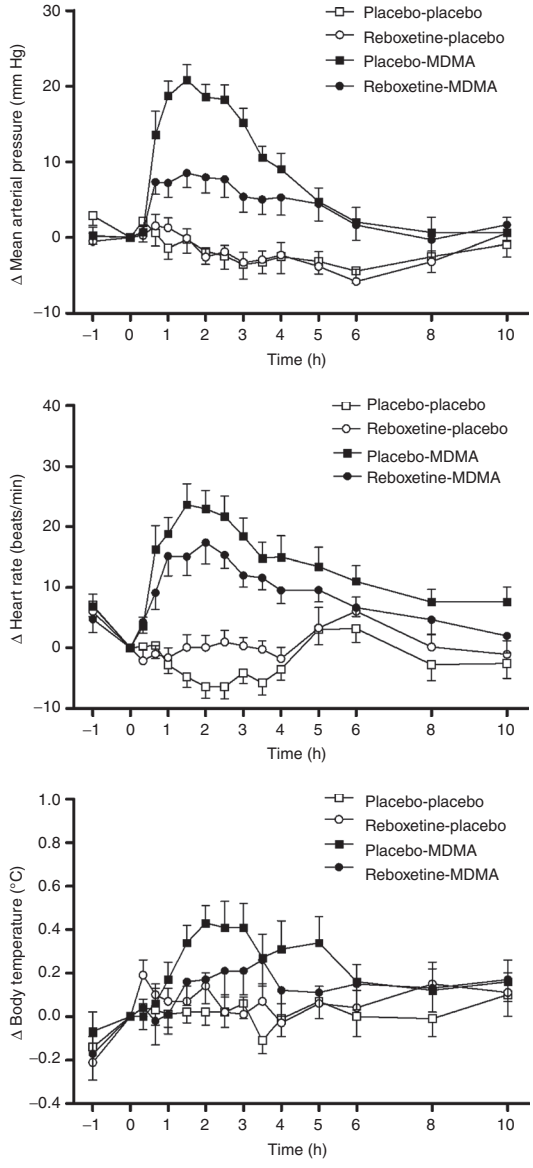

Figure 1 Physiologic effects. Values are mean ± SEM of changes from baseline in 16 subjects. Reboxetine was administered at t = −12 h and at t = −1 h. 3,4-Methylenedioxymethamphetamine (MDMA) was administered at t = 0 h. Reboxetine pretreatment reduced MDMA-induced elevations in blood pressure and heart rate.

Pharmacokinetics

The decrease in the pharmacodynamic response to MDMA after reboxetine pretreatment is not attributable to a pharmacokinetic interaction between reboxetine and MDMA because reboxetine was shown to increase exposure to MDMA. Reboxetine increased the maximum concentration

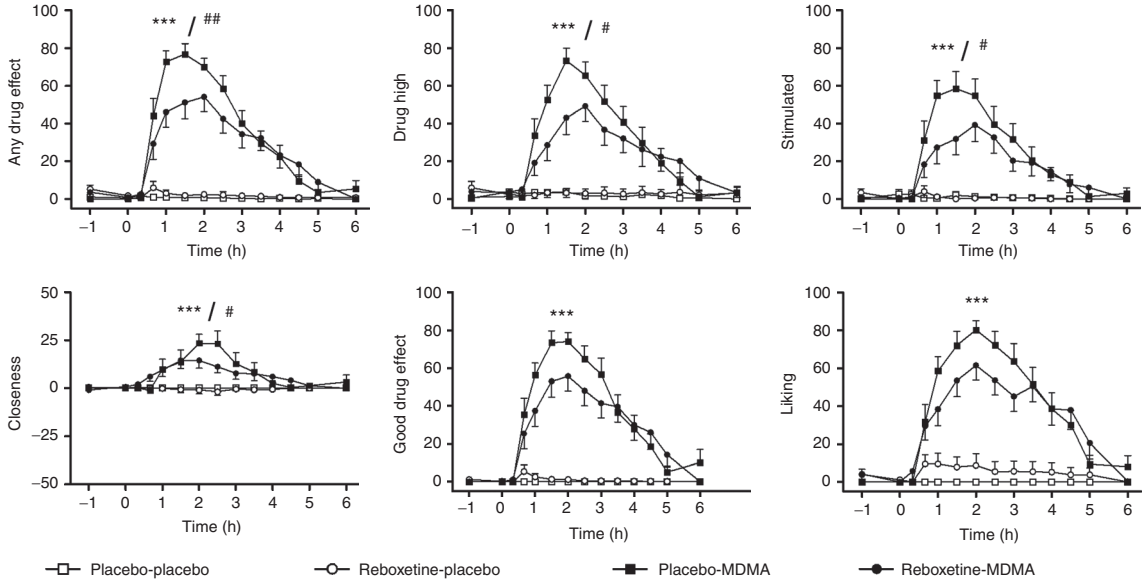

Figure 2 Time courses of subjective visual analog scale (VAS) ratings. Values are mean ± SEM of % maximal values in 16 subjects. Reboxetine decreased 3,4-methylenedioxymethamphetamine (MDMA)-induced elevations in scores for "any drug effect," "drug high," "stimulated," and "closeness to others." ***$P < 0.001$ for peak-score differences between placebo–placebo and placebo–MDMA. #$P < 0.05$ and ##$P < 0.01$ for peak-score differences between placebo–MDMA and reboxetine–MDMA.

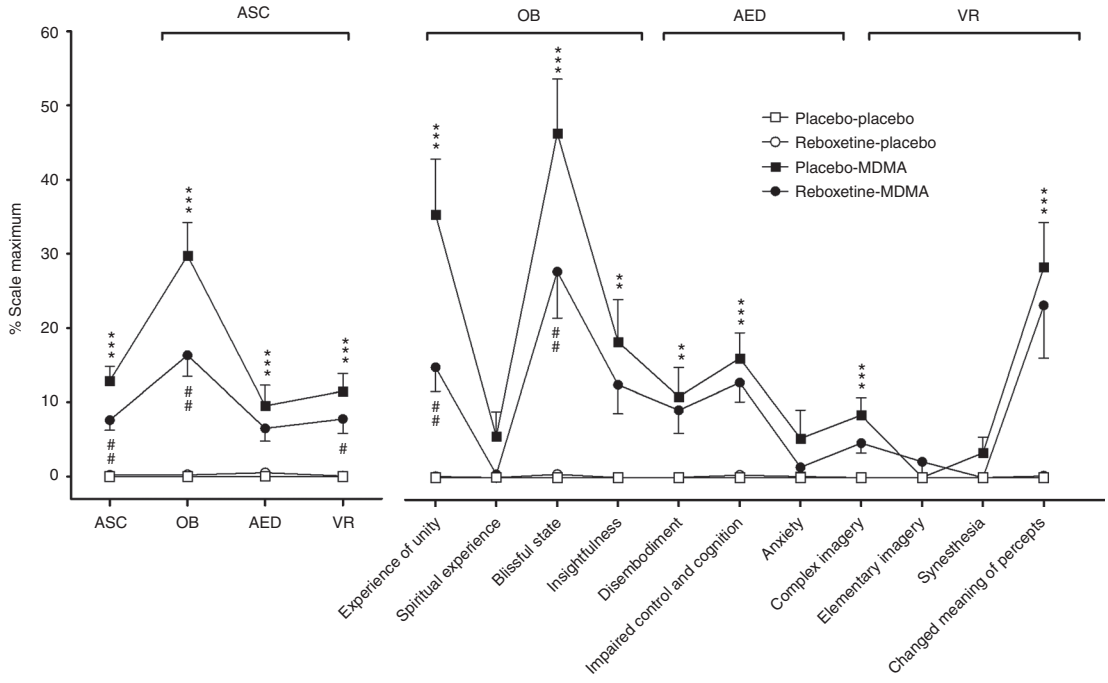

Figure 3 The 5-Dimensions Altered States of Consciousness (5D-ASC) scale. Values are mean ± SEM in 16 subjects. 3,4-Methylenedioxymethamphetamine (MDMA) elicited mainly "experience of unity," "a blissful state," and "changed meaning of percepts." Reboxetine reduced MDMA's effect in the OB dimension, including significant reductions in "experience of unity" and "blissful state." **$P < 0.01$ and ***$P < 0.001$ as compared to placebo–placebo. #$P < 0.05$ and ##$P < 0.01$ as compared to placebo–MDMA. AED, anxious ego dissolution; ASC, altered states of consciousness (sum of the scores for OB, AED, and VR); OB, oceanic boundlessness; VR, visionary restructuralization.

(C_{max}) of MDMA by 19 ± 6% ($F_{(1,15)} = 9.23$; $P < 0.01$) and the area under the plasma concentration–time curve $(AUC)_{0-24\,h}$ by 9 ± 4% ($F_{(1,15)} = 5.53$; $P < 0.05$) (**Figure 5a** and **Table 2**). Reboxetine also increased $AUC_{0-24\,h}$ and $AUC_{0-\infty}$ values of MDA by 50 ± 13% ($F_{(1,15)} = 15.98$; $P < 0.001$) and 66 ± 16% ($F_{(1,15)} = 19.03$; $P < 0.001$), respectively (**Figure 5b** and **Table 2**). Conversely, MDMA increased the C_{max} of reboxetine by 16 ± 6% ($F_{(1,15)} = 5.97$; $P < 0.05$) (**Figure 5c** and **Table 2**). The pharmacokinetic parameters of MDMA were not dependent on CYP2D6 phenotype.

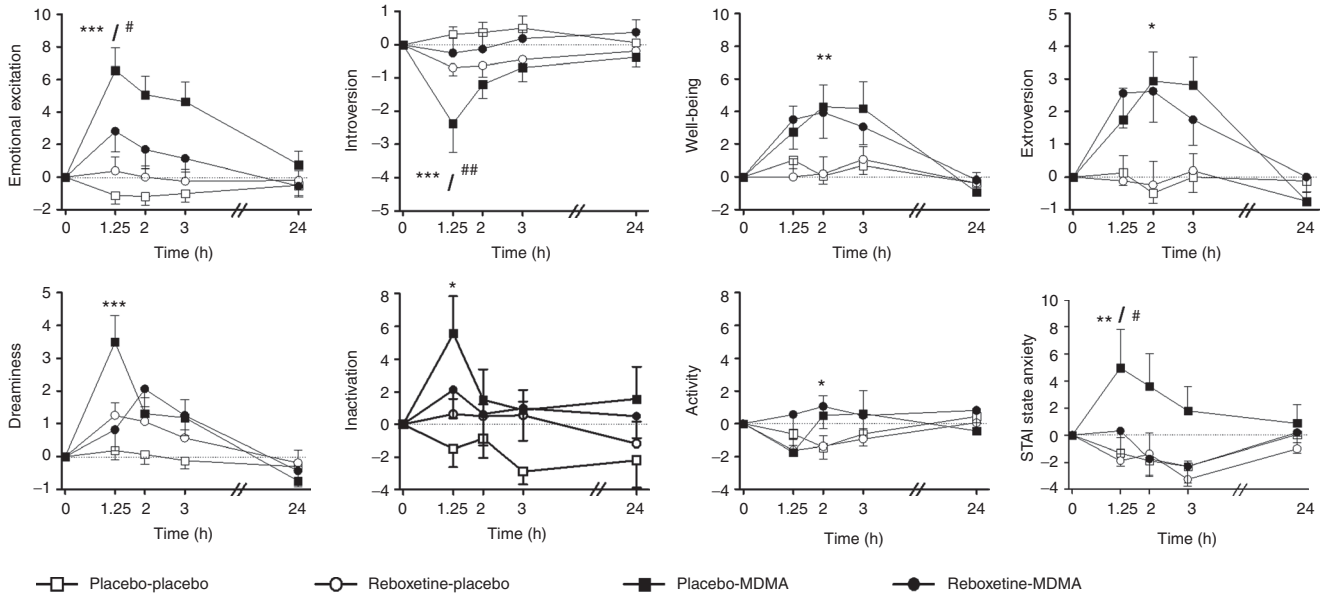

Figure 4 Mood effects in the Adjective Mood Rating Scale (AMRS) and the State-Trait Anxiety Inventory (STAI). Values are mean ± SEM of AMRS/STAI score changes from baseline in 16 subjects. Reboxetine reduced "emotional excitation," and STAI state anxiety produced by 3,4-methylenedioxymethamphetamine (MDMA) and prevented the MDMA-induced decrease in "introversion". $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ for peak-score differences between placebo–placebo and placebo–MDMA. $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ for peak-score differences between placebo–MDMA and reboxetine–MDMA.

Pharmacokinetic–pharmacodynamic relationship

Figure 5d,e shows MDMA's effects in terms of the plasma concentration. MDMA-induced changes in (Figure 5d) mean arterial pressure (MAP) and (Figures 5e) "any drug effect" returned to baseline within 8 h and 6 h, respectively, when MDMA concentrations were still high (clockwise hysteresis). Reboxetine pretreatment attenuated both physical and subjective responses to MDMA (Figure 5d,e).

Ex vivo binding studies

Plasma from subjects treated with reboxetine–placebo or reboxetine–MDMA inhibited *ex vivo* radioligand binding to NET but not to SERT or DAT (Table 1).

DISCUSSION

In this study, pretreatment with the selective NET inhibitor reboxetine prevented MDMA-induced increase in circulating levels of NE, which is a marker of sympathetic system activation, and significantly reduced the cardiovascular response to MDMA. Reboxetine also attenuated some, but not all, of the psychotropic effects of MDMA and reduced MDMA-induced drug high, stimulation, emotional excitation, and anxiety, as well as the blissful state and experience of unity elicited by MDMA. Reboxetine also ameliorated some of the adverse effects of MDMA such as tremor and restlessness. Overall, blockade of NET resulted in a pronounced decrease in the cardiovascular stimulant effects of MDMA and a moderate attenuation of its psychostimulant properties. In contrast, good drug effects and the sense of well-being associated with MDMA were not significantly altered by reboxetine pretreatment. The findings are consistent with a role for NET in the mediation of the sympathomimetic stimulant-like aspects of the MDMA effect.

The pharmacodynamic interaction observed in this study cannot be explained on the basis of a pharmacokinetic interaction between reboxetine and MDMA because reboxetine decreased the pharmacodynamic effects of MDMA even while it increased the C_{max} of MDMA by 20% and the $AUC_{0-24\,h}$ of MDMA and its active metabolite MDA by 10% and 50%, respectively. The potent CYP2D6 inhibitor paroxetine has previously been shown to increase the C_{max} and $AUC_{0-27\,h}$ of MDMA by 20% and 30%, respectively, and of MDA by 20% and 20%, respectively[31] (similar to the effect of reboxetine in our study), whereas it decreased MDMA metabolism to HHMA and HMMA.[31] The effects of paroxetine and reboxetine on MDMA metabolism can therefore be explained by CYP2D6 inhibition[32] and a shift of the metabolism from the major pathway (by reducing HHMA and HMMA formation) to a minor pathway (including an increase in MDA formation). However, we did not measure HHMA and HMMA levels in plasma, and this may be a limitation with respect to the conclusion regarding pathways. Furthermore, MDMA is itself a mechanism-based inhibitor of CYP2D6, and its pharmacokinetics is nonlinear.[33,34] In this study, we used an *ex vivo* binding assay to investigate whether the plasma samples taken from the subjects exhibited NET-binding properties. We confirmed that plasma from reboxetine-treated subjects displaced ^{3}H-nisoxetine from NET and that there was a trend toward this effect with regard to plasma from MDMA-treated subjects. Although both reboxetine and MDMA bind to NET *in vitro* and inhibit NE uptake,[6,35] MDMA is also a NET substrate and releases NE.[6] The pharmacodynamic interaction between reboxetine and MDMA observed in this study is consistent with

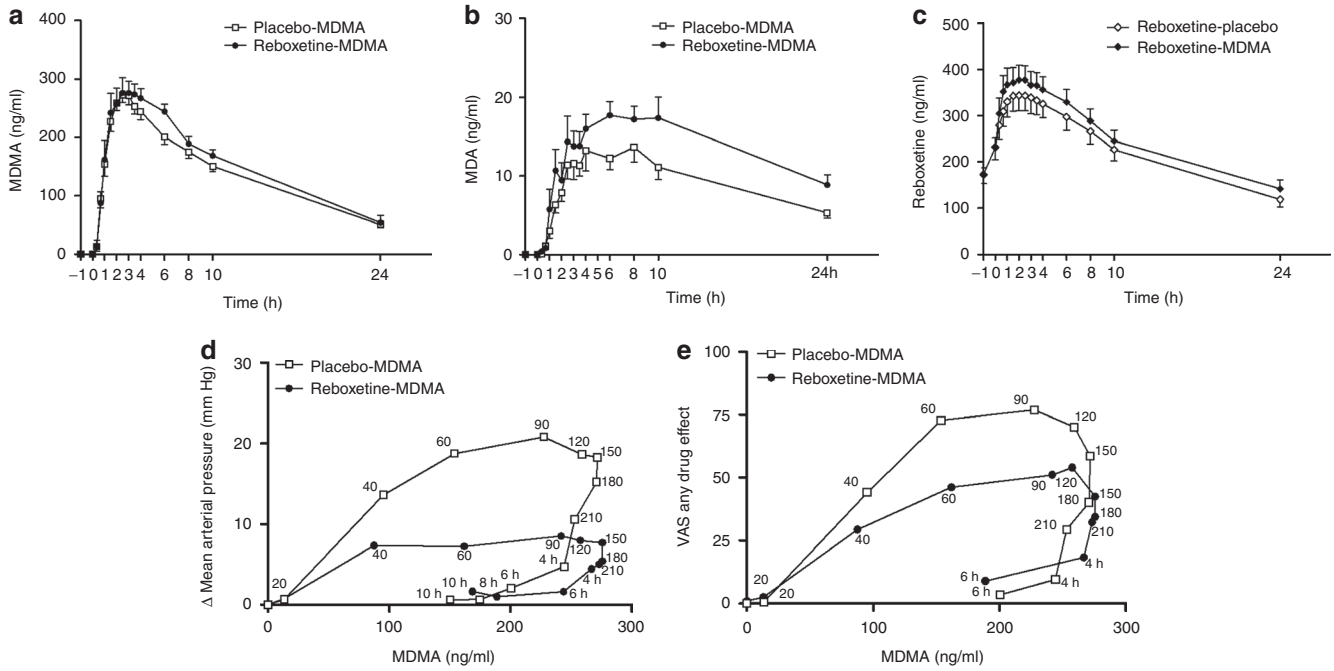

Figure 5 Pharmacokinetics of (**a**) MDMA, (**b**) MDA, and (**c**) reboxetine. The values are mean ± SEM in 16 subjects. Reboxetine was administered at $t = -12$ h and at $t = -1$ h. MDMA was administered at $t = 0$ h. Reboxetine increased the exposure to (**a**) MDMA (C_{max} and $AUC_{0-24\,h}$) and (**b**) MDA ($AUC_{0-24\,h}$). Conversely, (**c**) MDMA increased the C_{max} of reboxetine. (**d**,**e**) MDMA effects plotted against MDMA plasma concentrations. The values are the means of the change from baseline in 16 subjects, with SEM omitted for clarity. The time of sampling is noted next to each point in minutes or hours after MDMA administration. Less effect is seen at the same MDMA concentration at later time points. MDMA-induced changes in mean arterial pressure and "any drug effect" VAS score returned to baseline within 8 h and 6 h, respectively, even when MDMA concentrations remained high. This clockwise hysteresis indicates acute tolerance to the MDMA effect, possibly because of monoamine depletion, resulting in no effect until monoamine stores are refilled. Reboxetine pretreatment lowered both physical and subjective responses to MDMA. $AUC_{0-24\,h}$, area under the plasma concentration–time curve from 0 to 24 h; C_{max}, maximum plasma concentration; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; VAS, visual analog scale.

Table 2 Pharmacokinetic parameters of MDMA, MDA, and reboxetine

	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-24\,h}$ (ng/ml·h)	$AUC_{0-\infty}$ (ng/ml·h)
MDMA					
Placebo–MDMA	298.0 ± 12.3	2.6 ± 0.2	9.6 ± 0.8	3,357.1 ± 159.0	4,127.2 ± 229.2
Reboxetine–MDMA	351.0 ± 19.0**	2.8 ± 0.4	7.0 ± 0.3	3,629.1 ± 167.5*	4,106.2 ± 215.8
MDA					
Placebo–MDA	19.0 ± 2.1	4.7 ± 0.6	16.1 ± 1.8	227.4 ± 19.0	358.6 ± 25.0
Reboxetine–MDA	23.0 ± 2.7	7.0 ± 0.6	17.3 ± 1.9	327.7 ± 24.0**	572.2 ± 48.9***
Reboxetine					
Reboxetine–placebo	371.7 ± 33.9	3.3 ± 0.4	13.7 ± 0.7	5,704.4±599.6	8,292.5 ± 1,042.2
Reboxetine–MDMA	416.9 ± 31.1†	3.6 ± 0.5	13.2 ± 0.9	6,176.2 ± 553.2	9,103.4 ± 1,099.9

Values are mean ± SEM of 16 healthy subjects.

AUC, area under the plasma concentration–time curve; C_{max}, maximum plasma concentration; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; $T_{1/2}$, terminal elimination half-life; T_{max}, time to maximum plasma concentration.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to placebo–MDMA. †$P < 0.05$ compared to reboxetine–placebo.

inhibition of the MDMA-induced NET-mediated NE release by reboxetine.

The role for NET in the mechanism of action of MDMA, as suggested by the results of this study, is in line with both preclinical and clinical data from other studies, as outlined in the introduction. As compared to SERT and DAT, MDMA shows higher affinity to NET *in vitro*[5,6] and releases NE more potently than 5-HT or DA.[6,8] In humans, NET inhibition reduced cardiostimulant responses to cocaine[29] and both cardiostimulant and psychostimulant responses to D-amphetamine,[30] similar to the findings in our study with MDMA.

The SERT inhibitor citalopram has been shown to reduce the cardiovascular and subjective effects of MDMA in humans.[18,19] Citalopram reduced MDMA-induced increases in systolic blood pressure and emotional excitation by <50% and positive mood effects by >50%. In contrast, reboxetine reduced MDMA-

induced increases in systolic blood pressure and emotional excitation by >50% and positive mood effects by <50%. The two studies indicate that SERT-mediated 5-HT release is more important than NET-mediated NE release for MDMA-typical positive mood effects, whereas NET-mediated NE release primarily mediates the more stimulant-typical emotional excitation and cardiovascular response to MDMA. The effect of reboxetine on subjective responses to MDMA is similar to changes in the subjective effects of D-amphetamine after pretreatment with the NET inhibitor atomoxetine.[30] As in our study, NET inhibition reduced amphetamine-induced increases in subjective ratings of "stimulated" and "high" but not in "drug liking,"[30] thereby reinforcing the view that NET contributes mainly to the psychostimulant aspect of amphetamines.[36]

DA is commonly thought to mediate the reinforcing and rewarding effects of drugs of abuse. For example, the DAT/NET inhibitor methylphenidate has been shown to reduce intravenous amphetamine use in amphetamine-dependent patients.[37] The role of DA in the mediation of the acute subjective effects of amphetamine-type stimulants in humans is less clear. The DAT/NET inhibitor bupropion was shown to attenuate subjective responses to methamphetamine.[38] However, the effect of DAT inhibition on the acute response to MDMA has not been studied in humans. We have previously shown that the DA D_2 antagonist haloperidol reduces the positive mood elicited by MDMA and that haloperidol depresses mood also when given alone, as compared to the effect of placebo.[15] Similarly, DA D_2 receptor blockade did not affect subjective responses to D-amphetamine, according to the results of most studies.[13,14] Accordingly, DA may primarily mediate the reinforcing properties of psychostimulants but might not be the primary mediator of their acute effects.[8]

The exact mechanism by which monoamine transport inhibitors interact with MDMA-induced monoamine release is not known. The SERT, DAT, and NET inhibitor indatraline blocks MDMA-induced transmitter release according to simple competitive models.[7] Other inhibitors alter the efficiency of the MDMA-induced transmitter release in a noncompetitive manner, possibly by inducing conformational changes in the transporter protein.[7] A channel-like conformation of DAT, resulting in rapid DA efflux, has also been described for amphetamine-induced DA release.[39]

The present study adds to a better understanding of the mechanism of action of MDMA. Our finding that reboxetine reduces the subjective effects of MDMA (stimulant and drug high) is similar to the finding from another study that atomoxetine attenuates the subjective effects of D-amphetamine.[30] Taken together, these findings indicate that NET inhibitors may potentially be useful as treatments for stimulant addiction.[36,40] However, further clinical studies are needed to explore the therapeutic potential of NET inhibitors in stimulant dependence.

In summary, we showed that NE plays a critical role in the acute physiologic and subjective effects of MDMA in humans.

METHODS

Study design. We used a double-blind, placebo-controlled, randomized, crossover design with four experimental conditions (placebo–placebo, reboxetine–placebo, placebo–MDMA, and reboxetine–MDMA). The order of the four test sessions was counterbalanced. Washout periods between sessions were 10–14 days long. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Guidelines on Good Clinical Practice and was approved by the Ethics Committee of the Canton of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The study was registered at ClinicalTrials.gov (NCT00886886).

Study outline. Subjects completed a screening session, four test sessions with a next-day follow-up, and an end-of-study visit. Test sessions took place in a quiet hospital research ward with no more than two research subjects present per session. Prior to admission to the test sessions, the subjects were asked about potential health problems; drug tests and urine tests for pregnancy were also performed. An indwelling intravenous catheter was placed in the antecubital vein for blood sampling. Reboxetine (8 mg orally) or placebo was administered at 20:00 h the day before the test session and again at 7:00 h after a light meal on the day of the test. MDMA (125 mg orally) or placebo was administered at 8:00 h, 1 and 12 h after reboxetine. A standardized lunch was served at 12:00 h, and subjects were sent home at 18:00 h. On the day following each test session, the subjects returned to the research ward at 8:00 h for the assessment of adverse effects and blood sampling. During the test sessions, the subjects did not drink beverages containing caffeine or alcohol. They were reading, listening to music, or walking around in the research ward. For most of the time, they were sitting or lying comfortably. Outcome measures were assessed repeatedly before and after drug administration.

Subjects. Sixteen healthy subjects (eight men and eight women), 20–44 years of age (mean ± SD: 25.7 ± 5.5 years), were recruited on the university campus by word of mouth. Exclusion criteria included: age <18 or >45 years, pregnancy (urine pregnancy test before each test session), abnormal body mass index (<18.5 or >25 kg/m^2), personal or family (first-degree relative) history of psychiatric disorder (as assessed by the structured clinical interview for axis I and II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV),[41] supplemented by psychometric instruments),[42] regular use of medications, chronic or acute physical illness (as assessed by physical examination, electrocardiogram, standard hematological, and chemical blood analyses), smoking (more than 10 cigarettes/day), lifetime history of illicit drug use more than five times (except for tetrahydrocannabinol), illicit drug use within the past 2 months, and illicit drug use during the study (urine tests for drug use before test sessions using TRIAGE 8, Biosite, San Diego, CA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and, in particular, to limit alcohol use to one glass on the day before each test session. Three subjects were light smokers (fewer than 10 cigarettes/day). They maintained their usual smoking habit but were not allowed to smoke for 6 h after MDMA/placebo administration. Eleven subjects had previously used cannabis. Six subjects had illicit drug experiences (one to four times): one subject had tried cocaine, one had tried ecstasy, two had tried psilocybin, one had tried psilocybin and ecstasy, and one had tried ecstasy, psilocybin, and cocaine. The three subjects with ecstasy experience had all used the drug only once. All the subjects were phenotyped for CYP2D6 activity, using dextrometorphan as the probe drug. There were 10 extensive, 4 intermediate, and 2 poor CYP2D6 metabolizers in the study. All subjects gave their written informed consent before participating in the study, and they were paid for their participation.

Study drugs. (±) MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules (100 mg and 25 mg) by Bichsel Laboratories AG, Interlaken, Switzerland, in accordance with good manufacturing practice. Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute dose of 125 mg, corresponding to a dose of 1.85 ± 0.24 mg/kg body weight. This dose

of MDMA corresponds to a typical recreational dose of ecstasy, and comparable doses of MDMA have previously been used in controlled settings.[2–4,43] Reboxetine is a potent, selective, and specific NE uptake inhibitor.[35] Reboxetine (8 mg, Edronax; Pfizer, Zurich, Switzerland) and identical-looking placebo (lactose) capsules were similarly prepared by Bichsel Laboratories. Reboxetine (8 mg) or placebo was administered twice, 12 h and 1 h before MDMA (125 mg) or placebo. Similar dosing regimens have previously been used to manipulate the NE system function in healthy subjects.[44]

Pharmacodynamics

Psychometric scales: Subjective measures included VAS,[21] the AMRS,[45] the 5D-ASC,[46] and the STAI.[42]

VAS: VASs included "any drug effect," "drug high," "stimulated," "closeness to others," "good drug effect," and "liking."[3,20,21] VASs were presented as 100-mm horizontal lines marked "not at all" on the left and "extremely" on the right. The VAS for "closeness to others" was bidirectional (± 50 mm). VAS tests were administered 1 h before and at 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 6 h after MDMA/placebo administration.

5D-ASC: The 5D-ASC rating scale measures alterations in mood, perception, experience of self in relation to environment, and thought disorder. The instrument comprises five subscales (dimensions)[46] and eleven lower-order scales[47]: The 5D-ASC dimension OB (27 items) measures derealization and depersonalization associated with positive emotional states ranging from heightened mood to euphoric exaltation. The corresponding lower-order scales are "experience of unity," "spiritual experience," "blissful state," and "insightfulness." The dimension AED (21 items) summarizes ego disintegration and loss of self-control, phenomena associated with anxiety. The corresponding lower-order scales are "disembodiment," "impaired control of cognition," and "anxiety." The dimension "VR (18 items) consists of the lower-order scales "complex imagery," "elementary imagery," "audiovisual synesthesia," and "changed meaning of percepts." Two other dimensions of the scale were not used in our study. The global ASC score was constructed by adding the OB, AED, and VR scores. The 5D-ASC scale was administered 4 h after administration of MDMA or placebo.

AMRS: The 60-item Likert-scale short version of the AMRS[45] was administered 1 h before and at 1.25, 2, 3, and 24 h after MDMA or placebo. The AMRS contains subscales for activity, inactivation, extroversion and introversion, well-being, emotional excitation, anxiety–depression, and dreaminess.

STAI: The STAI state-anxiety scale[42] test was administered 1 h before and at 1.25, 2, and 3 h after MDMA or placebo.

Physiologic measures. Physiologic measures were assessed repeatedly, at −1, 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, and 10 h after administration of MDMA or placebo. Heart rate, systolic blood pressure, and diastolic blood pressure were measured using an OMRON M7 blood pressure monitor (OMRON Healthcare Europe, Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5–10 min, with the volunteer sitting in bed with the back supported. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Between measurements, subjects were allowed to engage in nonstrenuous activities. Core (tympanic) temperature was assessed using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY). The temperature of the room was maintained at 22.5 ± 0.5 °C.

Adverse effects. Adverse effects were assessed at 0, 3, and 24 h after administration of MDMA or placebo by using the List of Complaints.[2,48] The scale consists of 66 items, yielding a total adverse effects score (nonweighted sum of the item answers), reliably measuring physical and general discomfort. The scale has previously been shown to be sensitive to the adverse effects of MDMA.[2,22]

Blood collection for endocrine and pharmacokinetic measurements. Samples of whole blood for the determination of MDMA, MDA, and reboxetine levels were collected into lithium heparin monovettes at −1, 0, 0.33, 0.66, 1, 1.5, 2,3.5, 3, 3.5, 4, 6, 8, 10, and 24 h after administration of MDMA or placebo. Blood samples to determine concentrations of NE and epinephrine were taken 60 min after administration of MDMA or placebo. All blood samples were collected on ice and centrifuged within 10 min at 4 °C. Plasma was then stored at −70 °C until analysis.

Laboratory analyses

Catecholamines: The levels of free catecholamines (NE and epinephrine) were determined using a modified method of the RECIPE kit (ClinRep; RECIPE Chemicals and Instruments, Munich, Germany) (see **Supplementary Methods** online). The lower limit of quantification was 20 pmol/l, and interassay precisions (coefficient of variation (CV)) were <15%.

MDMA and MDA: Plasma concentrations of MDMA and its active metabolite, 3,4-methylenedioxyamphetamine (MDA), were determined using high-performance liquid chromatography with diode-array detection[49] (see **Supplementary Methods** online). The limit of quantification was 5 ng/ml for MDMA and 2 ng/ml for MDA. Interday precision values (CV) were 7 and 4%, and interday accuracy values were 96–106% and 100–103% for MDMA and MDA, respectively.

Reboxetine: Plasma reboxetine concentrations were analyzed using liquid chromatography–mass spectrometry (see **Supplementary Methods** online). The limit of quantification was 34.5 ng/ml. Interday precision (CV) values were 5.7 and 3.2%, and interday accuracy values were 98.5 and 101.8%, at 92 ng/ml and at 344 ng/ml, respectively.

Ex vivo *binding*: Plasma samples for investigating *ex vivo* binding were collected 60 min after administration of MDMA or placebo. We determined the potencies of plasma to inhibit ^{3}H-nisoxetine, ^{3}H-citalopram, and ^{3}H-WIN35, 428 binding to NET, SERT, and DAT, respectively (see **Supplementary Methods** online). K_i values were calculated as percentages of plasma sample dilutions required for obtaining 50% of maximum effect (10 μmol/l indatraline in human plasma was used to achieve 100% inhibition). Undiluted plasma samples were set as 100%. Therefore, a K_i of 10% indicates that a plasma sample diluted 10-fold displaced 50% of the radioligand.

Pharmacokinetics. Data for plasma concentrations of MDMA, MDA, and reboxetine were analyzed using noncompartmental methods (WinNonlin; Pharsight, Mountain View, CA). C_{max} and time to maximum concentration (T_{max}) were obtained directly from the concentration–time curves of observed values. The terminal elimination rate constant (λ_z) was estimated by log-linear regression after semilogarithmic transformation of the data, using at least three data points of the terminal linear phase of the concentration–time curve. Terminal elimination half-life ($t_{1/2}$) was calculated using λ_z and the equation $t_{1/2} = \ln_2/\lambda_z$. The $AUC_{0-24\,h}$ was calculated using the linear trapezoidal rule. The $AUC_{0-\infty}$ was determined by extrapolation of the $AUC_{0-24\,h}$, using λ_z.

Statistical analysis. Values were transformed to differences from baseline. Peak effects (E_{max}) were determined for repeated measures. E_{max} values were compared by one-way General Linear Models repeated measures analysis of variance with drug as a factor, using STATISTICA 6.0 (StatSoft, Tulsa, OK). Tukey *post hoc* comparisons were performed based on significant main effects of treatment. Additional analyses of variance were performed with drug order as an additional factor so as to exclude carryover effects. The criterion for significance was $P < 0.05$. MAP was calculated from diastolic blood pressure and systolic blood pressure using the formula MAP = DBP + (SBP−DBP)/3.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Paper Two: Role of Vesicular NE Release

Effects of the α_2-Adrenergic Agonist Clonidine on the Pharmacodynamics and Pharmacokinetics of 3,4-Methylene-dioxymethamphetamine in Healthy Volunteers

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Effects of the α_2-Adrenergic Agonist Clonidine on the Pharmacodynamics and Pharmacokinetics of 3,4-Methylenedioxymethamphetamine in Healthy Volunteers

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ABSTRACT

The mechanism of action of 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) involves the carrier-mediated and potentially vesicular release of monoamines. We assessed the effects of the sympatholytic α_2-adrenergic receptor agonist clonidine (150 μg p.o.), which inhibits the neuronal vesicular release of norepinephrine, on the cardiovascular and psychotropic response to MDMA (125 mg p.o.) in 16 healthy subjects. The study used a randomized, double-blind, placebo-controlled crossover design with four experimental sessions. The administration of clonidine 1 h before MDMA reduced the MDMA-induced increases in plasma norepinephrine concentrations and blood pressure but only to the extent that clonidine lowered norepinephrine levels and blood pressure compared with placebo. Thus, no interaction was found between the cardiovascular effects of the two drugs. Clonidine did not affect the psychotropic effects or pharmacokinetics of MDMA. The lack of an interaction of the effects of clonidine and MDMA indicates that vesicular release of norepinephrine, which is inhibited by clonidine, does not critically contribute to the effects of MDMA in humans. Although clonidine may be used in the treatment of stimulant-induced hypertensive reactions, the present findings do not support a role for α_2-adrenergic receptor agonists in the prevention of psychostimulant dependence.

Introduction

The sympathomimetic amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA; ecstasy; $C_{11}H_{15}NO_2$) releases norepinephrine (NE), serotonin [5-hydroxytryptamine (5-HT)], and dopamine from nerve terminals via their corresponding presynaptic monoamine transporters (Rudnick and Wall, 1992; Rothman et al., 2001; Verrico et al., 2007). The psychotropic and cardiostimulant effects of MDMA in humans seem to depend on the transporter-mediated release of NE and 5-HT. Both the subjective and cardiovascular responses to MDMA can be reduced by blocking the NE or 5-HT transporter by using selective transporter inhibitors (Liechti et al., 2000; Farré et al., 2007; Tancer and Johanson, 2007; Hysek et al., 2011). The release of NE has been shown to critically mediate the effects of psychostimulants (Rothman et al., 2001; Sofuoglu et al., 2009), including MDMA (Hysek et al., 2011; Newton, 2011). However, MDMA-induced increases in extracellular monoamines may also result from impulse-dependent vesicular/exocytotic release or transmitter uptake inhibition (Seiden et al., 1993; Florin et al., 1994; Hondebrink et al., 2011). The vesicular release of NE is under negative feedback control mediated by presynaptic α_2-adrenergic receptors (Buccafusco, 1992; Starke, 2001), and α_2 receptors are thereby involved in noradrenergic function, including vascular contraction, blood pressure control, body temperature regulation, arousal, and memory (Starke, 2001). Clonidine is an α_2-adrenergic receptor

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; AMRS, Adjective Mood Rating Scale; ANOVA, analysis of variance; AUEC, area under the effect-time curve; C_{max}, maximal plasma concentration; 5D-ASC, 5-Dimensions of Altered States of Consciousness; DBP, diastolic blood pressure; E_{max}, maximal effect; HPLC, high-performance liquid chromatography; 5-HT, 5-hydroxytryptamine (serotonin); MAP, mean arterial pressure; MDA, 3,4-methylenedioxyamphetamine; NE, norepinephrine; PK, pharmacokinetic; PD, pharmacodynamic; SBP, systolic blood pressure; STAI, State-Trait Anxiety Inventory; VAS, Visual Analog Scale; OB, oceanic boundlessness; AED, anxious ego dissolution; VR, visionary restructuralization; VIR, vigilance reduction.

agonist and sympatholytic drug that reduces noradrenergic activity by decreasing the impulse-mediated vesicular release of NE (Buccafusco, 1992; Philipp et al., 2002). In healthy subjects, clonidine dose-dependently suppressed plasma levels of NE (Veith et al., 1984), blood pressure (Mitchell et al., 2005), and cardiac output, lowered body temperature (Bexis and Docherty, 2005), and had sedative effects (Hall et al., 2001). These sympatholytic effects of clonidine are opposite to the clinical effects of sympathomimetic drugs, including MDMA. Therefore, clonidine has been recommended in the treatment of MDMA intoxication (Green et al., 1995; Liechti, 2003), and it is routinely used to control sympathetic activation during withdrawal from drugs of abuse.

In rats, clonidine blocked the behavioral response and hippocampal and prefrontal NE release after treatment with cocaine or low doses of amphetamine (Florin et al., 1994; Carey et al., 2008). Clonidine may therefore reduce stimulant-induced NE release and the associated behavioral effects of psychostimulants and may even be used in the treatment of stimulant addiction. In fact, clonidine prevented amphetamine-induced psychomotor stimulation (Vanderschuren et al., 2003), cue-induced cocaine seeking in rats (Smith and Aston-Jones, 2011), and drug craving in cocaine users (Jobes et al., 2011).

The effects of clonidine on the acute response to psychostimulants have not yet been evaluated in humans. In the present study, we assessed the interactive pharmacodynamic effects of clonidine and MDMA in healthy subjects. We expected that clonidine would reduce the effects of MDMA in humans to the extent that the clinical effects of MDMA are mediated by the exocytotic release of NE.

Materials and Methods

Study Design. We used a double-blind, placebo-controlled, randomized, crossover design with four experiential conditions (placebo-placebo, clonidine-placebo, placebo-MDMA, and clonidine-MDMA) in a balanced order. The washout periods between sessions were 10 to 14 days long. The study was conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The study was registered at http://clinicaltrials.gov (NCT01136278).

Study Procedures. The subjects completed a screening session, four test sessions, and an end-of-study visit. The test sessions were conducted in a quiet hospital research ward with no more than two research subjects present per session. Before undergoing the test sessions, the subjects were asked about potential health problems. Drug tests and urine tests to determine pregnancy were also performed. An indwelling intravenous catheter was placed in the antecubital vein for blood sampling. Clonidine (150 μg p.o.) or placebo was administered at 8:00 AM. MDMA (125 mg p.o.) or placebo was administered at 9:00 AM. A standardized lunch was served at 12:00 PM, and the subjects were sent home at 3:00 PM. Outcome measures were assessed repeatedly before and after drug administration.

Subjects. Sixteen healthy subjects (eight men and eight women) with a mean ± S.D. age of 25.4 ± 4.9 years were recruited on the University of Basel campus. The exclusion criteria included the following; 1) age <18 or >45 years, pregnancy determined by a urine test before each test session; 2) body mass index <18.5 kg/m^2 or >25 kg/m^2; 3) personal or family (first-degree relative) history of psychiatric disorder (determined by the structured clinical interview for Axis I and Axis II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Wittchen et al., 1997) supplemented by the SCL-90-R Symptom Checklist (Derogatis et al., 1976; Schmitz et al., 2000), Freiburg Personality Inventory (Fahrenberg et al., 1984), and Trait Scale of the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970); 4) the regular use of medications; 5) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematology, and chemical blood analyses; 6) smoking more than 10 cigarettes per day; 7) a lifetime history of using illicit drugs more than five times, with the exception of cannabis; 8) illicit drug use within the last 2 months; and 9) illicit drug use during the study determined by urine tests conducted before the test sessions by using TRIAGE 8 (Biosite, San Diego, CA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and limit alcohol use to one glass on the day before each test session. All of the subjects were nonsmokers. Twelve subjects had previously used cannabis. Five subjects reported using illicit drugs once, in which one subject had tried lysergic acid diethylamide, ecstasy, and psilocybin, two had

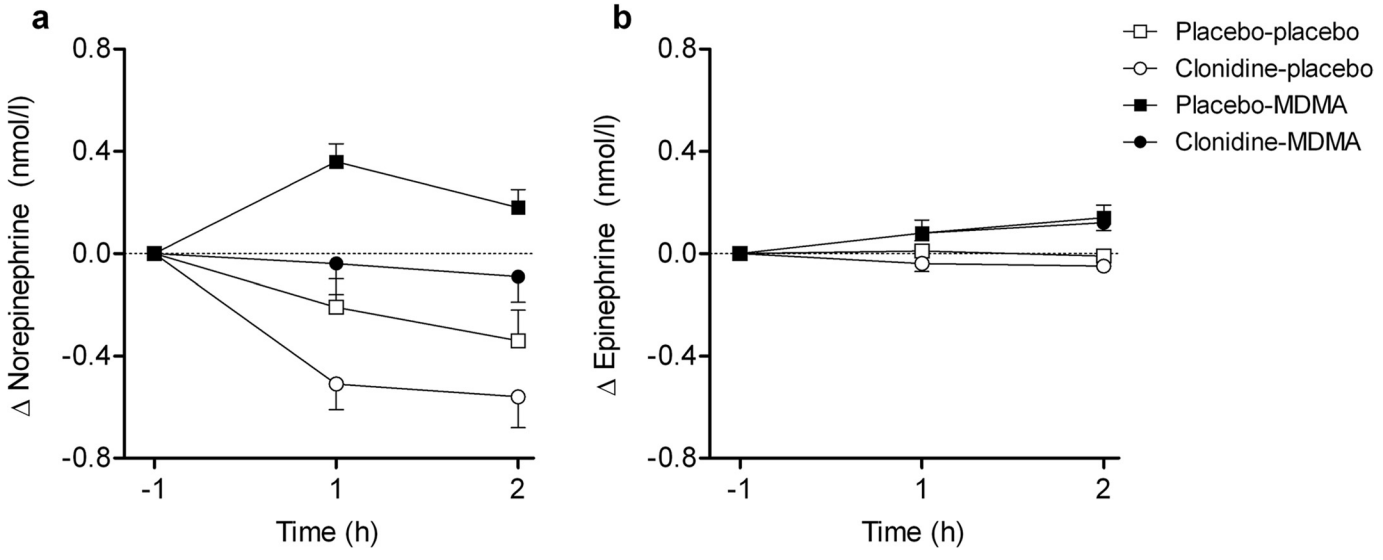

Fig. 1. Plasma concentration changes in norepinephrine (a) and epinephrine (b). Values are expressed as mean ± S.E.M. changes from baseline in 16 subjects. a, MDMA increased the plasma level of norepinephrine. Clonidine prevented the MDMA-induced increase in norepinephrine but also reduced the concentration of norepinephrine compared with placebo. b, the drug effects on plasma epinephrine levels were not significant.

tried cocaine and psilocybin, one had tried ecstasy, and one had tried psilocybin. All of the subjects were phenotyped for CYP2D6 activity by using dextromethorphan as the probe drug. Eight extensive, seven intermediate, and one poor CYP2D6 metabolizer were identified in the study. The female subjects were investigated during the follicular phase (days 2–14) of their menstrual cycle when the reactivity to amphetamines is expected to be similar to men (White et al., 2002). All of the subjects provided their written informed consent before participating in the study, and they were paid for their participation.

Drugs. (±)MDMA hydrochloride ($C_{11}H_{15}NO_2$; Lipomed AG, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules (100 and 25 mg). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute oral dose of 125 mg, corresponding to a dose of 1.88 ± 0.28 mg/kg body weight. Clonidine tablets (150 μg; Catapresan; Boehringer Ingelheim GmbH, Basel, Switzerland) were encapsulated within opaque gelatin capsules, and identical placebo (lactose) capsules were prepared. Clonidine (150 μg) or placebo was administered 1 h before MDMA (125 mg) or placebo administration. Oral medication administration was supervised by study personnel.

Pharmacodynamic Measurements. *Psychometric scales*. Subjective measures were assessed by using Visual Analog Scales (VAS) (Hysek et al., 2011), the Adjective Mood Rating Scale (AMRS) (Janke and Debus, 1978), the 5-Dimensions of Altered States of Consciousness (5D-ASC) (Dittrich, 1998; Studerus et al., 2010), and the STAI (Spielberger et al., 1970). VAS included any drug effect, good drug effect, bad drug effect, drug liking, drug high, stimulated, tiredness, closeness to others, and open (Farré et al., 2007; Tancer and Johanson, 2007; Kolbrich et al., 2008; Hysek et al., 2011). The VAS were presented as 100-mm horizontal lines marked "not at all" on the left and "extremely" on the right. The VAS for closeness to others and open were bidirectional (± 50 mm). The VAS were administered 1 h before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after MDMA or placebo administration. The 60-item Likert-type scale short version of the AMRS (Janke and Debus, 1978) was administered 1 h before and 1.25, 2, and 5 h after MDMA or placebo administration. The AMRS contains subscales for activity, inactivation, extroversion and introversion, well being, emotional excitation, anxiety-depression, and dreaminess. The 5D-ASC rating scale measures alterations in mood, perception, experience of self in relation to environment, and thought disorder (Studerus et al., 2010). The 5D-ASC rating scale comprises five subscales or dimensions (Dittrich, 1998) and 11 lower-order scales (Studerus et al., 2010). The 5D-ASC dimension oceanic boundlessness (27 items) measures derealization and depersonalization associated with positive emotional states ranging from heightened mood to euphoric exaltation. The dimension anxious ego dissolution (21 items) summarizes ego disintegration and loss of self-control, two phenomena associated with anxiety. The corresponding lower-order scales included: disembodiment, impaired control of cognition, and anxiety. The dimension visionary restructuralization (18 items) consists of the lower-order scales complex imagery, elementary imagery, audiovisual synaesthesia, and changed meaning of percepts. The dimension auditory alterations (16 items) subsumes auditory (pseudo) hallucinations, and the dimension vigilance reduction (12 items) describes states of drowsiness and impaired alertness and cognitive performance. The global ASC score was determined by adding the oceanic boundlessness, anxious ego dissolution, and visionary restructuralization scores. The 5D-ASC scale was administered 4 h after MDMA or placebo administration. The STAI state-anxiety subscale (Spielberger et al., 1970) was administered 1 h before and 1.25, 2, and 5 h after MDMA or placebo administration.

Physiologic measures. Physiologic measures were assessed repeatedly 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Heart rate, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were measured by using an OMRON M7 blood pressure monitor (OMRON Healthcare Europe, Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5 min. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Core (tympanic) temperature was assessed by using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY).

Adverse Effects. Adverse effects were assessed 1 h before and 3 and 24 h after MDMA or placebo administration by using the List of Complaints (Zerssen, 1976; Hysek et al., 2011). The scale consists of 66 items that yield a total adverse effects score, reliably measuring physical and general discomfort.

Laboratory Analyses. Samples of whole blood for the determination of MDMA and 3,4-methylenedioxyamphetamine (MDA; $C_{10}H_{13}NO_2$), the active metabolite of MDMA, were collected 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, and 6 h after MDMA or placebo administration. Blood samples to determine concentrations of NE and

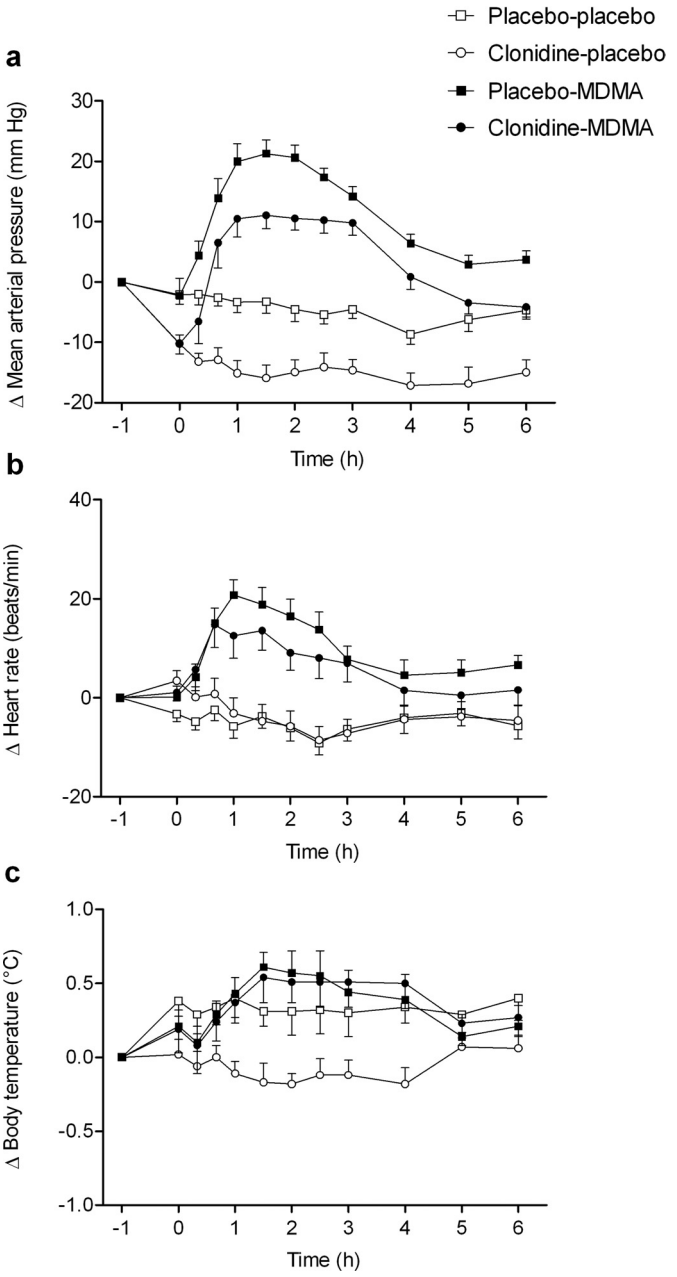

Fig. 2. Physiologic effects. Values are expressed as mean ± S.E.M. changes from baseline in 16 subjects. Clonidine was administered at $t = -1$ h. MDMA was administered at $t = 0$ h. a, clonidine reduced the MDMA-induced elevation in blood pressure to the same extent as it reduced blood pressure compared with placebo. b and c, clonidine had no significant effects on MDMA-induced elevations in heart rate (b) and body temperature (c).

epinephrine were taken at 1 h before and 1 and 2 h after MDMA or placebo administration. All blood samples were collected on ice and centrifuged within 10 min at 4°C. The plasma was then stored at −20°C until analysis. The plasma levels of free catecholamines (NE and epinephrine) were determined by high-performance liquid chromatography (HPLC) with an electrochemical detector as described previously (Hysek et al., 2011). The plasma concentrations of MDMA and MDA were determined by using HPLC coupled to tandem mass spectrometry. The analytes were extracted by protein precipitation using methanol (CH_4O) that contained 0.1 μg/ml MDMA-d5 ($C_{11}H_{10}D_5NO_2$), MDA-d4 ($C_{10}H_9D_4,NO_2$) (both from Lipomed, Arlesheim, Switzerland), duloxetine-d7 ($C_{18}H_{12}D_7NOS$; Toronto Research Chemicals Inc., North York, ON, Canada), and pholedrine ($C_{10}H_{15}NO$) (Sigma, Buchs, Switzerland). Chromatographic separation was performed on a Shimadzu HPLC system (Shimadzu, Reinach, Switzerland) that consisted of a HTS PAL autosampler (CTC Analytics, Zwingen, Switzerland), two Shimadzu LC-20 AD pumps controlled by a Shimadzu CBM-20A unit, a Shimadzu CTO-20AD column oven, and a six-port VICI valve (VICI, Schenkon, Switzerland). A Chromolith SpeedROD RP-18e column (50 × 4.6 mm; VWR, Dietikon, Switzerland) was used for the separation of the analytes. Eluent A (0.1% formic acid in water) and eluent B (0.1% formic acid in methanol) were used in the following gradient: 100% A for 0 to 1 min, 20 to 95% B for 1 to 4 min, 95% B for 4 to 5 min, and 100% A for 5 to 6 min. The mobile phases were delivered at a constant flow rate of 0.8 ml/min. The total run time was 6.0 min. The column oven was set at 35°C. The injection volume was 10 μl. Mass spectrometric detection was performed by using a triple quadrupole mass spectrometer (API4000; Applied Biosystems, Rotkreuz, Switzerland) operated in electrospray-ionization positive-ion mode. The samples were quantified by using peak area ratios. The assays were linear in the concentration range of 1 to 1000 ng/ml for MDMA and MDA. The performance of the method was monitored by using quality-control samples at the lower limit of quantification and at two or three concentrations. The interassay accuracy for the quality-control samples ranged from 97.5 to 100% for MDMA and from 95.3 to 103% for MDA. Interassay precision values ranged from 2.8 to 8.0% for MDMA and from 3.8 to 10.5% for MDA.

Pharmacokinetics and Pharmacokinetic-Pharmacodynamic Modeling. *Pharmacokinetics*. The data for the plasma concentrations of MDMA and MDA were analyzed by using noncompartmental methods. Maximal plasma concentration (C_{max}) and time to C_{max} were obtained directly from the concentration-time curves of the observed values. The terminal elimination rate constant (λ_z) for MDMA was estimated by log-linear regression after semilogarithmic transformation of the data, using the last two to three data points of the terminal linear phase of the concentration-time curve of MDMA. The terminal elimination half-life ($t_{1/2}$) was calculated by using λ_z and the equation $t_{1/2} = \ln_2/\lambda_z$. The area under the plasma concentration-time curve (0–6 h) was calculated by using the linear trapezoidal rule. Plasma concentrations were determined only up to 6 h after MDMA administration, because the aim of the study was to assess potential changes in plasma levels of MDMA during the time of the pharmacodynamic effects of MDMA. Determining the $t_{1/2}$ for MDA was not possible, because of its long $t_{1/2}$, which would require an extended sampling time.

PK-PD modeling. We evaluated the in vivo relationship between the MDMA concentration and the effect of MDMA on mean arterial

TABLE 1

Mean ± S.E.M. values and statistics of pharmacodynamic effects

Values are expressed as mean ± S.E.M. changes from baseline of 16 subjects.

		Placebo-Placebo	Clonidine-Placebo	Placebo-MDMA	Clonidine-MDMA	$F_{3,45}$	p
				Mean ± S.E.M.			
Circulating catecholamines							
Epinephrine, nM	E_{max}	0.01 ± 0.02	−0.02 ± 0.03†	0.17 ± 0.05	0.12 ± 0.03	4.16	<0.05
Norepinephrine, nM	E_{max}	−0.24 ± 0.10	−0.52 ± 0.10**††	0.41 ± 0.06**	−0.04 ± 0.08††	22.72	<0.001
Physiologic effect							
SBP, mm Hg	E_{max}	5.4 ± 3.1	−5.8 ± 2.2**†††	36.6 ± 2.48***	27.5 ± 3.3***	52.87	<0.001
	$AUEC_{0-6}$	736.8 ± 16.0	654.2 ± 21.5***†††	840.3 ± 17.1***	807.5 ± 18.4***†	98.25	<0.001
DPB, mm Hg	E_{max}	3.0 ± 1.8	−4.5 ± 2.5†††	20.8 ± 2.2***	15.3 ± 2.4***	26.48	<0.001
	$AUEC_{0-6}$	438.2 ± 9.7	371.9 ± 12.3***†††	505.8 ± 9.9***	479.7 ± 10.8***†	93.35	<0.001
MAP, mm Hg	E_{max}	2.2 ± 1.7	−6.4 ± 1.9*†††	25.0 ± 2.2***	18.2 ± 2.5***	49.97	<0.001
	$AUEC_{0-6}$	537.7 ± 10.5	466.0 ± 14.7***†††	616.9 ± 11.0***	589.0 ± 12.0***†	104.89	<0.001
Heart rate, beats/min	E_{max}	2.8 ± 2.5	5.9 ± 2.9	26.0 ± 3.2***	25.0 ± 5.6***	15.03	<0.001
	$AUEC_{0-6}$	402.3 ± 13.9	389.6 ± 12.4†††	472.3 ± 15.7***	460.0 ± 17.5***	22.16	<0.001
Body temperature, °C	E_{max}	0.7 ± 0.1	0.4 ± 0.1†	0.9 ± 0.1	0.9 ± 0.2	3.50	<0.05
	$AUEC_{0-6}$	222.3 ± 0.7	221.7 ± 0.6	223.0 ± 0.6	223.2 ± 0.6	3.61	<0.05
Visual Analog Scale (%max)							
Any drug effect	E_{max}	0.9 ± 0.9	16.8 ± 6.2†††	89.6 ± 4.0***	81.56 ± 6.9***	87.55	<0.001
Good drug effect	E_{max}	0.0 ± 0.0	3.2 ± 1.7†††	91.8 ± 4.4***	79.94 ± 7.2***	160.10	<0.001
Bad drug effect	E_{max}	0.0 ± 0.0	2.6 ± 2.2†	21.6 ± 5.8**	25.56 ± 6.6**	8.36	<0.001
Drug liking	E_{max}	0.0 ± 0.0	2.4 ± 2.1†††	91.1 ± 4.6***	80.94 ± 6.8***	154.30	<0.001
Drug high	E_{max}	0.0 ± 0.0	0.6 ± 0.4†††	85.6 ± 6.7***	75.38 ± 8.0***	95.74	<0.001
Stimulated	E_{max}	0.0 ± 0.0	0.4 ± 0.4†††	70.7 ± 7.9***	64.38 ± 9.3***	46.76	<0.001
Tiredness	E_{max}	24.7 ± 6.7	62.8 ± 8.1†††	55.4 ± 7.2**	53.81 ± 7.4*	7.16	<0.001
Closeness to others	E_{max}	0.4 ± 0.4	0.0 ± 0.0†††	23.4 ± 4.6***	24.06 ± 4.7***	20.35	<0.001
Open	E_{max}	0.0 ± 0.0	0.0 ± 0.0†††	30.3 ± 4.4***	30.2 ± 4.9***	33.71	<0.001
Adjective Mood Rating Scale (score)							
Emotional excitation	E_{max}	−1.0 ± 0.7	-0.3 ± 0.5†††	4.5 ± 1.1***	4.6 ± 0.9***	15.98	<0.001
Well being	E_{max}	0.8 ± 0.9	−0.4 ± 1.0†††	6.4 ± 1.5**	6.5 ± 1.1**	13.69	<0.001
Extroversion	E_{max}	1.6 ± 0.5	−0.8 ± 0.4*†††	3.3 ± 0.7	3.9 ± 0.8*	13.59	<0.001
Dreaminess	E_{max}	0.6 ± 0.5	1.9 ± 0.6††	4.5 ± 0.6***	3.4 ± 0.7**	13.64	<0.001
Activity	E_{max}	0.4 ± 0.6	−0.9 ± 1.0	2.1 ± 1.5	3.4 ± 1.0	4.68	<0.01
Inactivation	E_{max}	1.9 ± 1.4	10.7 ± 2.0**	8.8 ± 2.3*	7.9 ± 1.8*	6.07	<0.01
Anxiety-depression	E_{max}	−0.4 ± 0.4	0.4 ± 1.6	0.8 ± 0.4	2.1 ± 0.7**	4.98	<0.01
State-Trait Anxiety Inventory (state scale score)							
	E_{max}	0.1 ± 1.4	0.6 ± 1.2	3.7 ± 1.7	4.8 ± 2.1	3.23	<0.05
List of Complaints (total score)							
Acute adverse effects	at 3 h	0.6 ± 0.8	1.2 ± 0.6†††	10.3 ± 1.7***	12.7 ± 1.9***	24.48	<0.001
Subacute adverse effects	at 24 h	−1.1 ± 0.6	0.1 ± 0.6	2.1 ± 1.2*	3.9 ± 1.3***	7.08	<0.001

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, compared with placebo-placebo.
† $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$, compared with placebo-MDMA.

pressure (MAP) by using a soft-link PK-PD model (Meibohm and Derendorf, 1997). Blood pressure and plasma concentrations were assessed at the same time points. Because we observed clockwise hysteresis in the effect-concentration relationship over time, we used PK-PD data pairs within the ascending part of the individual curves up to the maximal effect (E_{max}) or C_{max}. Our estimation of E_{max}, which should represent the maximal response portion of the dose-response curve, may already have been affected by acute tolerance. However, E_{max} values of 100% (scale maximum) or stable high values were reached by most subjects despite possible tolerance. Based on the good brain penetration of MDMA and absence of a time lag, we assumed rapid equilibration between the plasma and central compartment (brain). A sigmoidal dose-response (variable slope) model was fitted to the pooled data of all individuals: $E = E_{max}/(1 + 10^{(\log EC50 - Cp) \times h})$ in which E is the observed effect, C_p is the plasma MDMA concentration, EC_{50} is the plasma concentration at which 50% of the maximal effect is reached, E_{max} is the maximal effect, and h is the Hill slope. The sigmoidal dose-response model provided the best fit to the data and a better fit than a simple E_{max} or linear model. Data pooling was used because only a few data pairs were available for each subject. Nonlinear regression was used to obtain parameter estimates.

Statistical Analyses. Values were transformed to differences from baseline. The E_{max} and AUEC values were determined for repeated measures and compared by one-way General Linear Models repeated-measures analysis of variance (ANOVA) with drug treatment as a factor, using STATISTICA 6.0 software (StatSoft, Tulsa, OK). Tukey post hoc comparisons were performed based on significant main effects of treatment. Additional two-way ANOVAs with the two drug factors, MDMA (MDMA versus placebo) and clonidine (clonidine versus placebo), were used to test for interactive versus additive effects of the two drugs on physiological measures or blood levels of catecholamines. Additional ANOVAs were performed, with drug order as an additional factor, to exclude carryover effects. The criterion for significance was $p < 0.05$. Mean arterial pressure was calculated from DBP and SBP by using the formula MAP = DBP + (SBP − DBP)/3.

Results

Neuroendocrine and Cardiovascular Effects. MDMA increased the level of circulating NE, an endocrine marker of sympathetic nervous system activation, and elevated blood pressure and heart rate compared with placebo (Figs. 1a and 2, a and b; Table 1). Clonidine prevented the MDMA-induced increase in plasma NE (Fig. 1a; Table 1). It also attenuated the blood pressure response to MDMA, although reflected only by the AUEC and not E_{max} (Fig. 2a; Table 1). Clonidine also decreased the level of circulating NE and blood pressure compared with placebo to a similar extent as the reduction in NE and pressure elevations induced by MDMA (Figs. 1a and 2; Table 1). Additional ANOVAs with the two drug factors, MDMA and clonidine, yielded significant main effects of MDMA and clonidine on E_{max} values of MAP ($F_{1,15} = 106.1$ and 18.1, respectively; both $p < 0.001$) but no MDMA × clonidine interaction ($F_{1,15} = 0.2$; $p = 0.7$), which is consistent with an additive effect of the two drugs. Likewise, the ANOVA of NE levels showed significant main effects of MDMA and clonidine ($F_{1,15} =$

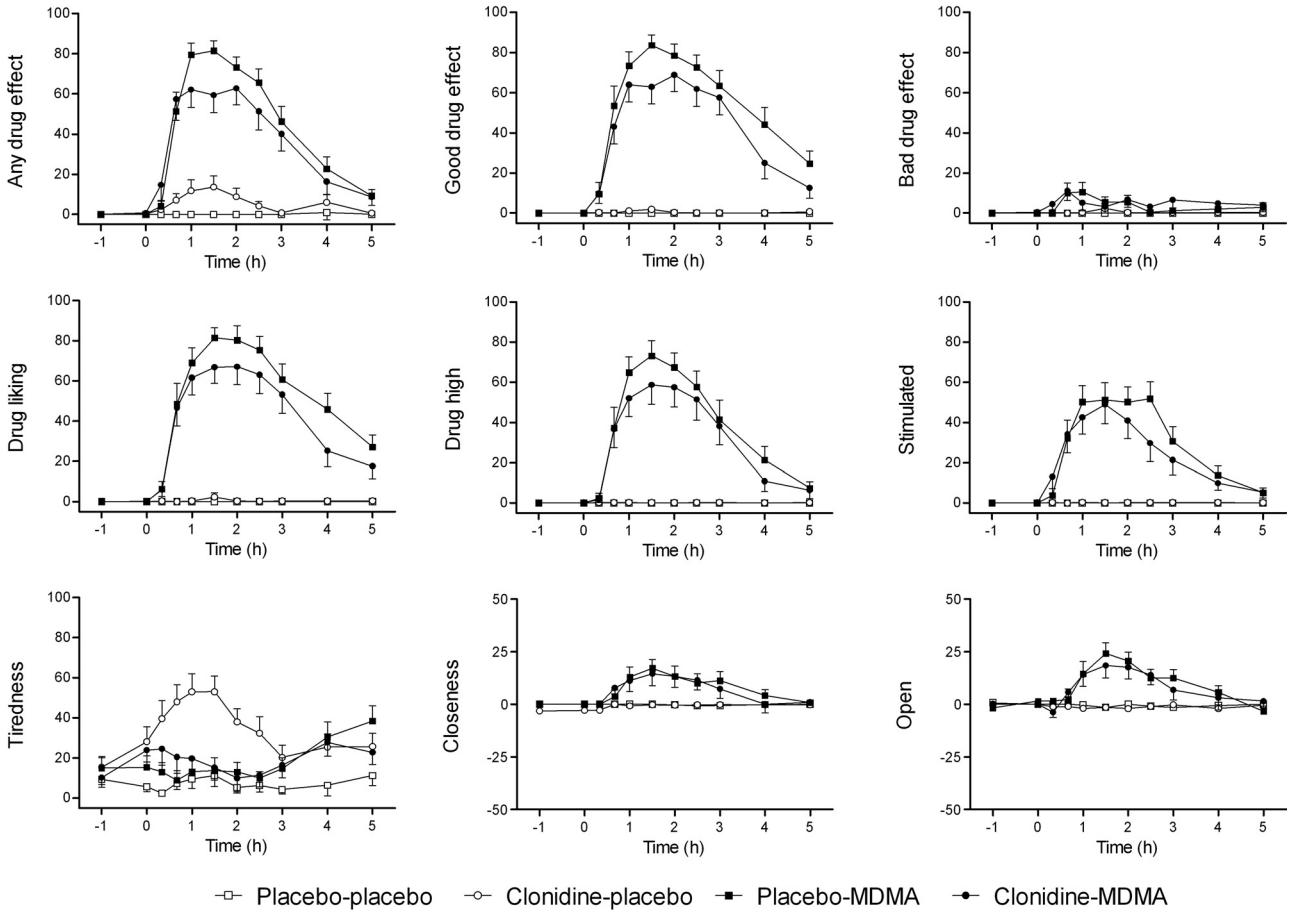

Fig. 3. Time course of subjective VAS ratings. Values are expressed as mean ± S.E.M. of percentage of maximal values in 16 subjects. MDMA increased scores on all scales. Although clonidine produced tiredness, it only weakly and nonsignificantly affected the pronounced subjective responses to MDMA.

41.5 and 34.2, respectively; both $p < 0.001$) but no MDMA × clonidine interaction ($F_{1,15} = 0.0$; $p = 1$). The circulating levels of epinephrine were not significantly altered by the drugs, and clonidine did not affect the increase in heart rate produced by MDMA (Fig. 2b; Table 1).

Psychotropic Effects. Overall, clonidine had no effect on the psychotropic response to MDMA. It did not significantly affect the MDMA-induced increases in VAS ratings of subjective effects or AMRS scores (Figs. 3 and 4; Table 1), although it weakly but nonsignificantly attenuated good drug effect, drug liking, and drug high produced by MDMA (Fig. 3). Clonidine alone increased the VAS score for tiredness and the AMRS score for inactivation compared with placebo (Figs. 3 and 4; Table 1). Clonidine had no effect on the robust changes produced by MDMA on the 5D-ASC rating scale (Fig. 5). Neither clonidine nor MDMA altered the state anxiety scale scores on the STAI (Table 1).

Adverse Effects. MDMA increased the total adverse effects score on the List of Complaints both 3 and 24 h after administration compared with placebo (Table 1). The adverse effects of clonidine and MDMA were additive (Table 1). Thus, clonidine did not affect the untoward effects of MDMA. The frequently reported adverse effects of MDMA included a lack of appetite ($n = 11$), restlessness ($n = 11$), thirst ($n = 10$), sweating ($n = 8$), and bruxism ($n = 8$). Tiredness was typically reported after administration of clonidine-placebo and clonidine-MDMA ($n = 11$ and 10, respectively). No severe adverse effects were reported.

Pharmacokinetics and PK-PD Relationship. Clonidine did not affect the plasma concentration-time curves of MDMA or MDA (Fig. 6, a and b; Table 2). The pharmacokinetic parameters of MDMA did not depend on CYP2D6 phenotype. However, the sample was small, and only one subject was a poor metabolizer. Figure 6, c and d shows the effects of MDMA on blood pressure in terms of plasma concentration. The hysteresis loop shows that the MDMA-induced changes in MAP returned to baseline within 6 h when MDMA concentrations were still high (clockwise hysteresis), which is consistent with acute pharmacodynamic tolerance (Fig. 6c). Clonidine reduced the MDMA-induced blood pressure response for all MDMA plasma concentrations, which is reflected by a downward shift in the concentration-pressure effect curve of MDMA (Fig. 6c). This shift was similar to the blood pressure-lowering effect of clonidine alone compared with placebo. Thus, the effects of the drugs were additive. Clonidine did not affect the EC_{50} value of the concentration-pressure effect curve of MDMA (Fig. 6d).

Discussion

In the present study, the α_2-adrenergic receptor agonist clonidine reduced the elevations in the plasma concentration of NE and increases in blood pressure in response to MDMA. However, clonidine decreased plasma NE levels and blood pressure to a similar extent as the decreases in the response to MDMA when its effects were compared with placebo. Thus, the sympatholytic effects of clonidine and the sympathomimetic effects of MDMA were additive with no interaction between the effects of MDMA and clonidine on the noradrenergic system. In addition, clonidine did not affect the psychotropic effects of MDMA, although clonidine was used in this study in a relatively high single dose that produced sympatholytic effects, including lower plasma NE levels, decreased blood pressure, and sedation on all psychometric scales compared with placebo,

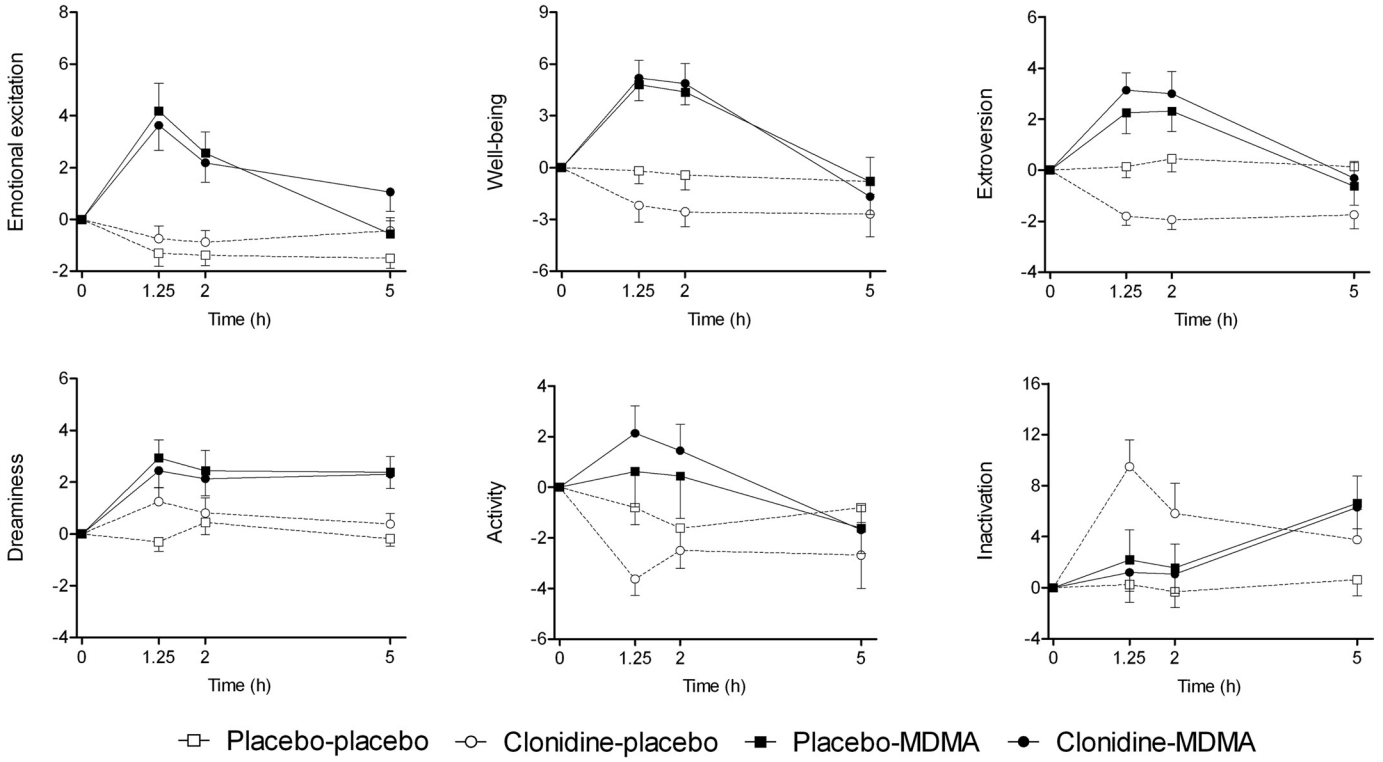

Fig. 4. Mood effects in the AMRS. Values are expressed as mean ± S.E.M. of AMRS score changes from baseline in 16 subjects. MDMA produced emotional excitation, well being, extroversion, and dreaminess. It increased activity at the beginning of the session and inactivation toward the end of the session. Clonidine produced inactivation but did not affect responses to MDMA on any of the scales.

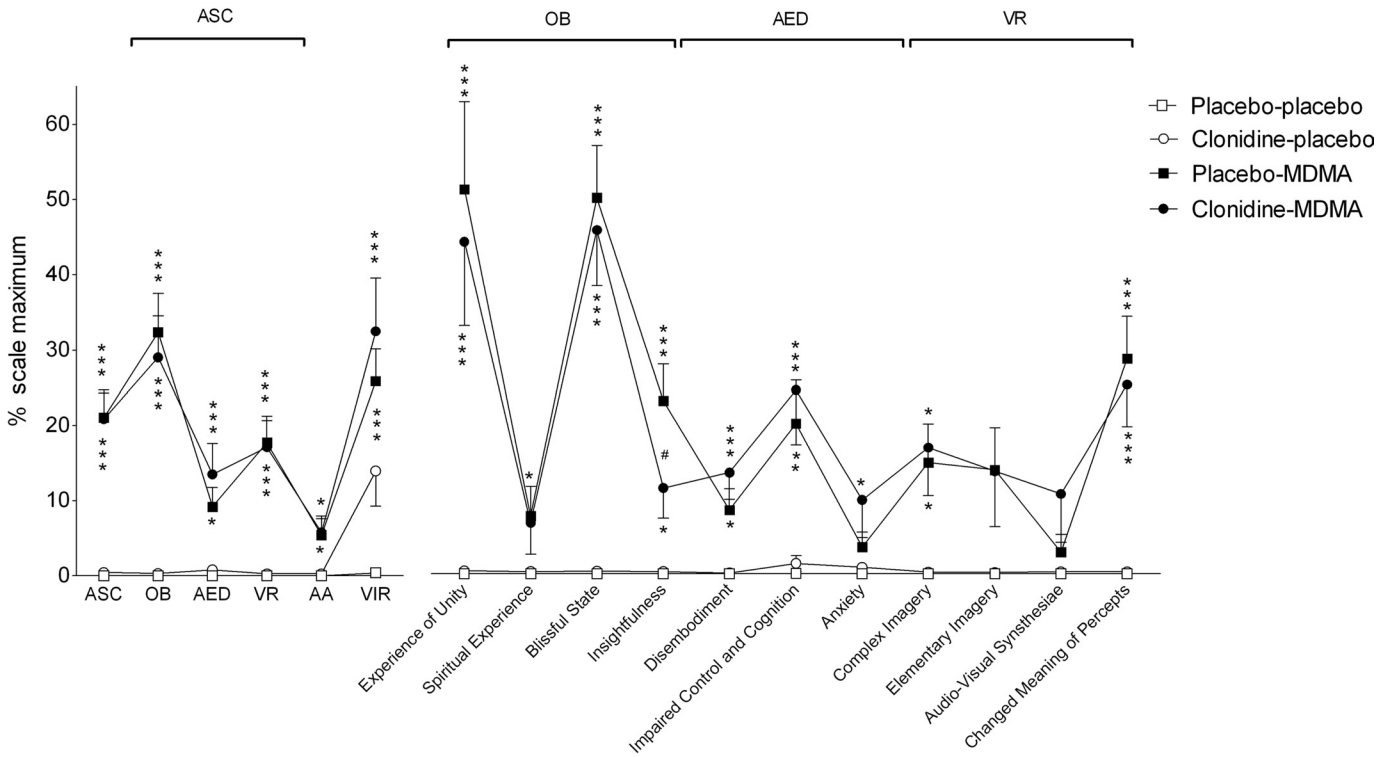

Fig. 5. 5D-ASC scale. Values are expressed as mean ± S.E.M. in 16 subjects. MDMA markedly increased scores on the oceanic boundlessness (OB), anxious ego dissolution (AED), visionary restructuralization (VR), and vigilance reduction (VIR) dimensions and on most subscales compared with placebo (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Clonidine did not change MDMA's effect on any of the ASC dimensions and reduced MDMA's effect on only one of the subscales (#, $p < 0.05$). The main effects of the drug in the analysis of variance were significant for the sum score and all dimensions of the scale [$F_{3,45} = 26.96$, 25.52, 9.70, 20.49, 5.20, and 13.70 for the ASC sum score, OB, AED, VR, auditory alterations (AA), and VIR, respectively; all $p < 0.001$ with the exception of VIR, $p < 0.01$].

which are findings consistent with previous studies (Keränen et al., 1978; Anavekar et al., 1982).

We used the sympatholytic drug clonidine to block the MDMA-induced impulse-dependent vesicular release of NE (Florin et al., 1994). The fact that clonidine did not interact with the clinical effects of MDMA in the present study indicates that the vesicular release of NE is not involved in the mediation of the effects of MDMA in humans. The finding indirectly supports the view that the effects of MDMA in humans primarily depend on the transporter-mediated release of NE, 5-HT, and possibly dopamine (Liechti et al., 2000; Hysek et al., 2011). A preclinical study that used microdialysis in rats showed that clonidine reduced the NE response only to a low dose of amphetamine, but clonidine became less effective as the dose of amphetamine increased (Florin et al., 1994). This result suggests that amphetamine acts primarily as a transporter-mediated NE releaser at higher doses (Florin et al., 1994). In contrast to our study, clonidine prevented behavior relevant to stimulant addiction, including amphetamine-induced psychomotor stimulation (Vanderschuren et al., 2003), cue-induced cocaine seeking in rats (Smith and Aston-Jones, 2011), and drug craving in cocaine users (Jobes et al., 2011). Altogether, the previous preclinical studies and our clinical findings indicate that amphetamines, including MDMA, do not increase NE impulse flow and their action in humans depends on transporter-mediated monoamine release that is not altered by clonidine.

The pharmacokinetic and other pharmacodynamic interactions between clonidine and MDMA need to be considered in the interpretation of the present findings. First, MDMA metabolism involves CYP2D6-mediated O-demethylation to 3,4-dihydroxymethamphetamine and N-demethylation to MDA by CYP2B6 and CYP3A4 (Segura et al., 2005). Clonidine is not known to affect cytochrome P450 function. As expected, clonidine did not alter the plasma-concentration time curves for MDMA or MDA in the present study. Second, both clonidine and MDMA bind to α_2-adrenergic receptors (Battaglia et al., 1988; Lavelle et al., 1999), and some α_2 agonistic actions in the peripheral NE system have been documented for MDMA in vitro (Lavelle et al., 1999). However, in contrast to clonidine, MDMA increased plasma NE levels and blood pressure in the present and previous studies (Dumont et al., 2009; Hysek et al., 2011), indicating that the α_2 agonistic effects of MDMA are not relevant for its main action in humans or are outweighed by the transporter-mediated release of NE and other monoamines.

Our study has a few limitations. First, only single doses of MDMA and clonidine were used. A dose-response study was not feasible because we did not want to expose our subjects to more than two doses of MDMA in a crossover design. The doses of both drugs were selected in the upper dose range, and both drugs produced marked effects. Unknown is whether clonidine affects the response to low doses of MDMA. Second, the clinical effects of clonidine may be attributable to actions at other binding sites. For example, clonidine binds to the imidazoline binding site with very high affinity (Buccafusco et al., 1995), and this binding site is also involved in the mediation of the pressure-lowering effects of clonidine (Ernsberger et al., 1990).

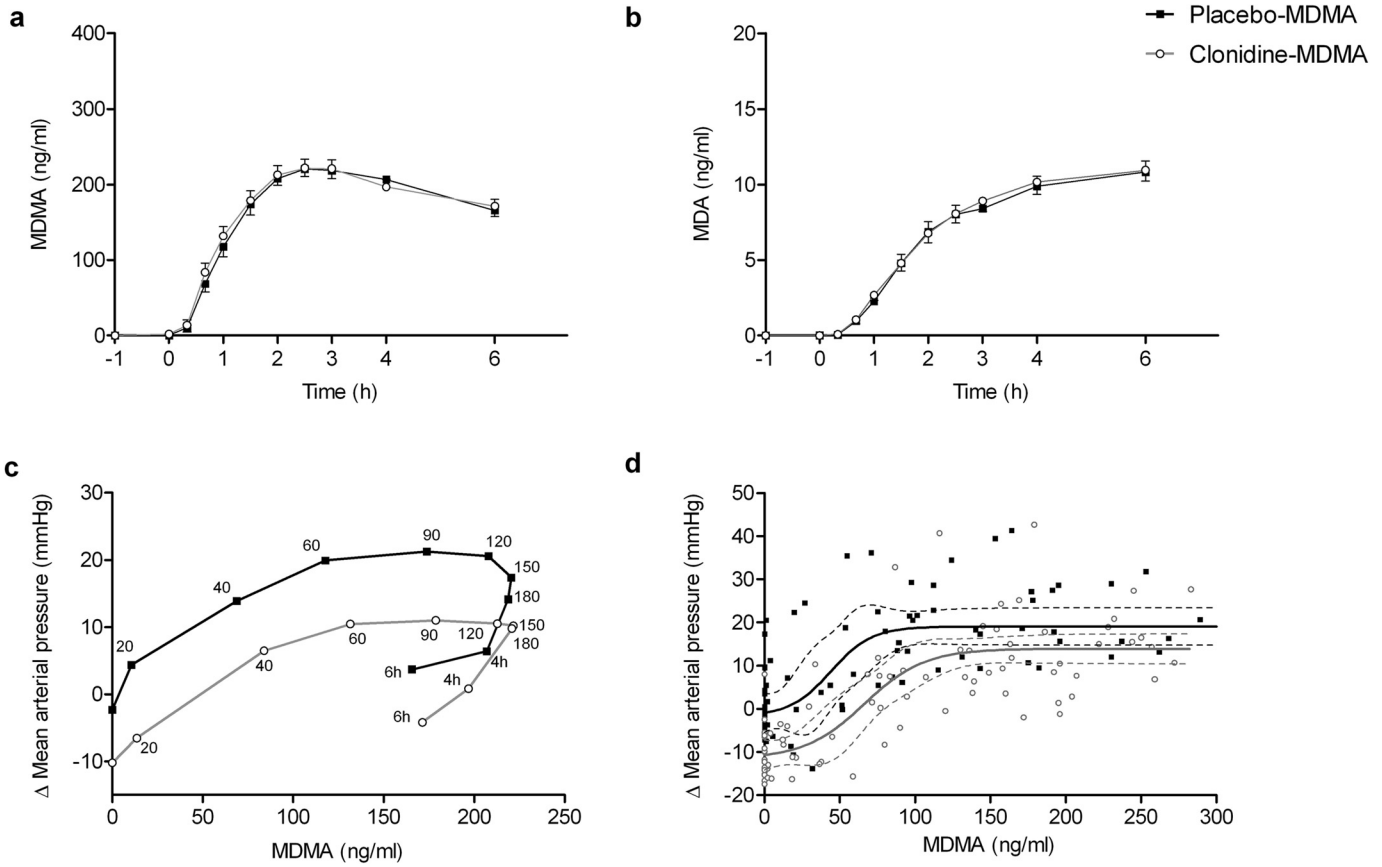

Fig. 6. a and b, pharmacokinetics of MDMA (a) and MDA (b). The values are expressed as mean ± S.E.M. in 16 subjects. Clonidine was administered at $t = -1$ h, and MDMA was administered at $t = 0$ h. c, MDMA effects on blood pressure plotted against simultaneous plasma MDMA concentrations. The values are expressed as the means of the changes from baseline in 16 subjects, with S.E.M. values omitted for clarity. The time of sampling is noted next to each point in minutes or hours after MDMA administration. Clonidine produced a downward shift in the pressure response-concentration curve of MDMA. The magnitude of the shift was similar to the pressure-lowering effect of clonidine alone compared with placebo. d, PK-PD relationship. Values are expressed as individual MDMA concentration-effect data pairs for ascending concentrations for placebo-MDMA (black squares) and clonidine-MDMA (gray circles). Notice the large interindividual variance in the response to MDMA. The solid lines show the fit of a sigmoid E_{max} model to the pooled observed data. Dashed lines indicate 95% confidence intervals of the estimation error. Clonidine produced a downward shift of the concentration-pressure effect curve of MDMA, indicating that it reduced the effect of MDMA at all concentrations. The magnitude of the downward shift was similar to the blood pressure-lowering effect of clonidine compared with placebo. Thus no interaction effect was found between clonidine and MDMA on blood pressure, and the effects of the two drugs were additive. Clonidine did not affect the EC_{50} value of the concentration-pressure effect curve of MDMA. The EC_{50} values (mean 95% confidence interval) were 44 ng/ml (14–74 ng/ml) and 66 ng/ml (41–91 ng/ml) for placebo-MDMA and clonidine-MDMA, respectively.

TABLE 2

Pharmacokinetic parameters of MDMA and MDA

Values are expressed as mean ± S.E.M. of 16 healthy subjects.

	C_{max}	t_{max}	$t_{1/2}$	AUC_{0-6}
	ng/ml	*h*		*ng/ml · h*
MDMA				
Placebo-MDMA	238 ± 10	2.5 ± 0.2	7.7 ± 0.7	1021 ± 35
Clonidine-MDMA	231 ± 11	2.5 ± 0.1	9.4 ± 0.9	1032 ± 51
MDA				
Placebo-MDMA	10.9 ± 0.7	5.2 ± 0.3	N.A.	42.3 ± 3.1
Clonidine-MDMA	11.2 ± 0.8	5.1 ± 0.4	N.A.	44.3 ± 3.4

t_{max}, time to maximum plasma concentration; $t_{1/2}$, terminal elimination half-life; AUC, area under the plasma concentration-time curve; N.A., not assessed.

The present study further characterized the pharmacodynamic and pharmacokinetic effects of a single dose of 125 mg of MDMA in healthy male and female subjects with no or only single previous MDMA use. MDMA produced cardiovascular-stimulant effects, positive mood, emotional stimulation, and extroversion, confirming previous studies in healthy subjects (Liechti et al., 2000; Dumont and Verkes, 2006; Hysek et al., 2010, 2011) and MDMA users (Farré et al., 2007; Tancer and Johanson, 2007; Kolbrich et al., 2008). The study documented the phenomenon of rapid acute tolerance to the effects of MDMA. The E_{max} in blood pressure was observed within 60 to 120 min, and C_{max} was reached within 120 to 180 min after MDMA administration. In addition, the plasma levels of MDMA remained close to C_{max} for several hours, whereas the pharmacodynamic effects returned to baseline more rapidly. Furthermore, half-maximal effects (EC_{50}) of MDMA on blood pressure were observed at plasma concentrations of MDMA of approximately 50 ng/ml, which was 4-fold lower than the C_{max} of MDMA. Acute tolerance to the effects of psychostimulant drugs, including MDMA, cocaine, and nicotine, has been described previously (Van Dyke et al., 1978; Porchet et al., 1987; Hysek et al., 2011). This pharmacodynamic tolerance could be attributable to receptor or transporter down-regulation or desensitization (Meibohm and Derendorf, 1997; Robertson et al., 2009), the more rapid distribution of drug to the brain than to venous blood (Porchet et al., 1987), or the functional depletion of presynaptic monoamine stores so that no more transmitter can be released despite high concentrations of MDMA.

In summary, our findings support the hypothesis that the

effects of MDMA in humans do not depend on the vesicular release of NE but on transporter-mediated monoamine release. The clinical implications of the present study are that clonidine could be of limited use in the treatment of hypertensive reactions in psychostimulant users. In contrast, the lack of an effect of clonidine on the euphoria produced by MDMA does not indicate a role for α_2-adrenergic receptor agonists in the prevention of psychostimulant dependence, despite their utility in the treatment of withdrawal from drugs of abuse.

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Authorship Contributions

Participated in research design: Hysek and Liechti.

Conducted experiments: Hysek, Brugger, Simmler, Bruggisser, Donzelli, Grouzmann, and Hoener.

Performed data analysis: Hysek, Simmler, and Liechti.

Wrote or contributed to the writing of the manuscript: Hysek and Liechti.

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Paper Three: Role of α_1 and β Adrenergic Receptors

Carvedilol Inhibits the Cardiostimulant and Thermogenic Effects of MDMA in humans

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RESEARCH PAPER

Carvedilol inhibits the cardiostimulant and thermogenic effects of MDMA in humans

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BACKGROUND AND PURPOSE

The use of ±3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') is associated with cardiovascular complications and hyperthermia.

EXPERIMENTAL APPROACH

We assessed the effects of the α_1- and β-adrenoceptor antagonist carvedilol on the cardiostimulant, thermogenic and subjective responses to MDMA in 16 healthy subjects. Carvedilol (50 mg) or placebo was administered 1 h before MDMA (125 mg) or placebo using a randomized, double-blind, placebo-controlled, four-period crossover design.

KEY RESULTS

Carvedilol reduced MDMA-induced elevations in blood pressure, heart rate and body temperature. Carvedilol did not affect the subjective effects of MDMA including MDMA-induced good drug effects, drug high, drug liking, stimulation or adverse effects. Carvedilol did not alter the plasma exposure to MDMA.

CONCLUSIONS AND IMPLICATIONS

α_1- and β-Adrenoceptors contribute to the cardiostimulant and thermogenic effects of MDMA in humans but not to its psychotropic effects. Carvedilol could be useful in the treatment of cardiovascular and hyperthermic complications associated with ecstasy use.

Abbreviations

AUC, area under the concentration–time curve; C_{max}, maximal plasma concentration; CYP, cytochrome P450; 5D-ASC, 5-Dimensions of Altered States of Consciousness; E_{max}, maximal effect; MDA, ±3,4-methylenedioxyamphetamine; MDMA, ±3,4-methylenedioxymethamphetamine; VAS, Visual Analogue Scale

Introduction

±3,4-Methylenedioxymethamphetamine (MDMA, 'ecstasy') is widely abused for its euphoric effects. The use of ecstasy is associated with hyperthermia (Henry *et al.*, 1992; Liechti *et al.*, 2005; Halpern *et al.*, 2011). MDMA-induced hyperthermia is a life-threatening disorder that may lead to rhabdomyolysis, disseminated intravascular coagulation, acute hepatic and renal failure and death (Henry *et al.*, 1992; Liechti *et al.*, 2005). Severe hyperthermia has typically been observed when ecstasy is used in crowded clubs, at high ambient temperatures or during physical activity (Henry *et al.*, 1992; Parrott, 2012). In laboratory animals, crowding, high ambient temperature, reduced water consumption and repeated dosing similarly enhanced MDMA-induced hyperthermia (Dafters, 1995; Docherty and Green, 2010). However, MDMA also

elevates body temperature under controlled laboratory conditions in humans in the absence of permissive factors (Liechti *et al.*, 2001; Freedman *et al.*, 2005; Dumont and Verkes, 2006; Parrott, 2012). The clinical treatment of sympathomimetic amphetamine toxicity is mainly supportive and includes volume repletion and sedation with benzodiazepines (Liechti *et al.*, 2005; Halpern *et al.*, 2011). The management of severe MDMA-related hyperpyrexia includes cooling and ventilation (Hall and Henry, 2006). Dantrolene, which acts peripherally at skeletal muscles to inhibit release of calcium from the sarcoplasmic reticulum, has also been used (Green *et al.*, 1995; Hall and Henry, 2006; Grunau *et al.*, 2010). However, dantrolene does not inhibit the thermogenic effects of MDMA (Rusyniak *et al.*, 2004) and the drug does not specifically interfere with the presumed mechanism of MDMA-induced hyperthermia. MDMA mainly releases 5-HT, NA and dopamine (Rudnick and Wall, 1992; Liechti and Vollenweider, 2001; Verrico *et al.*, 2007). Stimulation of both α_1- and β_3-adrenoceptors has been implicated in the thermogenic effects of MDMA (Sprague *et al.*, 2004a; 2005). Specifically, increasing NA levels through the inhibition of phenylethanolamine *N*-methyltransferase potentiated the hyperthermic effects of MDMA in rats (Sprague *et al.*, 2007). Combined pretreatment with the α_1-adrenoceptor antagonist prazosin plus the β_3-adrenoceptor antagonist SR59230A attenuated MDMA-induced elevations in core body temperature and creatine kinase levels in rats (Sprague *et al.*, 2004a). The α_1 and $\beta_{1,2,3}$ antagonist carvedilol similarly prevented the hyperthermic response to MDMA in rats (Sprague *et al.*, 2005). Moreover, carvedilol reversed established hyperthermia when it was administered 1 h after MDMA (Sprague *et al.*, 2005). Selective inhibition of β_3 receptors with low concentrations of SR59230A attenuated the slowly developing late hyperthermic response to MDMA, suggesting a role for β_3 receptors in this late response in mice (Bexis and Docherty, 2008). In contrast, α_1 blockade with prazosin induced an early hypothermic reaction to MDMA, consistent with a role for α_1-receptors in this early response to MDMA in mice (Bexis and Docherty, 2008). Finally, mice deficient in uncoupling protein 3, which is regulated by NA, were protected against the hyperthermic effects of MDMA (Mills *et al.*, 2003) and methamphetamine (Sprague *et al.*, 2004b). Altogether, the preclinical data suggest that MDMA-induced hyperthermia results from noradrenergic activation of mitochondrial uncoupling that involves both α_1- and β_3-adrenoceptors (Mills *et al.*, 2004; Rusyniak *et al.*, 2005). Additionally, α_1-receptors contribute to the vasoconstriction of skin blood vessels, impairing heat dissipation, which enhances hyperthermia induced by MDMA (Pedersen and Blessing, 2001).

Psychostimulants, including MDMA, also produce hypertension and tachycardia. Myocardial ischaemia and stroke are complications of the sympathomimetic action of cocaine and ecstasy (Brody *et al.*, 1990; Liechti *et al.*, 2005; Bruggisser *et al.*, 2010; Halpern *et al.*, 2011). Selective β-adrenoceptor blockers are commonly used in the treatment of myocardial infarction or acute hypertension but are not recommended if psychostimulants are involved because of the risk of unopposed α_1-receptor stimulation (Hoffman, 2008). Indeed, propranolol potentiated cocaine-induced coronary vasoconstriction (Lange *et al.*, 1990) and worsened cocaine-associated hypertension (Ramoska and Sacchetti, 1985). β blockade also did not affect the blood pressure response to MDMA (Hysek *et al.*, 2010). In contrast, α- and β-adrenoceptor blockade with labetalol (Boehrer *et al.*, 1993; Sofuoglu *et al.*, 2000b) and carvedilol (Sofuoglu *et al.*, 2000a) dose-dependently prevented the haemodynamic response to cocaine in humans. Labetalol also had no negative effect on cocaine-induced coronary vasoconstriction (Boehrer *et al.*, 1993). Combined α- and β-blockers may therefore be the treatment of choice for stimulant-associated hypertension and myocardial ischaemia.

Because carvedilol has been shown to prevent MDMA-induced hyperthermia and rhabdomyolysis in rats (Sprague *et al.*, 2005) and the cardiostimulant response to cocaine in humans (Sofuoglu *et al.*, 2000a), we evaluated the effects of carvedilol on the cardiovascular and hyperthermic response to MDMA in healthy subjects.

Methods

Study design

We used a double-blind, double-dummy placebo-controlled, randomized, crossover study design with four experiential conditions (placebo-placebo, carvedilol-placebo, placebo-MDMA and carvedilol-MDMA) in a balanced order. The washout periods between the sessions were at least 10 days. The study was conducted at the University Hospital of Basel in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland, and Swiss Agency for Therapeutic Products (Swissmedic). The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health. The study was registered at ClinicalTrials.gov (NCT01270672). The reduction in the MDMA-induced increase in blood pressure by carvedilol was the predefined primary outcome of this clinical trial.

Study procedures

The subjects completed a screening visit, four test sessions and an end-of-study visit. The test sessions were conducted in a quiet hospital research ward with no more than two research subjects present per session. The mean (SD) room temperature was 23.3°C (0.7°C). At the beginning of each test session, an indwelling i.v. catheter was placed in the antecubital vein for blood sampling. Carvedilol (50 mg) or placebo was administered at 8 h 00 min. MDMA (125 mg) or placebo was administered at 9 h 00 min. A standardized lunch was served at 12 h 00 min, and the subjects were sent home at 15 h 00 min.

Subjects

Sixteen healthy subjects (eight men, eight women) with a mean (SD) age of 24.2 (2.2) years and a mean body weight of 67 (13) kg were recruited from the university campus. The allocation to treatment order was performed by drawing from blocks of eight different balanced drug treatment sequences by two pharmacists not involved in the study. Each code was stored in a sealed envelope until the termination of the study. Data from all 16 subjects were available for the final analysis.

The exclusion criteria included the following: (i) age <18 or >45 years; (ii) pregnancy determined by a urine test before each test session; (iii) body mass index <18.5 kg·m^{-2} or >25 kg·m^{-2}; (iv) personal or family (first-degree relative) history of psychiatric disorder [determined by the structured clinical interview for Axis I and Axis II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Wittchen *et al.*, 1997) supplemented by the SCL-90-R Symptom Checklist (Derogatis *et al.*, 1976; Schmitz *et al.*, 2000)]; (v) regular use of medications; (vi) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard haematology and chemical blood analyses; (vii) smoking more than seven cigarettes per day; (viii) a lifetime history of using illicit drugs more than five times, with the exception of cannabis; (ix) illicit drug use within the last 2 months; and (x) illicit drug use during the study, determined by urine tests conducted before the test sessions using TRIAGE 8 (Biosite, San Diego, CA, USA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and limit alcohol use to one glass on the day before each test session. All of the subjects were non-smokers. All of the subjects, with the exception of one, had previously used cannabis. Four subjects reported using illicit drugs, in which three subjects had tried amphetamine once and one had tried ecstasy once and amphetamine three times. All of the subjects were phenotyped for cytochrome P450 (CYP) 2D6 activity using dextromethorphan as the probe drug. Nine extensive, six intermediate and one poor CYP2D6 metabolizer were identified in the study. The female subjects were investigated during the follicular phase (day 2–14) of their menstrual cycle when the reactivity to amphetamines is expected to be similar to men (White *et al.*, 2002). All of the subjects provided their written informed consent before participating in the study, and they were paid for their participation.

Drugs

±MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was prepared as gelatine capsules (100 and 25 mg of the salt). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single oral dose of 125 mg, corresponding to a dose of 1.93 ± 0.36 mg·kg^{-1} body weight. Carvedilol tablets (50 mg, Dilatrend, Roche Pharma AG, Basel, Switzerland) were encapsulated within opaque gelatine capsules, and identical placebo (lactose) capsules were prepared. An oral dose of carvedilol (50 mg) was used that has previously been shown to attenuate the smoked cocaine-induced increases in heart rate and blood pressure in humans (Sofuoglu *et al.*, 2000a). At this dose, carvedilol is expected to inhibit both α_1- and β-adrenoceptors (Tham *et al.*, 1995; Sofuoglu *et al.*, 2000a). Carvedilol or placebo was administered 1 h before MDMA or placebo administration so that the maximal plasma concentration (C_{max}) of carvedilol was reached (Morgan, 1994) shortly before the C_{max} of MDMA occurred. Oral medication administration was supervised by study personnel.

Pharmacodynamic measurements

Vital signs. Vital signs were assessed repeatedly 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h after MDMA or placebo administration. Heart rate, systolic blood pressure and diastolic blood pressure were measured using an OMRON M7 blood pressure monitor (Omron Healthcare Europe, Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5 min. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Core (tympanic) temperature was assessed using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY, USA).

Plasma catecholamines. Blood samples to determine the concentrations of NA and adrenaline were taken 1 h before and 1 and 2 h after MDMA or placebo administration. All of the blood samples were collected on ice and centrifuged within 10 min at 4°C. The plasma was then stored at –20°C until analysis. The plasma levels of free catecholamines (NA and adrenaline) were determined by HPLC with an electrochemical detector as described previously (Hysek *et al.*, 2011).

Psychometric scales. Subjective measures were repeatedly assessed using Visual Analogue Scales (VASs; (Hysek *et al.*, 2011) 1 h before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5 and 6 h after MDMA or placebo administration. The VASs included 'any drug effect', 'good drug effect', 'bad drug effect', 'drug liking', 'drug high' and 'stimulated' (Farre *et al.*, 2007; Kolbrich *et al.*, 2008; Hysek *et al.*, 2011). The VASs were presented as 100-mm horizontal lines marked 'not at all' on the left and 'extremely' on the right. Additionally, the 5-Dimensions of Altered States of Consciousness Scale [5D-ASC; (Dittrich, 1998; Studerus *et al.*, 2010)] was applied 4 h after MDMA or placebo administration. The 5D-ASC rating scale measures alterations in mood, perception and experience of self in relation to the environment and thought disorder (Studerus *et al.*, 2010). The 5D-ASC dimension 'oceanic boundlessness' (27 items) measures derealization and depersonalization associated with positive mood. The dimension 'anxious ego dissolution' (21 items) summarizes ego disintegration and loss of self-control, phenomena associated with anxiety. The dimension 'visionary restructuralization' (18 items) describes perceptual alterations. Two other dimensions of the scale were not used in our study. The total ASC score was determined by adding the scores of the three dimensions.

Adverse effects. Adverse effects were assessed 1 h before and 3 and 24 h after MDMA or placebo administration using the List of Complaints (Zerssen, 1976; Hysek *et al.*, 2011). The scale consists of 66 items that yield a total adverse effects score, reliably measuring physical and general discomfort.

Pharmacokinetic measurements

Samples of plasma for the determination of MDMA and ±3,4-methylenedioxyamphetamine (MDA), the active metabolite of MDMA, were collected 1 h before and 0 (just before), 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4 and 6 h after MDMA or placebo administration. The plasma concentrations of MDMA and MDA were determined using HPLC coupled to tandem MS as described previously (Hysek *et al.*, 2012).

Data analysis

Pharmacokinetic analysis. The data for the plasma concentrations of MDMA and MDA were analysed using non-

compartmental methods. C_{max} and time to C_{max} were obtained directly from the concentration–time curves of the observed values. The area under the plasma concentration–time curve $(AUC)_{0-6\,h}$ was calculated using the linear trapezoidal rule. Plasma concentrations were only determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in plasma levels of MDMA during the time of the pharmacodynamic effects of MDMA.

Statistical analysis. Values were transformed to differences from baseline. The maximal effect (E_{max}) values were determined for repeated measures and analysed by two-way General Linear Models repeated-measures ANOVA with the two drug factors MDMA (MDMA vs. placebo) and carvedilol (carvedilol vs. placebo) using STATISTICA 6.0 software (StatSoft, Tulsa, OK, USA). Tukey's *post hoc* comparisons were performed based on significant main effects or interactions. Additional ANOVAs were performed, with drug order as an additional factor, to exclude carry-over effects. The criterion for significance was $P < 0.05$. A sample-size estimation based on previous data (Hysek *et al.*, 2011; 2012) showed that eight subjects would be needed to detect a relevant change in the primary study outcome with 80% power using a within-subjects study design.

Results

Vital signs and circulating catecholamines

MDMA significantly increased blood pressure, heart rate and body temperature compared with placebo (Table 1 and Figure 1). Carvedilol significantly inhibited the MDMA-induced increases in blood pressure, heart rate and body temperature (Table 1 and Figure 1). Carvedilol alone also moderately lowered blood pressure and heart rate compared with placebo. The effect of carvedilol on the pressure and hyperthermic response to MDMA was more pronounced than the effect of carvedilol alone compared with placebo, corroborated by the significant carvedilol × MDMA interaction in the two-way ANOVA. Carvedilol alone increased the plasma concentration of NA compared with placebo. MDMA also tended to increase circulating NA compared with placebo, but the effect was not significant. The co-administration of carvedilol and MDMA significantly increased both circulating adrenaline and NA (Table 1 and Figure 2).

Subjective effects

Carvedilol did not affect the psychotropic response to MDMA. It did not alter the pronounced MDMA-induced increases in the VAS (Table 1 and Figure 3) or 5D-ASC ratings of subjective drug effects (Table 1). Carvedilol alone had no subjective effects.

Adverse effects

MDMA increased the total adverse effect score on the List of Complaints, both 3 and 24 h after drug administration compared with placebo (Table 1). Carvedilol had no effect on the MDMA-induced increase in the total score. However, fewer subjects reported palpitations and hot flushes after carvedilol and MDMA co-treatment (n = 2 and n = 2, respectively) compared with MDMA treatment alone (n = 6 and n = 5, respectively). Frequent adverse effects of MDMA and carvedilol-MDMA were thirst (n = 10 and n = 11, respectively), lack of appetite (n = 9 and n = 7, respectively), sweating (n = 8 and n = 7, respectively), restlessness (n = 7 and n = 5, respectively) and bruxism (n = 7 and n = 7, respectively). No severe adverse effects were reported.

Pharmacokinetics and pharmacokinetic–pharmacodynamic relationship

The decrease in the cardiovascular and thermogenic response to MDMA after carvedilol pretreatment was not attributable to a pharmacokinetic interaction between carvedilol and MDMA. Carvedilol did not affect the C_{max} or $AUC_{0-6\,h}$ of MDMA or MDA (Table 2 and Figure 4A). The effect of MDMA on blood pressure in relation to the plasma concentration of MDMA is illustrated by the hysteresis curves in Figure 4B. Carvedilol produced a pronounced downward shift in the E_{max} of the systolic pressure response to MDMA and a rightward shift in the C_{max} of MDMA in the concentration-effect curve (Figure 4B). The pharmacokinetic parameters of MDMA did not depend on CYP2D6 phenotype or the dextromethorphan : dextrorphan ratio in our small study sample.

Discussion

The α_1- and $\beta_{1,2,3}$-adrenoceptor antagonist carvedilol reduced the cardiostimulant and hyperthermic response to MDMA in healthy subjects. Carvedilol similarly reduced MDMA-induced hyperthermia in rats (Sprague *et al.*, 2004a; 2005). Additional studies in rats and mice showed that the transient and early hypothermic effect of MDMA are enhanced by blocking α_1-receptors (Bexis and Docherty, 2008), whereas the late hyperthermic response to MDMA is blunted by blocking β_3-receptors (Sprague *et al.*, 2004a; Bexis and Docherty, 2008). Moreover, α_1-receptors mediate peripheral vasoconstriction and heat dissipation, which are impaired by MDMA (Pedersen and Blessing, 2001). Administration of $\beta_{1,2}$-receptor antagonists had no effect on the thermogenic response to MDMA in rats (Sprague *et al.*, 2005) or humans (Hysek *et al.*, 2010). These data suggest a role for both α_1- and β_3-receptors in MDMA-induced hyperthermia. Carvedilol should be considered for the treatment of hyperthermia associated with ecstasy use because it effectively reduced MDMA-induced hyperthermia in both animals and humans and reversed established hyperthermia in rats (Sprague *et al.*, 2005).

In addition to adrenoceptors, other sites have been implicated in stimulant-induced hyperthermia. MDMA primarily induces the release of 5-HT, NA and dopamine through their respective presynaptic monoamine transporters (Rudnick and Wall, 1992; Rothman *et al.*, 2001; Verrico *et al.*, 2007). MDMA binds to α_2-adrenoceptors, 5-HT_{2A}-receptors, H_1-histamine and trace amine-1 receptors (Battaglia *et al.*, 1988; Bunzow *et al.*, 2001). The 5-HT_{2A}-receptor antagonist ketanserin inhibited the thermogenic effects of MDMA in rats (Shioda *et al.*, 2008), mice (Di Cara *et al.*, 2011) and humans (Liechti *et al.*, 2000). In both mice and humans, ketanserin administered alone lowered body temperature compared with vehicle and placebo, respectively (Liechti *et al.*, 2000; Di Cara *et al.*, 2011).

Table 1

Values and statistics of pharmacodynamic changes

	Placebo-placebo Mean (SEM)	Carvedilol-placebo	Placebo-MDMA	Carvedilol-MDMA	MDMA		Carvedilol		Carvedilol × MDMA	
					$F_{1,15}$	P	$F_{1,15}$	P	$F_{1,15}$	P
Physiological effects										
Systolic blood pressure (mmHg)	4.7 (1.8)	−8.1 (2.0)***$^{\#\#\#}$	28.1 (3.2)***	6.5 (2.2)$^{\#\#\#}$	59.30	<0.001	72.33	<0.001	5.02	<0.05
Diastolic blood pressure (mmHg)	−1.0 (1.4)	−8.1 (1.6)*$^{\#\#\#}$	15.3 (1.6)***	9.3 (1.9)***$^{\#}$	151.10	<0.001	16.40	<0.001	1.70	NS
Heart rate (beats min^{-1})	5.8 (3.0)	−5.0 (2.5)*$^{\#\#\#}$	26.2 (3.9)***	5.5 (3.0)$^{\#\#\#}$	15.44	<0.001	38.84	<0.001	18.64	<0.001
Body temperature (°C)	0.24 (0.06)	0.32 (0.06)$^{\#\#}$	0.69 (0.10)***	0.40 (0.06)$^{\#}$	13.78	<0.01	3.29	NS	7.65	<0.05
Circulating catecholamines										
Adrenaline (nM)	−0.03 (0.03)	0.08 (0.05)	0.23 (0.06)	0.70 (0.15)***$^{\#\#\#}$	19.63	<0.001	14.04	<0.01	14.20	<0.01
Noradrenaline (nM)	−0.34 (0.14)	1.85 (0.36)***$^{\#\#}$	0.29 (0.14)	2.58 (0.40)***$^{\#\#\#}$	4.33	0.055	59.86	<0.001	0.04	NS
Visual Analogue Scale (%max)										
Any drug effect	2.4 (1.4)	7.1 (3.4)$^{\#\#\#}$	64.8 (7.5)***	69.6 (7.6)***	94.67	<0.001	1.50	NS	0.00	NS
Good drug effect	1.4 (1.4)	0.0 (0.0)$^{\#\#\#}$	71.1 (7.6)***	76.8 (7.2)***	112.69	<0.001	0.40	NS	1.01	NS
Bad drug effect	0.3 (0.3)	2.5 (1.1)	13.6 (5.1)	25.3 (9.1)**	13.70	<0.01	1.51	NS	0.92	NS
Drug liking	1.6 (1.4)	0.0 (0.0)$^{\#\#\#}$	74.8 (7.1)***	75.9 (7.9)***	106.20	<0.001	0.01	NS	0.20	NS
Drug high	1.7 (1.7)	0.0 (0.0)$^{\#\#\#}$	59.4 (9.0)***	66.3 (8.7)***	56.46	<0.001	0.49	NS	1.15	NS
Stimulated	2.0 (2.0)	0.4 (0.4)$^{\#\#\#}$	57.8 (9.2)***	61.7 (8.9)***	47.78	<0.001	0.10	NS	0.38	NS
5D-ASC Scale										
Total ASC score	8.7 (8.7)	0.0 (0.0)$^{\#\#}$	747 (177)**	894 (227)***	20.06	<0.001	0.55	NS	0.68	NS
Oceanic boundlessness	7.6 (7.6)	0.0 (0.0)$^{\#\#\#}$	436 (119)**	531 (152)***	13.91	<0.01	0.86	NS	1.12	NS
Anxious ego dissolution	0.7 (0.7)	0.0 (0.0)$^{\#}$	192 (75)*	161 (55)	13.53	<0.01	0.12	NS	0.11	NS
Visionary restructuralization	0.4 (0.4)	0.0 (0.0)$^{\#}$	119 (33)*	202 (54)***	16.39	<0.001	3.88	NS	3.94	0.07
List of complaints (total score)										
Acute adverse effects (at 3 h)	−0.2 (0.3)	0.9 (0.4)$^{\#\#\#}$	8.4 (1.5)***	9.9 (2.0)***	46.96	<0.001	0.37	NS	0.08	NS
Subacute adverse effects (at 24 h)	0.1 (0.3)	1.1 (0.8)	5.3 (1.6)*	4.9 (1.5)*	25.96	<0.001	0.08	NS	0.47	NS

Values are expressed as mean (SEM) changes from baseline of 16 subjects. ASC, Altered States of Consciousness; NS, not significant.
*P < 0.05, **P < 0.01, ***P < 0.001, compared with placebo-placebo. $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001, compared with placebo-MDMA.

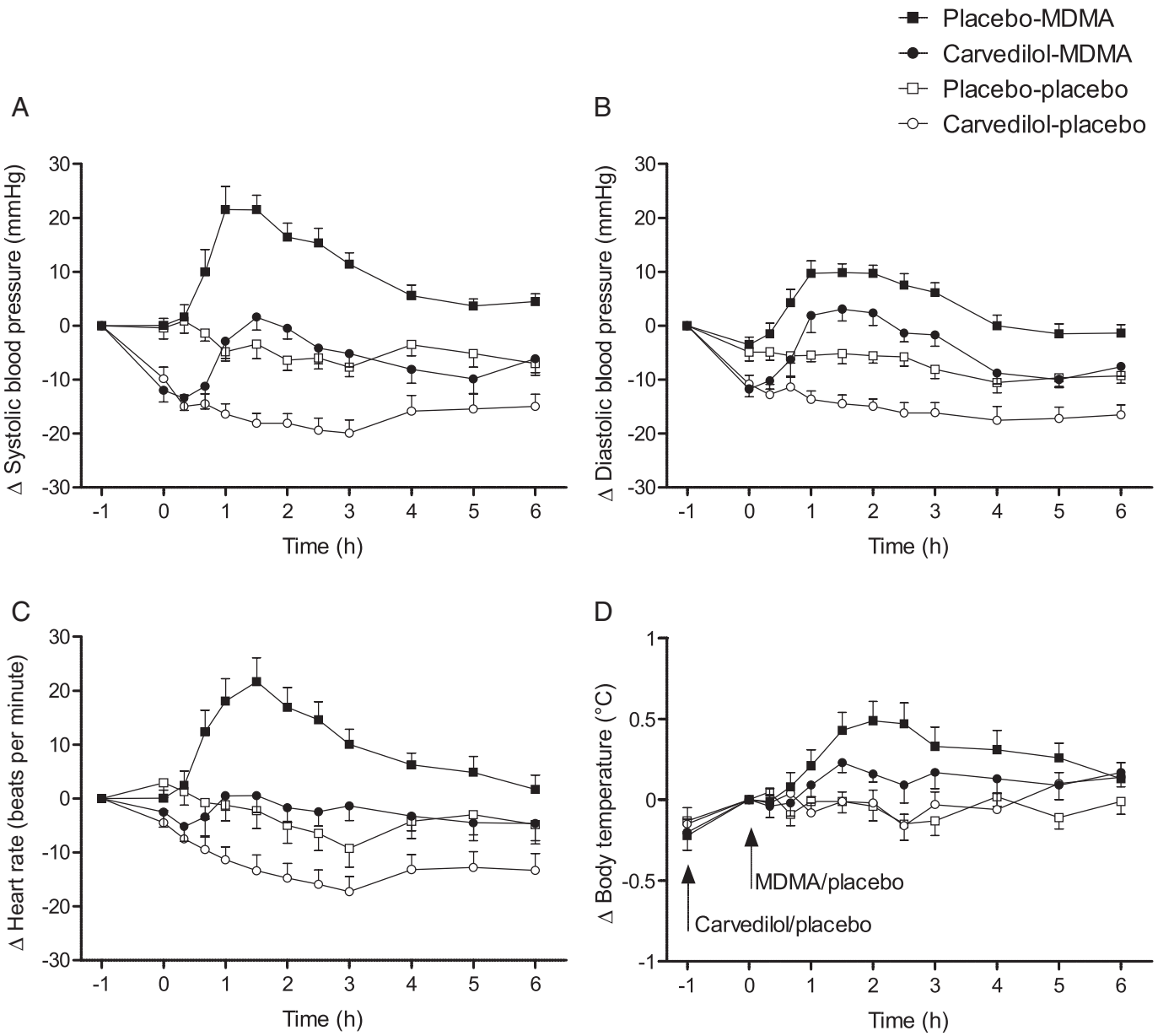

Figure 1

Physiological effects of carvedilol and MDMA. Carvedilol reduced MDMA-induced elevations in systolic (A) and diastolic (B) blood pressure, heart rate (C) and body temperature (D). Carvedilol was administered at t = –1 h. MDMA was administered at t = 0 h. The values are expressed as mean ± SEM changes from baseline in 16 subjects.

Thus, no interactive effect of ketanserin and MDMA on body temperature was observed, in contrast to carvedilol and MDMA in the present study. Furthermore, ketanserin has α_1-adrenoceptor-blocking properties (Brogden and Sorkin, 1990), and its ability to reduce MDMA-associated hyperthermia may be explained, at least partially, by α_1-receptor antagonism. A recent study showed that mice that lack trace amine-1 receptors did not exhibit the early hypothermic response to MDMA, indicating a role for this receptor in the early hypothermic effects of MDMA (Di Cara *et al.*, 2011). D_1- and D_2-dopamine receptors, α_2-adrenoceptors and 5-HT_1-receptors do not appear to be involved in the effects of MDMA on body temperature, demonstrated by preclinical (Docherty and Green, 2010; Di Cara *et al.*, 2011) and clinical (Liechti and Vollenweider, 2000; Hysek *et al.*, 2010; 2012) studies.

Recreational users of ecstasy report subjective increases in body temperature, sweating and hot flushes (Parrott *et al.*, 2008). Hot flushes and sweating were also reported after administration of MDMA in the present and in previous studies (Liechti *et al.*, 2001; Freedman *et al.*, 2005). Carvedilol did not reduce the number of subjects who reported MDMA-induced subjective sweating but reduced the number of subjects reporting flushes. Interestingly, in another laboratory

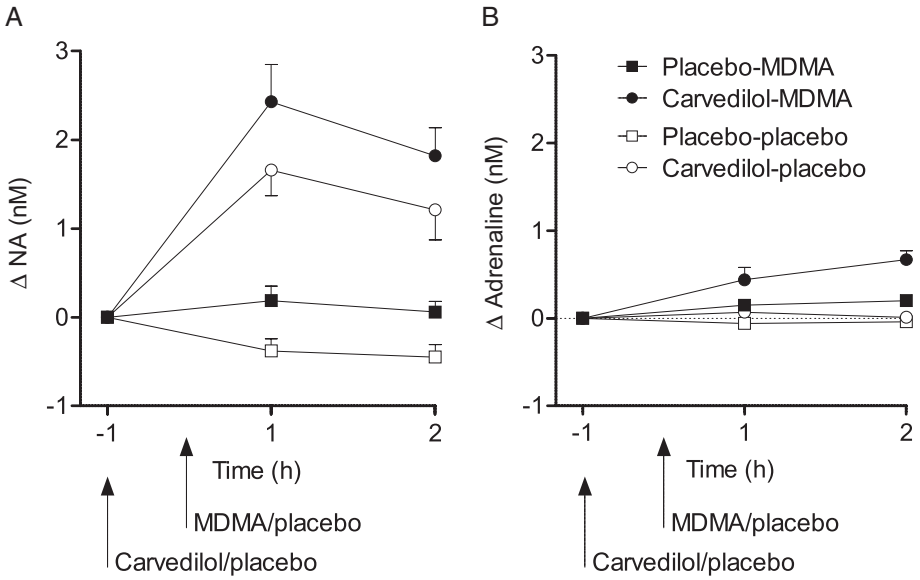

Figure 2

Effects of carvedilol and MDMA on circulating catecholamines. Carvedilol alone increased the plasma levels of noradrenaline (A) compared with placebo. MDMA alone produced a similar non-significant increase in noradrenaline. Co-administration of carvedilol and MDMA increased the concentrations of circulating noradrenaline (A) and adrenaline (B) compared with placebo. The values are expressed as mean ± SEM changes from baseline in 16 subjects.

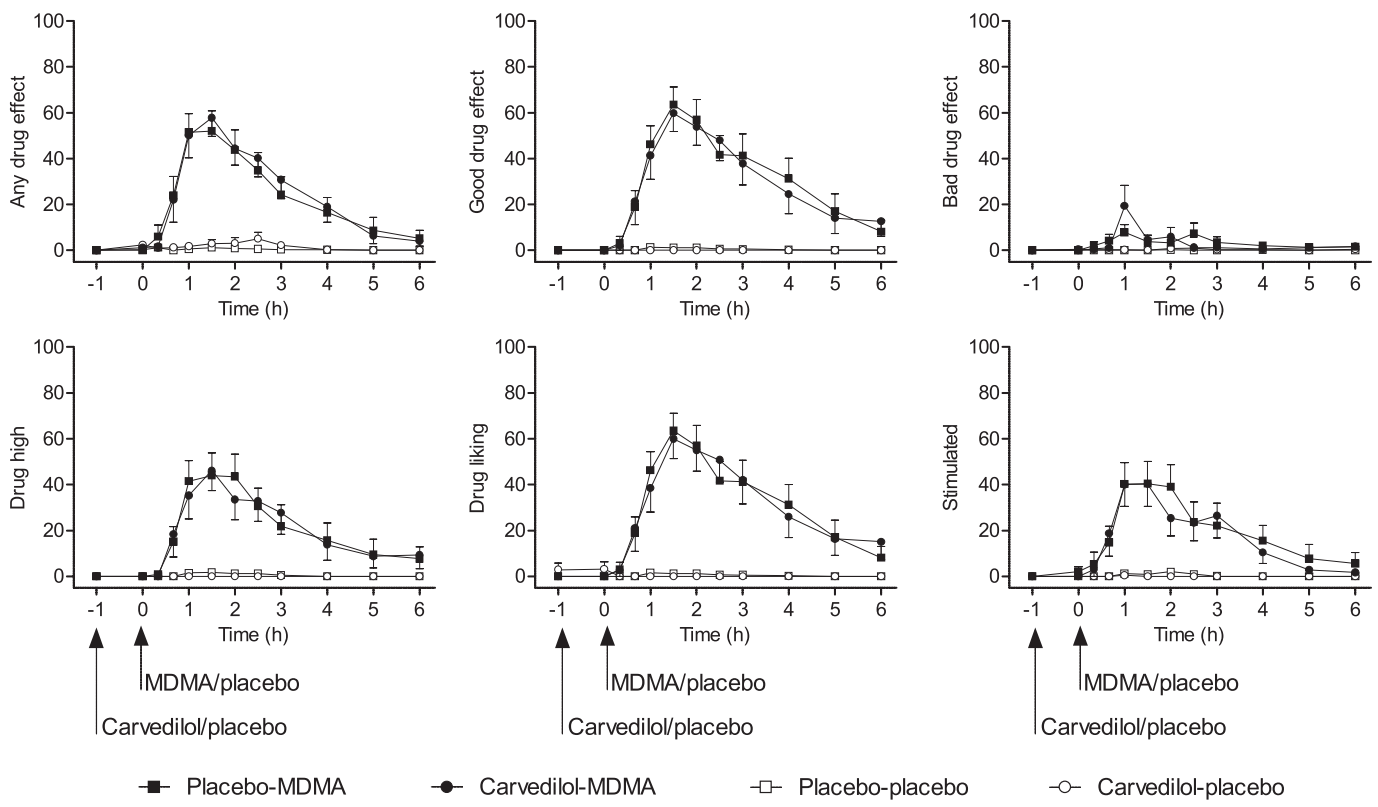

Figure 3

Time course of subjective drug effects on Visual Analogue Scale ratings. MDMA increased scores on all scales. Carvedilol did not affect any of the MDMA-induced increases in Visual Analogue Scale ratings. Carvedilol was administered at t = –1 h. MDMA was administered at t = 0 h. The values are expressed as mean ± SEM percentage of maximal values in 16 subjects.

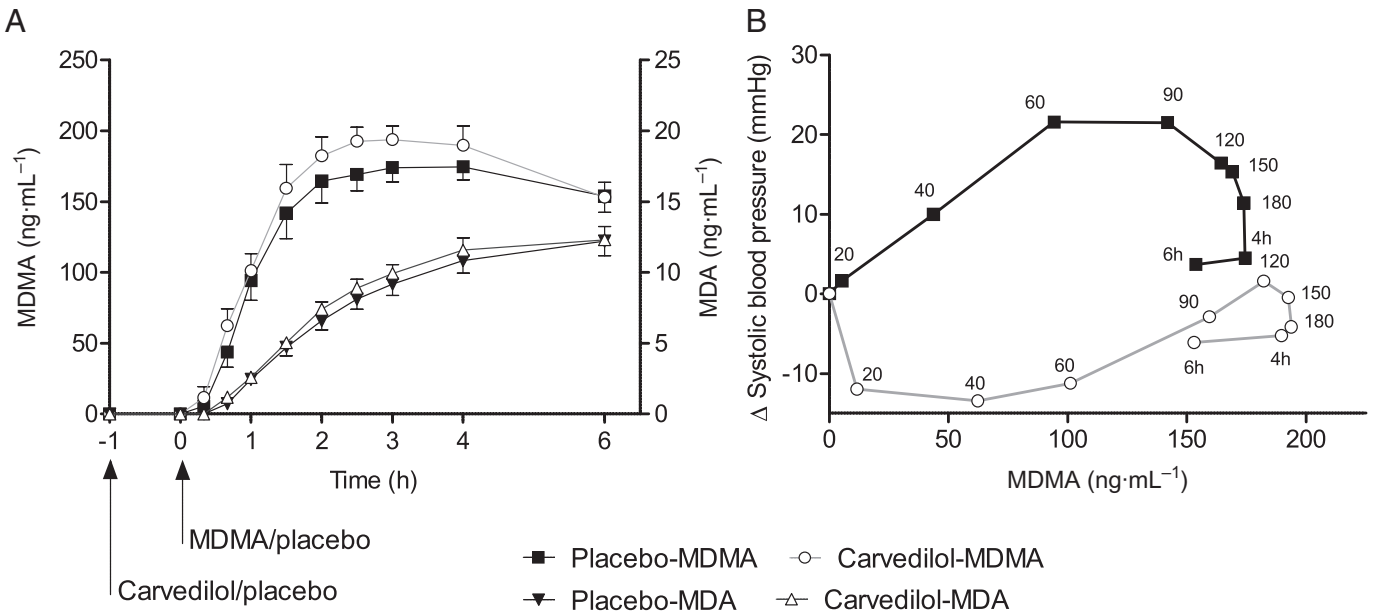

Figure 4

Pharmacokinetics (A) and pharmacokinetic–pharmacodynamic relationship (B). Carvedilol non-significantly increased the exposure to MDMA and MDA (A). The values are expressed as mean ± SEM in 16 subjects. Carvedilol was administered at t = –1 h. MDMA was administered at t = 0 h. MDMA effects on systolic blood pressure plotted against MDMA plasma concentration (B). The values are expressed as means of the changes from baseline in 16 subjects. The time of sampling is noted next to each point in min or h after MDMA administration. Carvedilol produced a downward and rightward shift of the concentration-blood pressure response curve of MDMA (B).

Table 2

Pharmacokinetic parameters of MDMA and MDA

	C_{max} (ng·mL^{-1})	T_{max} (h)	$AUC_{0-6\,h}$ (h·ng·mL^{-1})
MDMA			
Placebo-MDMA	214 (12)	2.9 (0.3)	866 (47)
Carvedilol-MDMA	224 (12)	2.8 (0.2)	921 (45)
MDA			
Placebo-MDMA	12.3 (1.0)	5.5 (0.2)	46.3 (3.5)
Carvedilol-MDMA	12.5 (0.9)	5.0 (0.4)	49.3 (2.8)

Values are mean (SEM) of 16 healthy subjects. AUC, area under concentration–time curve; C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration.

study, MDMA did not influence the perceptions of warmth and cold but delayed the onset of sweating at a warm ambient temperature along with an MDMA-induced increase in body temperature (Freedman *et al.*, 2005).

Carvedilol also reduced the cardiostimulant response to MDMA, including blood pressure and heart rate. The α- and β-blockers carvedilol and labetalol have similarly been shown to inhibit the blood pressure response to cocaine in humans (Boehrer *et al.*, 1993; Sofuoglu *et al.*, 2000a,b). Blockade of β-receptors alone did not reduce the pressure response to cocaine (Ramoska and Sacchetti, 1985) or MDMA (Hysek *et al.*, 2010) in humans and enhanced cocaine-induced coronary vasoconstriction (Lange *et al.*, 1990). In rats, the blockade of α_1-receptors inhibited both the pressure response and vasoconstriction in isolated vessels in response to cocaine (Mo *et al.*, 1999). The data indicate that dual α,β-blockers, but not selective β-blockers, should be used in the treatment of psychostimulant-associated hypertension and myocardial ischaemia. The data indicate that carvedilol could be useful in the treatment of both psychostimulant-induced hypertension and hyperthermia.

Circulating catecholamine levels were increased by both MDMA and carvedilol. Plasma adrenaline is mainly derived from the adrenals, whereas plasma NA stems largely from transmitters released by sympathetic nerves and the escape of NA into the circulation (Esler *et al.*, 1990; Eisenhofer *et al.*, 1995). Circulating NA is therefore considered an indicator of sympathetic system activation. We observed a marked increase in plasma NA concentrations after carvedilol administration. This compensatory sympathoadrenal response with enhanced levels of catecholamines has previously been documented after α_1- or α- and β-adrenoceptor blockade (Omvik *et al.*, 1992; Mazzeo *et al.*, 2001). The MDMA-induced increase in circulating NA in the present study did not reach statistical significance compared with previous work (Dumont *et al.*, 2009; Hysek *et al.*, 2011; 2012). It is possible that the peak effect was missed because we took only two samples. The catecholamine response was enhanced when MDMA was administered following carvedilol. A similar potentiation of the exercise-induced increases in plasma catecholamines has been shown following blockade of α_1-adrenoceptors or α- and β-adrenoceptors (Berlin *et al.*, 1993).

Preclinical and clinical studies suggest that NA contributes to the mediation of the subjective effects of MDMA and other psychostimulants (Sofuoglu and Sewell, 2009; Hysek *et al.*, 2011; Newton, 2011). For example, MDMA is more potent in releasing NA than 5-HT or dopamine from monoamine-preloaded human embryonic kidney cells transfected with the corresponding human monoamine transporters (Verrico *et al.*, 2007). Additionally, doses of stimulants that produce amphetamine-type subjective effects in humans correlated with their potency to release NA (Rothman *et al.*, 2001). Furthermore, the NA transporter inhibitor reboxetine attenuated the cardiovascular and subjective response to MDMA in humans, indicating a role for MDMA-induced transporter-mediated NA release in the psychostimulant effects of MDMA (Hysek *et al.*, 2011). Similarly, atomoxetine attenuated the effects of amphetamine in humans (Sofuoglu *et al.*, 2009). Clonidine, which blocks the vesicular release of NA, did not affect the psychological effects of MDMA in humans (Hysek *et al.*, 2012). Although these data suggest a role for transporter-mediated NA release in the psychotropic effects of psychostimulants, how and which postsynaptic adrenoceptors are involved are still unclear. Carvedilol did not alter the subjective effects of MDMA in the present study. Similar to our results, carvedilol and labetalol did not affect the subjective responses to cocaine in humans at doses of cocaine that effectively inhibited the cardiostimulant effects of the drug (Sofuoglu *et al.*, 2000a,b). The available clinical data do not support a critical role for α_1- and $\beta_{1,2,3}$-receptors in the subjective effects of psychostimulants. Alternatively, the carvedilol concentrations in humans may not have been high enough to produce sufficient adrenoceptor occupancy in the brain. Carvedilol is lipophilic and enters the brain (Elsinga *et al.*, 2005). However, carvedilol is a substrate of the efflux transporter P-glycoprotein in the blood-brain barrier (Elsinga *et al.*, 2005; Bachmakov *et al.*, 2006), and P-glycoprotein activity is known to limit brain exposure to carvedilol (Elsinga *et al.*, 2005).

Preclinical studies indicate that α_1-receptors are involved in the mechanism of action of psychostimulants, including MDMA. For example, pretreatment with the α_1-receptor antagonist prazosin inhibited locomotor stimulation induced by cocaine (Wellman *et al.*, 2002), amphetamine (Vanderschuren *et al.*, 2003) and MDMA (Fantegrossi *et al.*, 2004; Selken and Nichols, 2007) in rats and mice. Additionally, α_1-receptor activation in the ventral tegmental area contributed to the amphetamine-induced release of dopamine in the nucleus accumbens (Pan *et al.*, 1996). Injection of prazosin directly into the ventral tegmental area also blocked the locomotor response to MDMA in rats (Selken and Nichols, 2007). Furthermore, administration of prazosin in the rat prefrontal cortex also blocked amphetamine-induced dopamine release in the nucleus accumbens and hyperactivity (Forget *et al.*, 2011). Finally, α_1-adrenoceptor knockout mice do not show increased amphetamine-induced dopamine release in the nucleus accumbens (Auclair *et al.*, 2002) or behavioural sensitization to amphetamine or cocaine (Drouin *et al.*, 2002). In contrast to α_1-antagonism, the β-blocker propranolol enhanced both cocaine-induced locomotion and the cocaine-induced increase in dopamine in the nucleus accumbens (Harris *et al.*, 1996). Altogether, the preclinical studies indicate that α_1-adrenoceptors, but not β-receptors, play a role in the hyperlocomotion and dopaminergic neurochemical response to psychostimulants. However, the role of adrenoceptors in the reinforcing effects of psychostimulants is unclear. For example, prazosin reduced the self-administration of cocaine (Wee *et al.*, 2008) and nicotine (Forget *et al.*, 2011) in rats. In contrast, prazosin had no effect on cocaine self-administration in rhesus monkeys (Woolverton, 1987). The β-blocker propranolol also inhibited cocaine self-administration in rats (Harris *et al.*, 1996). Carvedilol lowered the number of cocaine self-administrations in humans at a low but not high dose (Sofuoglu *et al.*, 2000a). At low doses, carvedilol preferentially blocks β-receptors (Tham *et al.*, 1995; Sofuoglu *et al.*, 2000a) and active metabolites of carvedilol may contribute to the β- but not the α-adrenoceptor blocking effects of the drug (Spahn-Langguth and Schloos, 1996). The antagonism of α_1-adrenoceptors by carvedilol may not have been sufficient in the brain to attenuate the subjective effects of MDMA and we cannot exclude a role for these receptors. The efficacy of carvedilol to reduce cocaine use or abstinence in addicted patients is currently being investigated in ongoing clinical trials [(Sofuoglu and Sewell, 2009) clinicaltrials.gov identifier: NCT00566969 and NCT01171183]. Further trials have investigated the effects of selective α_1-blockers on the acute response to MDMA (NCT01386177) and cocaine (NCT01062945) and abstinence from cocaine use (NCT00880997).

Pharmacokinetic interactions between carvedilol and MDMA need to be considered in the interpretation of the present findings, because both drugs are metabolized by CYP2D6 (Graff *et al.*, 2001; O'Mathuna *et al.*, 2008). We therefore assessed the potential effects of carvedilol on the pharmacokinetics of MDMA. We found that carvedilol non-significantly increased the plasma exposure to MDMA or MDA. Thus, the reduced haemodynamic and thermogenic effects of MDMA after carvedilol pretreatment did not result from lower plasma levels of MDMA or MDA. We did not assess the plasma concentrations of carvedilol. MDMA inhibits CYP2D6 (O'Mathuna *et al.*, 2008). CYP2D6 inhibition has been shown to increase the exposure to carvedilol but not its pharmacodynamic or adverse effects in humans (Graff *et al.*, 2001).

Our laboratory study has a few limitations. The study design is limited by the use of single doses. We did not use a dose–response study because we did not want to expose the subjects to more than two doses of MDMA in a within-subject design. However, moderate to highly effective doses of both drugs were selected. The primary goal of the study was to investigate the role of adrenoceptors in the mechanism of action of MDMA in humans. Therefore, the study provides only indirect support for the use of carvedilol in the treatment of stimulant toxicity, in which carvedilol would be administered following the ingestion of ecstasy or other stimulants. Furthermore, the MDMA-induced increase in body temperature in our study was moderate, and we do not know whether carvedilol would also be effective in cases of severe hyperthermia following ecstasy use. Finally, thyroid function may modulate the thermogenic effects of MDMA (Martin *et al.*, 2007; Sprague *et al.*, 2007) and thyroid function parameters were not assessed in this study.

In conclusion, carvedilol inhibited the MDMA-induced increase in blood pressure and body temperature under con-

trolled laboratory conditions. The results demonstrate that α_1- and/or $\beta_{1,2,3}$-adrenoceptors contribute to the cardiostimulant and thermogenic effects of MDMA in humans. The absence of an effect of carvedilol on the psychotropic response to MDMA does not support a role for α- and β-adrenoceptors in the mediation of the subjective effects of MDMA in humans. Combined α- and β-blockers could be useful in the treatment of intoxications with MDMA or other psychostimulants including other amphetamine derivatives or cocaine.

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Conflict of interest

None.

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Paper Four: Role of the β Adrenergic Receptor

Effects of a β Blocker on the Cardiovascular Response to MDMA (Ecstasy)

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Effects of a β-blocker on the cardiovascular response to MDMA (Ecstasy)

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ABSTRACT

Background MDMA (3,4-methylenedioxymethamphetamine, 'Ecstasy') produces tachycardia and hypertension and is rarely associated with cardiovascular and cerebrovascular complications. In clinical practice, β-blockers are often withheld in patients with stimulant intoxication because they may increase hypertension and coronary artery vasospasm due to loss of β_2-mediated vasodilation and unopposed α-receptor activation. However, it is unknown whether β-blockers affect the cardiovascular response to MDMA.

Methods The effects of the non-selective β-blocker pindolol (20 mg) on the cardiovascular effects of MDMA (1.6 mg/kg) were investigated in a double-blind placebo-controlled crossover study in 16 healthy subjects.

Results Pindolol prevented MDMA-induced increases in heart rate. Peak values (mean±SD) for heart rate were 84±13 beats/min after MDMA vs 69±7 beats/min after pindolol-MDMA. In contrast, pindolol pretreatment had no effect on increases in mean arterial blood pressure (MAP) after MDMA. Peak MAP values were 115±11 mm Hg after MDMA vs 114±11 mm Hg after pindolol-MDMA. Pindolol did not change adverse effects of MDMA.

Conclusion The results of this study indicate that β-blockers may prevent increases in heart rate but not hypertensive and adverse effects of MDMA.

INTRODUCTION

MDMA (3,4-Methylenedioxymethamphetamine) is the main compound contained in 'Ecstasy' pills. Acute adverse effects of MDMA include hyperthermia leading to rhabdomyolysis and multiorgan failure, hyponatraemic cerebral oedema, acute liver failure, serotonin syndrome and acute panic reactions.[1 2] Rarely, MDMA has been associated with vascular events such as myocardial infarction, subarachnoid and intracranial haemorrhage and cerebral infarction.[3 4] These vascular complications may arise from cardiostimulant and hypertensive effects of MDMA.[5] β-Adrenergic antagonists are commonly used in the treatment of myocardial ischaemia and hypertension. However, in the case of intoxications with cocaine or other stimulants, the use of β-blockers is controversial because β-blockade is thought to worsen hypertension and coronary artery vasospasm through unopposed α-receptor activation.[6–8] It is unknown whether—and, if so, how—β-blockers affect cardiovascular responses to MDMA. We assessed the effects of the non-selective β-blocker pindolol on the haemodynamic effects of MDMA in healthy subjects.

METHODS

Participants

The use of MDMA in healthy subjects was authorised by the Swiss Federal Health Office. Sixteen male volunteers (age 25±4 years, range 20–36) were included in the study. Subjects were recruited from University Hospital staff or were students at the Medical School of the University of Zurich. All volunteers provided written consent after being informed about the aims and design of the study and potential risks associated with MDMA and pindolol use. Subjects were screened to be physically and mentally healthy according to medical history, physical examination, ECG and blood analyses, and were screened by a structured psychiatric interview based on a computerised diagnostic expert system.[9] Exclusion criteria were personal or family histories of mental diagnostic and statistical manual of mental disorders (DSM IV) axis I disorders, hypertension, cardiovascular or neurological disorders, use of medications and prior illicit drug use (except tetrahydrocannabinol-containing products) on more than five occasions. All subjects engaged in regular physical exercise. Apart from sporadic use of cannabis, one subject reported a single previous experience with a hallucinogenic drug (psilocybin), two subjects had previously used both MDMA and a hallucinogen, and seven subjects were drug-naïve. Subjective (primary outcome) and neurocognitive results from the present study have previously been reported.[10] Here we present the previously unpublished cardiovascular and adverse effects (secondary outcomes) of the same study subjects[10] with one additional subject.

Study design and setting

A double-blind placebo-controlled single-dose crossover design was used with four treatment conditions (placebo–placebo, pindolol–placebo, placebo–MDMA or pindolol–MDMA) and a 2-week washout time between sessions. This design has the main advantage that subjects act as their own control. Treatment order was pseudo-random and counterbalanced to avoid time order effects. The duration of the trial for an individual subject was 6–10 weeks. Placebo or pindolol was given to the subjects at 09.00 h on each of the four study days. Sixty minutes later MDMA or placebo was administered. Blood pressure, heart rate and body temperature were measured at 0, 30, 60, 90, 120, 150, 180, and 210 min after pindolol–placebo administration (−60, −30, 0, 30, 60, 90, 120 and 150 min after MDMA–placebo administration). Blood pressure and heart rate were registered by an ERKA ambulatory blood pressure measuring system

(ERKA.OS 90-2, Kallmeyer Medizintechnik GmbH, Bad Tölz, Germany) in the non-dominant arm after a resting time of 5 min with the volunteer sitting in an arm chair with the back supported. Measures were taken once per time point. Between measurements, subjects were allowed to engage in non-strenuous activities such as reading, listening to music or walking around in the testing room. Most of the time subjects were sitting in an armchair or lying on a couch. Body temperature was measured with an axillary thermometer (Terumo C202 Terumo Corp, Tokyo, Japan). Acute adverse effects were assessed 135 min after pindolol−placebo (75 min after MDMA−placebo) administration by the List of Complaints.[5 11] This scale consists of 66 items yielding a total adverse effects score (non-weighted sum of the item answers) reliably measuring physical and general discomfort. The scale has previously been shown to be sensitive to the effects of pharmacological pretreatments on the adverse effects of MDMA.[12 13] Subjective and cognitive drug effects were measured as reported elsewhere.[10]

Substances

(±)-MDMA hydrochloride (Lipomed, Arlesheim) was obtained from the Swiss Federal Health Office. Subjects received MDMA at a dose of 1.6 mg/kg (mean±SD dose 122±14 mg). This dose of MDMA corresponds to a typical recreational dose of Ecstasy and produces robust psychological and physiological effects.[5] Pindolol (Visken, Novartis Pharma, Basel, Switzerland) was used in a dose of 20 mg. Pindolol is a non-selective β-blocker with intrinsic activity and additional serotonergic $5HT_1$-receptor-blocking properties. We selected pindolol for this study and a dose of 20 mg because this dose produces approximately 40% brain $5\text{-}HT_{1A}$ receptor occupancy,[14] and we were also interested in the role of $5\text{-}HT_1$ receptors in the mediation of the subjective effects of MDMA based on behavioural studies in rats.[15–17] Pindolol is commonly used in doses of 5–30 mg per day divided into two daily doses in the treatment of arterial hypertension. Thus, a single dose of 20 mg of pindolol corresponds to a moderate to high therapeutic dose. Pindolol pretreatment slightly attenuated positive derealisation associated with MDMA and did not alter MDMA-induced impairment of cognitive performance as described in detail elsewhere.[10]

Data analysis

All analyses were performed with STATISTICA Version 6.0 (StatSoft Inc, Tulsa, USA). We determined the peak effect in the 150 min after MDMA−placebo administration (time points 60–210 min) and the area under the curve (AUC) of the effects versus time curve calculated by the trapezoidal rule for each value (time points 60–210 min). These individual peak effects and AUC values for each outcome variable were analysed by one-way repeated measures analysis of variance (ANOVA) with treatment condition (placebo−placebo, pindolol−placebo, placebo−MDMA and pindolol−MDMA) as within-subject factor. Post hoc comparisons were performed using Tukey tests based on significant main effects of treatment condition in the omnibus ANOVA. The absence of treatment order and carryover effects was confirmed by ANOVA with treatment order (1–4) as within-subject factor. Treatment effects were also analysed over time with two-way repeated measures ANOVA with treatment condition and time as within-subject factors followed by Tukey tests based on significant treatment by time interactions in the omnibus ANOVA. We controlled for deviations from multivariate normality using Mauchley tests of sphericity. Greenhouse and Geisser corrections were used where necessary to adjust for deviations from multivariate normality. These analyses yielded similar results to those using peak and AUC values. The criterion for significance was set at $p<0.05$. Mean arterial blood pressure (MAP) was calculated from diastolic blood pressure (DBP) and systolic blood pressure (SBP) using the following formula: MAP=DBP+(SBP−DBP)/3. A decrease in MAP of 5 mm Hg was considered clinically relevant and similar to the previously reported one for the effects of citalopram on MDMA-induced increases in blood pressure.[12] A sample size of 15 achieves 97% power to detect a difference of 5 between the null hypothesis mean of −5 and the alternative hypothesis mean of 0 with a known SD of the difference of 5[12] and with a significance level (α) of 0.05 using a two-sided one-sample t test.

RESULTS

Cardiovascular effects and body temperature

The results are shown in figure 1. All 16 subjects completed all four study sessions. ANOVA showed that the four treatment conditions overall resulted in significantly different peak levels of heart rate and MAP across sessions (main effects: $F(3,45)=28.7$, $p<0.001$; $F(3,45)=47.9$, $p<0.001$; respectively). MDMA significantly increased peak values for heart rate by (mean±SD) 15±10 beats/min ($p<0.01$) compared with placebo. Pindolol prevented the MDMA-induced increase in heart rate ($p<0.001$ for placebo−MDMA vs pindolol−MDMA) but had no effect on heart rate when given alone compared with placebo. MDMA significantly increased peak MAP by 16±8 compared with placebo ($p<0.001$). Pindolol had no effect on the peak MAP response to MDMA. The MDMA-induced increase in peak body temperature (0.2±0.3°C compared with placebo) was not significant. Pindolol had no effect on MDMA-induced elevations in body temperature.

Adverse effects

MDMA significantly increased acute adverse effects scores (main effect of treatment: $F(3,45)=10.3$, $p<0.001$, post hoc test: $p<0.001$ for MDMA vs placebo). The most frequently reported acute side effects of MDMA were impaired balance, lack of appetite, thirst, feelings of restlessness or restless legs, difficulty concentrating and feeling cold or warm. None of the subjects reported chest pain. Pindolol did not change the adverse effects of MDMA.

DISCUSSION

The β-blocker pindolol prevented MDMA-induced tachycardia but not hypertension or other adverse effects associated with MDMA. Pindolol is an antagonist at central serotonin $5\text{-}HT_1$ receptors.[14] As described in detail elsewhere,[10] pindolol moderately attenuated MDMA-induced increases in positive mood, dreaminess, derealisation and mania-like experience, indicating a possible role for serotonergic $5\text{-}HT_1$ receptors in the mediation of these mood effects of MDMA. In contrast, pindolol had no effect on MDMA-induced cognitive performance impairment.[10] In addition, the effect of pindolol pretreatment on the subjective response to MDMA was weak compared with that of the serotonin uptake transporter blocker citalopram,[12 18] which is thought to block the interaction of MDMA with the serotonin transporter so inhibits the release of serotonin from presynaptic nerve terminals.

We are not aware of reports on the effects of β-blockers on the haemodynamic effects of amphetamines including MDMA. The β-blocker propranolol decreases heart rate[19 20] and decreases[20] or increases[19] blood pressure in patients with acute cocaine intoxication. In a placebo-controlled study, the α-β-blocker carvedilol increased both heart rate and blood pressure in response to

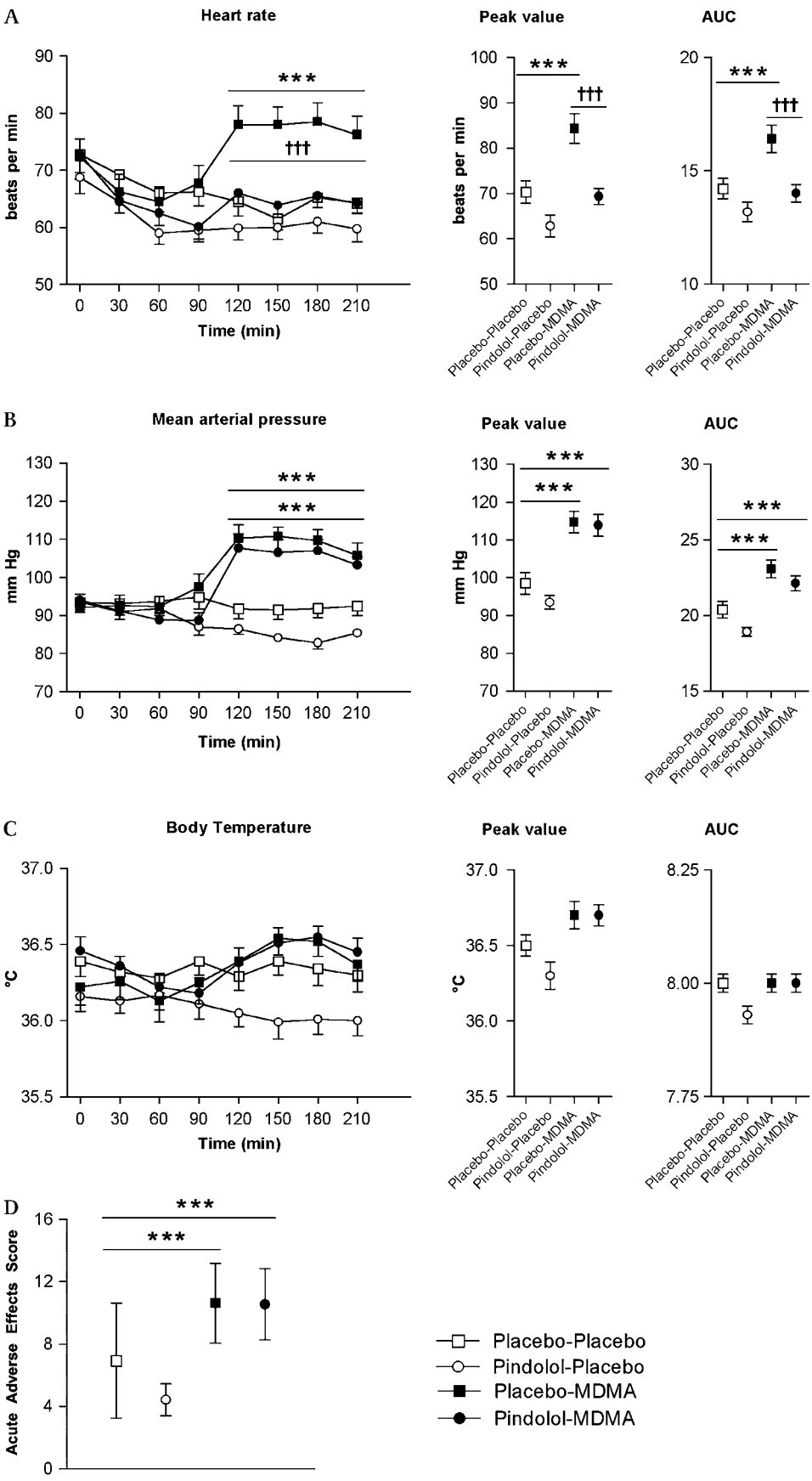

Figure 1 Graphs from left to right show drug effects over time, peak values (60—210 min) and area under the curve values (AUC from 60 to 210 min$\times 10^{-3}$). (A) Pindolol pretreatment prevented the MDMA-induced increase in heart rate. (B) Pindolol had no effect on MDMA-induced increases in mean arterial blood pressure. (C) Pindolol had no effect on the non-significant increase in body temperature associated with MDMA. (D) Pindolol did not change adverse effects associated with MDMA. *$p<0.05$ and ***$p<0.001$ placebo-MDMA vs placebo-placebo, †$p<0.05$ and †††$p<0.001$ placebo-MDMA vs pindolol-MDMA. Values represent mean±SE of 16 subjects.

smoked cocaine when carvedilol was used at a low dose which preferentially blocks β-receptors.[21] At a higher dose, which blocks both α- and β-receptors, carvedilol decreased all haemodynamic effects of cocaine.[21] The α-β-blocker labetolol, which has a higher relative affinity for the α-receptor than carvedilol, dose-dependently prevented all haemodynamic effects of smoked or

intranasal cocaine.[6 22] Furthermore, propranolol, but not labetalol, potentiated cocaine-induced coronary vasoconstriction.[6 7] Together these studies indicate that β-blockade without α-blockade has no effect or may even increase cocaine-induced hypertension, possibly due to unopposed α-receptor stimulation and increased vasoconstriction. Our results extend these findings and suggest that β-blockade affects MDMA-induced tachycardia but does not influence the blood pressure and adverse effects of MDMA. Severe MDMA toxicity such as multiorgan failure results from hyperthermia and not solely from tachycardia.[1] Heart rate is an easily determined marker of the severity of MDMA poisoning and β-blockade may mask this MDMA effect, during which time serious MDMA toxicity develops.

The present study has several limitations. Pindolol is a non-selective β-receptor blocker with intrinsic activity, unlike the β_1-selective β-blockers that are mostly used today and that may interact differently with MDMA. Pindolol was used because this compound also blocks serotonergic 5-HT_1 receptors and the primary aim of this study was to investigate the role of 5-HT_1 in the subjective effects of MDMA in humans. This focus was also the reason why pindolol was given before MDMA. Treatment after MDMA would have more closely mirrored the clinical situation where treatment for cardiovascular stimulation associated with intoxication with Ecstasy would be initiated following ingestion of MDMA. Treatment with a β-blocker after MDMA administration may result in less effective blockade of the effects of MDMA due to the delayed availability of the blocker at the site of action, but is unlikely to result in a qualitatively different pharmacodynamic interaction. Only single doses of pindolol and MDMA were used in the present study. However, the significant interactive effects of pindolol and MDMA on heart rate indicate that effective doses of both compounds were used. Nevertheless, different doses could interact differently. We do not know how the haemodynamic changes observed in our study would translate into actual risk changes for vascular complications. For example, the beneficial effects of β-blockers on heart rate and cardiac oxygen consumption may outweigh the potential harm of theoretically unopposed α-stimulation.[23] Finally, the present study was performed using pure MDMA in healthy subjects who were not engaged in physical activities and were seated in a quiet research environment. In contrast, recreational users of MDMA may be dancing and are likely to ingest other substances in addition to MDMA including cocaine or other amphetamines and may also show significant co-morbidity.[2] The findings from this study can therefore not be generalised to the treatment of patients with cardiovascular complications associated with recreational MDMA use. Nevertheless, our results indicate that β-blockers would not be expected to worsen the cardiovascular and adverse effects of MDMA. In addition, subjects on β-blocker medication are likely to show similar blood pressure responses to MDMA as those without medication.

In conclusion, β-blockers may prevent tachycardia but not blood pressure responses or adverse effects associated with MDMA. The role of α-β-blockade in the treatment of MDMA intoxications needs further evaluation. Furthermore, MDMA stimulates the sympathetic nervous system centrally rather than peripherally,[1 24 25] so centrally-acting sedative agents (eg, benzodiazepines) should be used as first-line treatments in cases of MDMA or other stimulant intoxication.[1 26 27]

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Competing interests None.

Ethics approval This study was conducted with the approval of the ethics committee of the University Hospital of Zurich.

Contributors MEL and FXV conceived the study and obtained research funding. FXV supervised the conduct of the trial and data collection. CMH and MEL analysed and interpreted the data. CMH and MEL wrote the paper. MEL takes responsibility for the paper as a whole.

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Paper Five: Role of the α_1 Adrenergic Receptor

Adrenergic α_1 receptors contribute to the acute effects of MDMA in humans

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ORIGINAL CONTRIBUTION

Adrenergic α_1 receptors contribute to the acute effects of MDMA in humans

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Running title: MDMA and doxazosin

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Running title: Doxazosin and MDMA

Abstract

Preclinical studies implicate a role for the noradrenergic α1 receptor in the effects of psychostimulants, including 3,4-methylendioxymethamphetamine (MDMA, ecstasy). This study evaluated the effects of the noradrenergic α1 receptor antagonist doxazosin on the acute pharmacodynamic and pharmacokinetic response to MDMA in 16 healthy subjects. Doxazosin (8 mg/day) or placebo was administered for three days before MDMA (125 mg) or placebo using a randomized, double-blind, placebo-controlled four-session cross-over design. Doxazosin reduced MDMA-induced elevations in blood pressure, body temperature, and positive mood but enhanced tachycardia associated with MDMA. Adrenergic α1 receptors contribute to the acute cardiostimulant and euphoric effects of MDMA in humans. Noradrenergic α1 receptor antagonists could be useful in the treatment of stimulant addiction.

Keywords: MDMA, 3,4-methylenedioxymethamphetamine, norepinephrine, doxazosin, α_1 adrenergic receptor

Introduction

3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") is a popular recreational drug with entactogenic and psychostimulant properties. MDMA binds to presynaptic monoamine transporters and releases serotonin, norepinephrine (NE), and dopamine.1-3 Carrier-mediated release of both serotonin and NE have been shown to contribute to the psychotropic effects of MDMA in humans.4-8 NE release has been implicated in particular as a mediator of the cardio- and psycho-stimulant response to MDMA.6,9 In particular, the selective norepinephrine transporter inhibitor reboxetine reduced MDMA-induced increases in plasma NE and the acute cardiovascular and psychostimulant effects of MDMA in healthy subjects.9 In addition, MDMA-induced increases in plasma NE were also associated with increases in good drug effects and drug liking.6 These findings implicate NE as a mediator of the effects of MDMA and of other psychostimulants.10-12 However it is unclear which adrenergic receptors are involved. Preclinical studies indicate that adrenergic $\alpha 1$ receptors regulate aspects of psychostimulant addiction including stimulant-induced locomotor activation,13-17 release of dopamine in the mesolimbic system,15,18,19 self-administration20 and drug seeking.20,21

The role of adrenergic $\alpha 1$ receptors in the acute effects of MDMA is not known. A recent clinical study showed that the $\alpha 1$-receptor antagonist doxazosin attenuated positive subjective effects of cocaine in humans.12 Therefore, we assessed the effect of doxazosin on the acute pharmacodynamic and pharmacokinetic effects MDMA in healthy volunteers. We hypothesized that doxazosin would reduce the MDMA-induced increase in blood pressure and the positive mood effects of MDMA.

Materials and Methods

Study Design

This was a double-blind, double-dummy placebo-controlled, randomized, crossover study with four experiential sessions (placebo-placebo, doxazosin-placebo, placebo-MDMA, and doxazosin-MDMA) in a balanced order. The washout periods between sessions were at least 7 days long. The study was conducted at the University Hospital of Basel in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland, and the Swiss Agency for Therapeutic Products (Swissmedic). The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health. All of the subjects provided written consent before participating in the study, and they were paid for their participation. The study was registered at ClinicalTrials.gov (NCT01386177). The reduction in the MDMA-induced increase in blood pressure by doxazosin was the predefined primary outcome of this trial. The reduction in MDMA-induced positive mood by doxazosin was a prespecified secondary outcome.

Study Procedures

The subjects completed a prescreening telephone interview, a screening visit, four test sessions, and an end-of-study visit. The test sessions were conducted in a quiet hospital research ward with no more than two

research subjects present per session. The mean±SD room temperature was 23.5±0.5 ºC. Subjects arrived at the study facilities at 8:00 AM on the test day. An indwelling i.v. catheter was placed in an antecubital vein for blood sampling. MDMA (125 mg) or placebo was administered at 9:00 AM. A standardized lunch was served at 12:00 PM, and the subjects were sent home at 3:00 PM.

Participants

Sixteen healthy subjects (eight men, eight women) with a mean±SD age of 25.8±3.3 years were recruited from the campus of the University of Basel. The allocation to treatment order was performed by drawing from blocks of eight different balanced drug treatment sequences by a pharmacist not involved in the study. Each code was stored in a sealed envelope until the termination of the study. Data from all 16 subjects were available for the final analysis. The exclusion criteria included the following: 1) age <18 or >45 years, 2) pregnancy determined by a urine test before each test session; 3) body mass index <18.5 kg/m2 or >25 kg/m2; 4) personal or family (first-degree relative) history of psychiatric disorder (determined by the structured clinical interview for Axis I and II disorders according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition22 supplemented by the SCL-90-R Symptom Checklist;23,24 5) regular use of medications; 6) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematology and chemical blood analysis; 7) smoking more than seven cigarettes per day; 8) a lifetime history of using illicit drugs more than five times, with the exception of cannabis; 9) illicit drug use within the last two months; and 10) illicit drug use during the study, determined by urine tests conducted before the test sessions using TRIAGE 8 (Biosite, San Diego, CA, USA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and limit alcohol use to one glass on the day before each test session. All of the subjects were non-smokers. Thirteen subjects had previously used cannabis. Nine subjects reported using illicit drugs, in which three subjects had tried ecstasy up to two times, one subject had tried amphetamine once, one subject had tried lysergic acid diethylamide once, three subjects had tried nitrous oxide up to three times, and one subject had tried nitrous oxide and methylphenidate once. All of the subjects were phenotyped for cytochrome P450 (CYP) 2D6 activity using dextromethorphan as the probe drug. Thirteen extensive, two intermediate, and one poor CYP2D6 metabolizer were identified in the study. The female subjects were investigated during the follicular phase (day 2-14) of their menstrual cycle when the reactivity to amphetamines is expected to be similar to men.25

Drugs and dosing

±MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was prepared as gelatine capsules (100 and 25 mg of the salt). Identical placebo (mannitol) capsules were prepared. MDMA was administered in a single oral dose of 125 mg, corresponding to a mean±SD dose of 1.91±0.39 mg/kg body weight. This dose of MDMA corresponds to a typical recreational dose of ecstasy, and comparable doses of MDMA have previously been used in similar studies. Doxazosin was selected because it reduced the effects of cocaine in a clinical study12 and can be administered once daily and uptitrated with good tolerability. Doxazosin tablets (4 mg; Cardura continued-release; Pfizer, Zurich, Switzerland) were encapsulated within opaque gelatine capsules, and identical placebo (mannitol) capsules were prepared. A first dose of 4 mg of doxazosin was administered 3 days before MDMA or placebo (-64 h) at 5:00 PM, a second dose of 8 mg was administered 2 days before MDMA or

placebo (-40 h) at 5:00 PM, and a third dose of 8 mg was administered the day before MDMA or placebo administration (-16 h) at 5:00 PM. The subjects were reminded by a phone call or phone text message to ingest the capsules, and medication containers were checked to confirm that the first two doses of doxazosin were administered. The last administration was supervised by study personnel at the research facility. This administration schedule accounted for the long tmax of 8-10 h of the continuous-release formulation of doxazosin and reduced the risk of hypotension.26 Based on similar dosing regimes in healthy subjects,26,27 the estimated peak plasma concentration of doxazosin at the time of the administration of MDMA was 30 ± 5 ng/ml, similar to the concentration with steady state-dosing with 4 mg.26

Pharmacodynamics

Vital signs

Vital signs were assessed repeatedly 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Heart rate, systolic blood pressure, and diastolic blood pressure were measured using an OMRON M7 blood pressure monitor (OMRAN Healthcare Europe; Hoofddorp, The Netherlands) in the dominant arm after a resting time of 5 min. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Core (tympanic) temperature was assessed using a GENUIS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY, USA).

Psychometric scales

Subjective measures were assessed by using the Adjective Mood Rating Scale (AMRS),28 Visual Analog Scales (VAS),9,29,30 and the 5-Dimensions of Altered States of Consciousness Scale (5D-ASC).31,32 The 60-item Likert-scale short version of the AMRS28 was administered 1 h before and 1.25, 2, 5, and 25 h after MDMA or placebo administration. The AMRS contains subscales for heightened mood, self-confidence, activity, emotional excitation, extroversion, and dreaminess. The VAS were presented as 100 mm horizontal lines marked “not at all” on the left and “extremely” on the right. The VAS were administered 1 h before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 6 h after MDMA or placebo administration. The 5D-ASC rating scale measures alterations in mood, perception, experience of self in relation to environment, and thought disorder.32 The 5D-ASC dimension “oceanic boundlessness” (27 items) measures derealization and depersonalization associated with positive mood. The dimension “anxious ego dissolution” (21 items) summarizes ego disintegration and loss of self-control, phenomena associated with anxiety. The dimension “visionary restructuralization” (18 items) describes perceptual alternations. The dimension “auditory alterations” (16 items) subsumes auditory (pseudo) hallucinations, and the dimension “vigilanze reduction” (12 items) describes impaired alertness and cognitive performance. The total ASC score was determined by adding the scores of the firs three dimensions. The 5D-ASC was administered 4 h after MDMA or placebo administration.

Adverse effects

Adverse effects were assessed 1 h before and 3 and 24 h after the administration of MDMA or placebo using the List of Complaints.33 The scale consists of 66 items that yield a total adverse effects score, reliably measuring physical and general discomfort.

Plasma catecholamines

Blood samples to determine the concentration of NE and epinephrine were taken 1 h before and 1 and 2 h after the administration of MDMA or placebo. All of the blood samples were collected on ice and centrifuged within 10 min at 4 °C. The plasma was then stored at -20°C until analysis. The plasma levels of free catecholamines (NE and epinephrine) were determines by HPLC with an electrochemical detector as described previously.9

Pharmacokinetics

Samples of whole blood for the determination of MDMA, ±3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxymethamphetamine (HMMA) were collected into lithium heparin monovettes 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, and 6 h after MDMA or placebo administration. Plasma concentrations of MDMA and MDA were determined using HPLC coupled to tandem MS as described previously.6,7 Plasma concentrations of HMMA were determined after enzymatic hydrolysis of the glucuronide conjugates. Deglucuronidation was performed by adding 37 nM sodium metabisulfite, 9.25 nM EDTA, and ≥ 500 units of β-glucuronidase Type HP 2 (Sigma G7017, Sigma-Aldrich, Buchs, Switzerland) to the plasma and incubation at 37 °C for 16 h. HMMA sample processing was then identical to MDMA and MDA analysis.

Data Analysis

The data for the plasma concentrations of MDMA, MDA, and HMMA were analyzed using non-compartmental models. Maximal plasma concentration (Cmax) and time to maximal plasma concentration (Tmax) were obtained directly from the observed concentration-time curves. The area under the plasma time curve (AUC)0-6h was calculated using the linear trapezoidal rule. Plasma concentrations were only determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in plasma levels of MDMA during the time of pharmacodynamic effects of MDMA.

For the repeatedly measured clinical data values, peak effects (Emax) and the area under the effect curve (AUEC)0-6h were determined. The Emax and AUEC0-6h values were analyzed by two-way General Linear Models repeated-measures analysis of variance (ANOVA) with the two drug factors MDMA (MDMA vs. placebo) and doxazosin (doxazosin vs. placebo) using STATISTICA 6.0 software (StatSoft, Tulsa, OK, USA). Tukey's post hoc comparisons were performed based on significant main effects or interactions. Additional ANOVAs were performed with drug order as an additional factor, to exclude carry-over effects. The criterion for significance was $p<0.05$. A sample-size estimation based on previous data7,9 showed that 8 subjects would be needed to detect a relevant change in the primary outcome with 80% power using a within-subjects study design.

RESULTS

Pharmacodynamics

MDMA significantly increased blood pressure, heart rate, and body temperature. Doxazosin significantly decreased the MDMA-induced increase in blood pressure although it had no effect on blood pressure when given alone. Doxazosin alone non-significantly increased heart rate compared to placebo and

further enhanced the heart rate elevation produced by MDMA (Fig. 1, Table 1). Doxazosin also reduced the increase in body temperature following MDMA as evidenced by a significant doxazosin × MDMA interaction on the AUC0-6h for body temperature (Fig. 1, Table 1). However, the posthoc comparison between placebo-MDMA and doxazosin-MDMA did not reach significance.

In the AMRS, MDMA produced stimulant and positive mood effects including increases in hightened mood, self-confidence, activity, emotional excitation, extroversion, and dreaminess (Fig. 2, Table 1). MDMA also increased VAS ratings for any drug effect, good drug effect, drug liking, drug high, and stimulated, but not for bad drug effects (Fig. 3, Table 1). Doxazosin significantly reduced MDMA-induced hightened mood and tended to reduce increase in activity produced by MDMA (Fig. 2, Table 1). Doxazosin also tended to attenuate MDMA-induced increases in self-confidence in the AMRS and VAS ratings for any drug effect, good drug effects, and drug liking associated with MDMA. However these trend effects of doxazosin were not significant (Fig. 2 and 3, Table 1). Doxazosin did not alter the effects of MDMA in the 5D-ASC (Fig.1S).

MDMA increased the total LC adverse effect score both 3 and 24 h after drug administration compared with placebo (Table 1). Doxazosin did not alter adverse effects of MDMA. Frequently reported acute adverse effects of placebo-MDMA and doxazosin-MDMA were lack of appetite (n=12 and n=13, respectively), thirst (n=10 and n=11, respectively), difficulties concentrating (n=9 and n=9), bruxism (n=7 and n=10, respectively), and sweating (n=6 and n=5, respectively). There were no severe adverse effects.

MDMA increased levels of circulating epinephrine and this effect was not altered by doxazosin. MDMA non-significantly increased plasma levels of NE compared to placebo. Doxazosin significantly increased plasma levels of NE and effects with MDMA were additive (Fig. 2S, Table 1).

Pharmacokinetics

Pharmacokinetics of MDMA and its metabolites MDA and HMMA are shown in Table 2 and Figure 4. Doxazosin did not affect Cmax, AUC0-6h, or Tmax of MDMA or HMMA (Fig. 4A and 4C, Table 2). Doxazosin lowered MDA exposure as evidenced by slight reductions in Cmax and AUC0-6h (Fig 4B, Table 2). Reduced CYP2D6 activity (i.e., a higher dextromethorphan:dextrophan urine concentration ratio) was associated with a larger AUC0-6h of MDMA and a smaller AUC0-6h of HMMA (R_s =0.61, $p<0.01$ and R_s =-0.57, $p<0.05$, respectively).

Discussion

In this present study the α1 adrenoceptor antagonist doxazosin decreased the MDMA-induced increases in blood pressure, body temperature, and hightened mood.

The α1 receptor antagonist prazosin has similarly been shown to reduce the blood pressure response to MDMA34 or NE in rats.35 In humans, a ten-day treatment with lower daily dose of doxazosin (4 mg) also tended to reduce systolic blood pressure elevations by cocaine.12 Together, the findings confirm a central role for α1 adrenergic receptors in the regulation of stimulant-induced hypertension. However, selectively blocking α1 adrenergic receptors resulted in compensatory tachycardia and enhanced heart rate increases in response to MDMA. Opposite to the effects of doxazosin on the cardiostimulant effects of MDMA, adrenergic β receptor blockers lowered tachycardia but enhanced the pressure response to cocaine36 or MDMA37 However,

combined α-β adrenergic receptor blockers such as carvedilol or labetalol have been shown to inhibit both the blood pressure and heart rate response to MDMA38 or cocaine39-41 in humans. Therefore, combined α-β adrenergic receptor blockers should be used in the treatment of sympathomimetic toxicity associated with psychostimulants.

In our study, MDMA increased body temperature in line with previous works.7,9,30,42 Preclinical studies indicate that the MDMA-induced rise in body temperature involves both adrenergic α1-receptor-mediated peripheral cutaneous vasoconstriction with impaired heat dissipation43 and β3 receptor-mediated heat generation by mitochondrial uncoupling.43,44 Consistent with preclinical data, doxazosin only partly attenuated the MDMA-induced increase in body temperature in the present study while the combined α1 and β1,2,3 adrenergic receptor antagonist carvedilol has been shown to more effectively reduce the hyperthermic response to MDMA in animals45 or in humans.38

In our study doxazosin significantly reduced MDMA-induced increases in hightened mood and there was also a non-significant trend reduction in activity and self-confidence associated with MDMA. These findings indicate a possible role for adrenergic α1 receptors in the mediation of the mood-enhancing and stimulant effects of MDMA in humans and extend our previous findings of the role for NE in the psychostimulant effects of MDMA.7,9 Simlilary, doxazosin has been shown to reduce subjective liking of the acute effects of cocaine and associated feelings of being stimulated.12 Interestingly, doxazosin reduced only the effects of a low dose of cocaine and effects of higher doses of cocaine were not significantly altered by doxazosin12. We used only one relatively high dose of MDMA in the present study and most of the mood effects of MDMA including drug liking and feelings of being stimulated were only non-significantly lowered by doxazosin. It is therefore possible that doxazosin would have had a stronger effect on the subjective responses to lower doses of MDMA as observed for cocaine. Nevertheless, the findings of the present study support the view that adrenergic α1 receptor stimulation contributes to the positive subjective effects of psychostimulants, including MDMA.

The findings are also in line with preclinical data. Prazosin blocked locomotor stimulation induced by cocaine,14 amphetamine,16,17 or MDMA.13 In addition, α1 receptor knock-out mice did not show amphetamine- or cocaine-induced locomotor activity.15 Prazosin also reduced nicotine self-administration20 and nicotine-20 or cocaine-seeking behavior21 in rats. However, α1 adrenergic blockade did not affect cocaine self-administration in rhesus monkeys.46 Adrenergic α1 receptor stimulation by stimulant-induced NE also enhances the effects of psychostimulants on the dopamine system47 which is thought to mainly mediate the rewarding and reinforcing effects of drugs of abuse. For example, the amphetamine-induced release of dopamine in the nucleus accumbens was inhibited by administration of an adrenergic α1 receptor blocker into the ventral tegmental area,19 the frontal cortex17 or systemically.17

In our study, both MDMA and doxazosin increased plasma concentrations of circulating catecholamines. Circulating epinephrine plasma levels are mainly derived from the adrenal medulla whereas NE entering plasma represents an overflow by sympathetic nerves.48,49 Circulating NE is therefore considered an indicator of sympathetic system activation. Doxazosin reduces vascular resistance which results in an antihypertensive effect. In our healthy young subjects this effect of doxazosin was offset by an increase in NE plasma concentration and a higher heart rate compared to placebo. A similar baroreceptor-mediated reflexive increase in

NE has previously been described following α1-50,51 or α1-β adrenergic receptor blockade.38 Interestingly, the sympathomimetic drug MDMA elevated NE or epinephrine levels to a lower extent than blockade of the α1 receptor, the important target for NE in the organs innervated by the sympathetic nervous system.

Doxazosin did not affect plasma exposure to MDMA or HMMA but increased exposure to MDA, the minor but active metabolite of MDMA. MDMA is mostly degraded to HMMA involving CYP2D6. Consistently, we found that subjects with lower CYP2D6 activity in the dextromethorphan test exhibited higher MDMA and lower HMMA exposure. The metabolism of MDMA to MDA involves CYP3A4 and CYP2B6.52 The slightly reduced levels of MDA following doxazosin pretreatment could be explained by competitive inhibition of CYP3A4 because doxazosin may also be metabolized by this enzyme according to the manufacturer (Pfizer, AG, Switzerland). Although, MDA is an active metabolite its concentrations in the plasma are low6,9 and the doxazosin effect on the response to MDMA are not explained by this pharmacokinetic interaction.

Several caveats of the present study should be recognized. First, we only administered single doses of MDMA and doxazosin. A dose-response study was not feasible because we did not want to exposure our subjects to more than two doses of MDMA in a crossover design. We administered moderate to high doses of both drugs. As discussed above it is possible that doxazosin would have more pronounce effects on lower doses of MDMA. Second, doxazosin may poorly penetrate the blood-brain barrier.53 Although we used a higher dose of doxazosin than the one previously shown to be effective to reduce the stimulant effects of cocaine12 it is possible that the α1 adrenergic receptor occupancy by doxazosin in the CNS was to low to reduce the psychoactive effects of the high dose of MDMA. Another α1 adrenergic blocker such as prazosin may therefore be more effective in reducing the CNS effects of psychostimulants.

In conclusion, adrenergic α1 receptors contribute substantially to the blood pressure and partially to the thermogenic and subjective effects of MDMA in humans. Adrenergic α1 receptors may be an interesting target for the treatment of stimulant dependence.

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TABLE 1 Pharmacodynamic drug effects

			Placebo-placebo (mean ± SEM)	Doxazosin-placebo (mean ± SEM)	Placebo-MDMA (mean ± SEM)	Doxazosin-MDMA (mean ± SEM)	main effect of MDMA		main effect of Doxazosin		Doxazosin×MDMA interaction	
							$F_{1,15}$=	p<	$F_{1,15}$=	p<	$F_{1,15}$=	p<
Vital signs												
	MAP (mm Hg)	E_{max}	90.3±1.4	90.5±1.8$^{\#\#\#}$	110.9±2.9***	103.4±2.5***$^{\#}$	81.0	0.001	3.6	NS	4.6	0.05
		$AUEC_{0-6h}$	500.9±6.9	500.0±7.7$^{\#\#\#}$	579.9±6.4***	542.2±10.3***$^{\#\#}$	136.6	0.001	9.2	0.01	6.8	0.05
	Heart rate (beats/min)	E_{max}	73.6±2.1	80.4±2.5$^{\#\#}$	93.4±3.4***	102.4±4.1***$^{\#}$	52.0	0.001	16.7	0.001	0.4	NS
		$AUEC_{0-6h}$	398.7±11.1	426.6±10.7$^{\#\#\#}$	466.0±12.3***	496.5±17.3***$^{\#}$	51.3	0.001	11.5	0.01	0.1	NS
	Body temperature (°C)	ΔE_{max}	0.23±0.05	0.39±0.07	0.56±0.09*	0.41±0.11	4.8	0.05	0.0	NS	3.0	NS
		$\Delta AUEC_{0-6h}$	-0.52±0.28	0.68±0.36	1.00±0.48*	0.18±0.63	1.0	NS	0.4	NS	6.3	0.05
Adjective mood rating scale												
	Emotional excitation	ΔE_{max}	-0.5±0.5	-0.6±0.7$^{\#\#\#}$	5.4±1.2***	5.3±1.2***	34.3	0.001	0.1	NS	0.0	NS
		$\Delta AUEC_{0-6h}$	-3.6±2.0	-6.0±2.4$^{\#\#\#}$	10.7±3.0***	10.1±2.7***	32.8	0.001	0.5	NS	0.2	NS
	Activity	ΔE_{max}	0.3±0.5	-0.2±0.3$^{\#\#\#}$	3.9±1.1**	2.4±0.9	18.5	0.001	2.0	NS	1.2	NS
		$\Delta AUEC_{0-6h}$	-1.5±1.9	-2.6±1.2$^{\#\#}$	7.3±3.4*	1.1±2.5	6.6	0.05	2.3	NS	4.0	0.06
	Hightened mood	ΔE_{max}	0.1±0.4	0.3±0.4$^{\#\#\#}$	4.0±0.7***	2.4±0.5**	20.9	0.001	5.4	0.05	4.3	0.06
		$\Delta AUEC_{0-6h}$	-3.3±2.1	-2.7±1.9$^{\#\#}$	9.1±2.8***	1.3±2.4$^{\#}$	9.1	0.01	5.9	0.05	5.2	0.05
	Extroversion	ΔE_{max}	0.0±0.4	0.2±0.5$^{\#\#\#}$	4.3±0.9***	3.3±0.8**	24.7	0.001	0.8	NS	1.2	NS
		$\Delta AUEC_{0-6h}$	-4.7±1.7	-2.8±2.0$^{\#\#}$	8.4±2.7**	5.8±3.4*	16.2	0.001	0.0	NS	1.0	NS
	Self-confidence	ΔE_{max}	-0.3±0.4	-0.4±0.4$^{\#\#}$	2.1±0.6**	1.8±0.7*	17.7	0.001	0.3	NS	0.1	NS
		$\Delta AUEC_{0-6h}$	-5.2±1.8	-6.0±2.0$^{\#\#}$	3.4±2.9*	-0.7±2.1	12.0	0.001	1.3	NS	1.4	NS
	Dreaminess	ΔE_{max}	-0.1±0.2	0.3±0.3$^{\#\#\#}$	3.9±0.7***	3.8±0.6***	32.5	0.001	0.1	NS	0.5	NS
		$\Delta AUEC_{0-6h}$	-2.2±1.0	-0.7±1.1$^{\#\#\#}$	9.7±2.5***	10.4±2.3***	24.5	0.001	0.8	NS	0.1	NS
Visual analogue scales												
	Any drug effect	ΔE_{max}	6.8±5.5	8.9±3.7$^{\#\#\#}$	82.7±4.8***	82.6±4.5***	283.8	0.001	0.0	NS	0.1	NS
		$\Delta AUEC_{0-6h}$	13.2±12.4	9.3±4.4$^{\#\#\#}$	223.6±25.5***	193.4±22.9***	74.7	0.001	1.4	NS	3.1	NS
	Good drug effect	ΔE_{max}	7.7±5.7	5.2±2.5$^{\#\#\#}$	86.8±4.0***	85.3±4.3***	321.5	0.001	0.2	NS	0.0	NS
		$\Delta AUEC_{0-6h}$	13.8±12.8	4.1±2.5$^{\#\#\#}$	266.0±26.8***	231.6±30.2***	69.2	0.001	2.4	NS	3.8	NS
	Bad drug effect	ΔE_{max}	0.0±0.0	2.0±1.4	15.8±6.8	14.3±6.0	10.7	0.01	0.0	NS	0.1	NS
		$\Delta AUEC_{0-6h}$	0.0±0.0	2.5±2.0	14.4±5.9	14.5±5.8	8.6	0.01	0.1	NS	0.1	NS
	Drug liking	ΔE_{max}	7.3±5.7	7.8±3.8$^{\#\#\#}$	86.8±3.9***	85.8±4.6***	269.7	0.001	0.0	NS	0.1	NS
		$\Delta AUEC_{0-6h}$	13.6±12.8	6.5±3.4$^{\#\#\#}$	273.7±24.0***	252.6±32.1***	82.6	0.001	0.7	NS	0.6	NS
	Drug high	ΔE_{max}	5.8±5.6	3.1±1.8$^{\#\#\#}$	83.9±4.5***	82.6±4.7***	320.3	0.001	0.2	NS	0.0	NS
		$\Delta AUEC_{0-6h}$	12.0±11.9	3.1±2.5$^{\#\#\#}$	206.4±24.0***	201.0±27.8***	68.2	0.001	0.2	NS	0.0	NS
	Stimulated	ΔE_{max}	6.7±5.6	1.8±1.3$^{\#\#\#}$	73.2±7.8***	80.3±5.5***	130.1	0.001	0.0	NS	3.4	NS
		$\Delta AUEC_{0-6h}$	13.3±11.9	2.8±1.9$^{\#\#\#}$	174.4±25.0***	184.1±26.7***	50.9	0.001	0.0	NS	1.6	NS
List of complaints												
	Acute adverse effects	3h	1.3±0.4	2.9±0.7$^{\#\#\#}$	8.4±1.3***	9.2±1.2***	45.4	0.001	3.8	NS	0.5	NS
	Sub-acute adverse effects	24h	0.7±0.3	1.9±0.6$^{\#\#}$	6.5±1.6***	6.6±1.2***	17.5	0.001	1.6	NS	0.5	NS
Circulating catecholamines												
	Epinephrine (nmol/L)	E_{max}	0.13±0.02	0.15±0.02$^{\#\#\#}$	0.39±0.04***	0.50±0.07***	45.3	0.001	3.1	NS	1.6	NS
	Norepinephrine (nmol/L)	E_{max}	1.66±0.20	3.56±0.40***$^{\#\#}$	2.17±0.14	4.40±0.45***$^{\#\#\#}$	5.1	0.05	43.3	0.001	0.4	NS

Values are mean±SEM (n=16). *for P<0.05, **for P<0.01, and ***for P<0.001 compared to placebo-placebo. #for P<0.05, ##for P<0.01, ###for P<0.001 compared to placebo-MDMA. MAP, mean arterial pressure. NS, not significant.

TABLE 2 Pharmacokinetics of MDMA, MDA, and HMMA

		C_{max} (ng/ml)	T_{max} (h)	AUC_{0-6} (ng/ml h)
MDMA				
	Placebo-MDMA	247±12	2.5±0.3	1029±49
	Doxazosin-MDMA	243±12	2.9±0.2	992±50
MDA				
	Placebo-MDMA	14.0±1.4	5.7±0.2	51.9±4.6
	Doxazosin-MDMA	12.3±1.1*	5.4±0.3	44.3±3.2**
HMMA				
	Placebo-MDMA	168±22	1.9±0.2	717±97
	Doxazosin-MDMA	169±25	2.1±0.2	730±104

C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; AUC, area under the plasma concentration-time curve. *p<0.05 and **p<0.01 compared to placebo-MDMA. Values are mean± SEM (n=16).

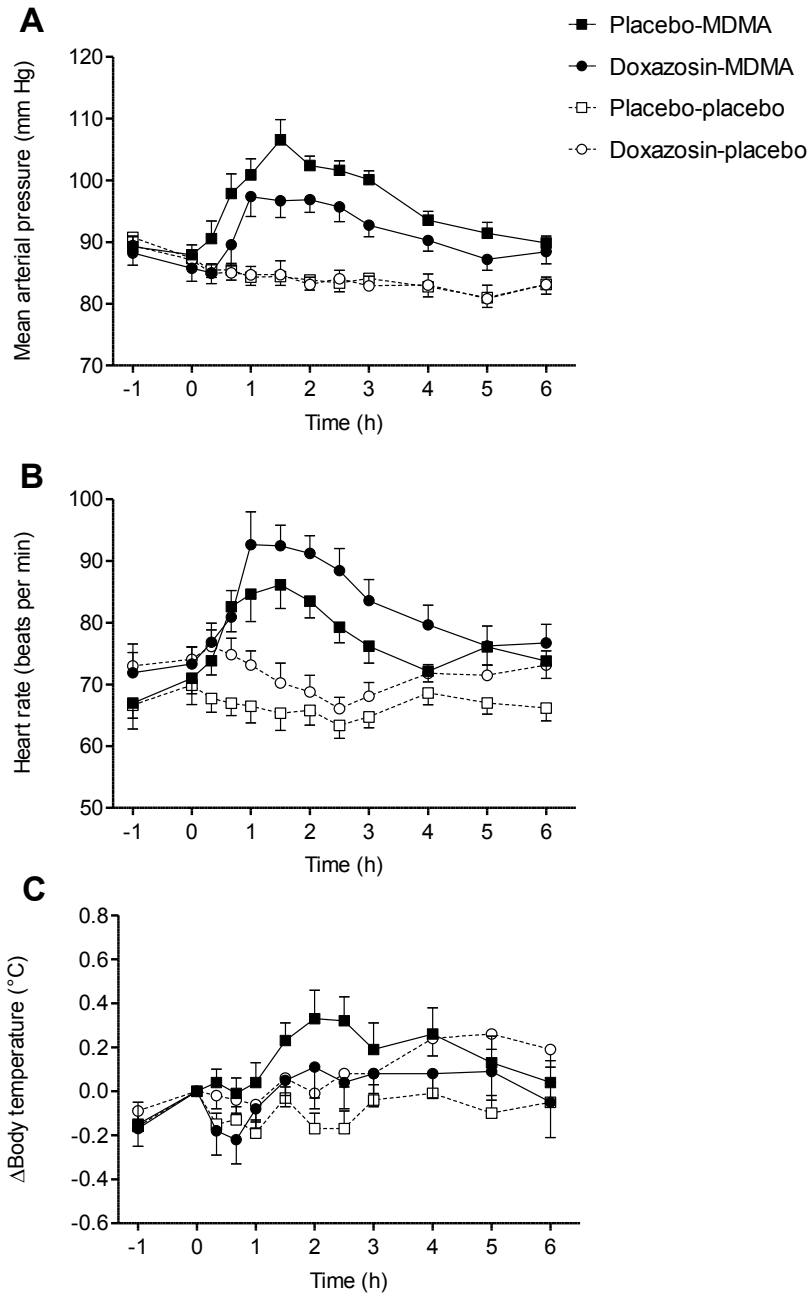

FIGURE 1. Doxazosin reduced MDMA-induced increase in mean arterial blood pressure (A) but enhanced the heart rate increase produced by MDMA (B). Doxazosin reduced the MDMA-induced elevation in body temperature (C). Values are expressed as mean±SEM (n=16).

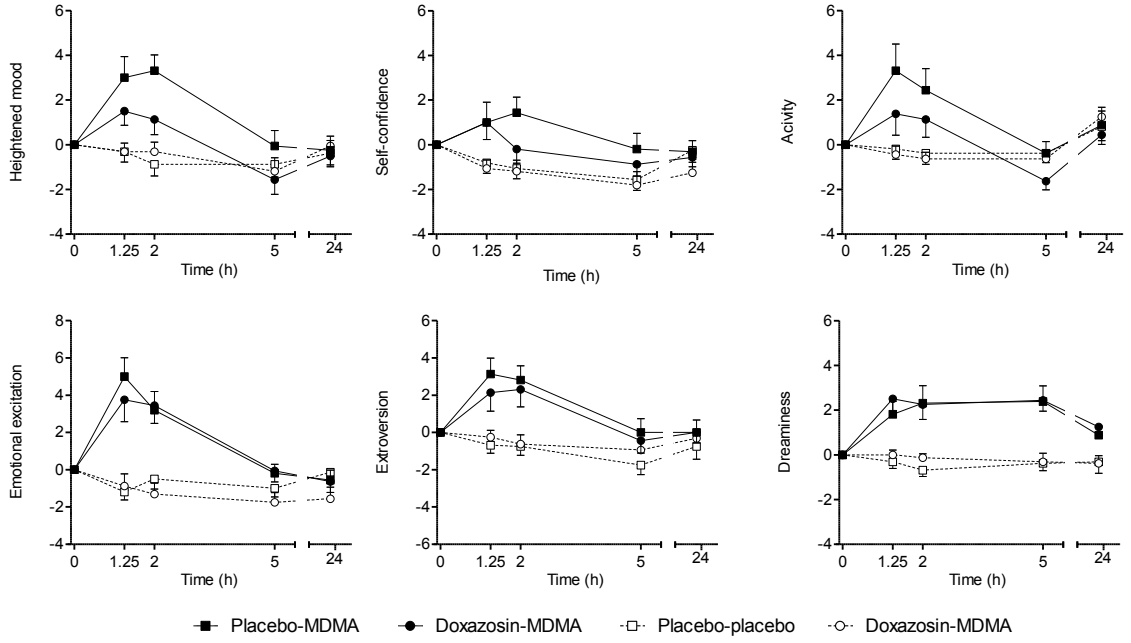

FIGURE 2. Subjective drug effects in the Adjective Mood Rating Scale (AMRS). Doxazosin decreased MDMA-induced increases in heightened mood and tended to reduce increased self-confidence and activity ratings produced by MDMA. Values represent mean±SEM (n=16).

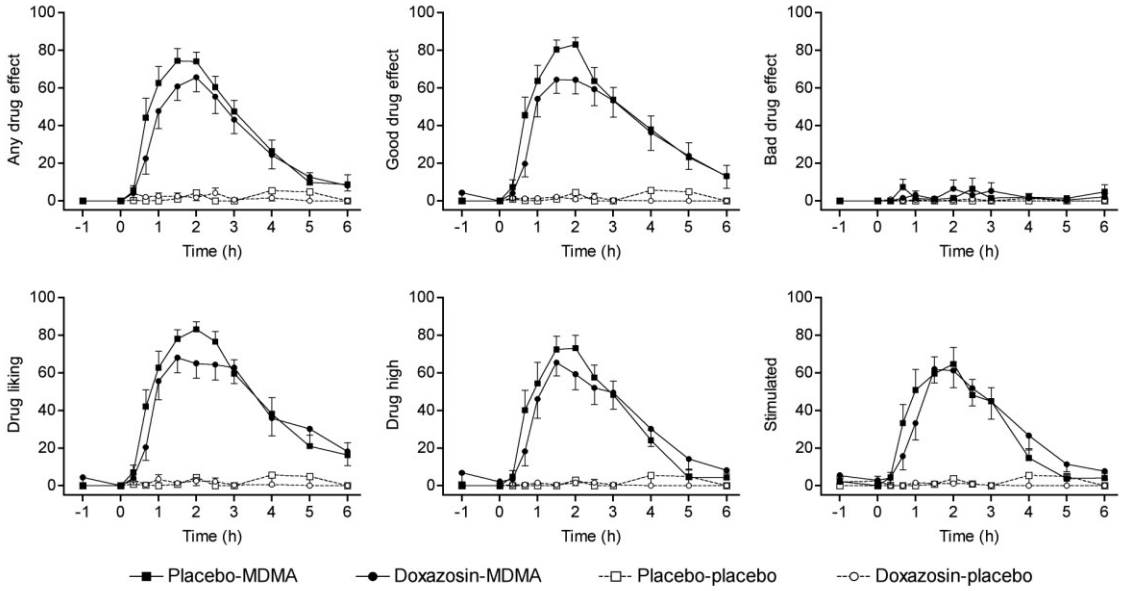

FIGURE 3. Visual Analog Scale ratings of subjective drug effects. Doxazosin non-significantly lowered MDMA-induced increases in any drug effect, good drug effect, and drug liking. Values represent mean±SEM (n=16).

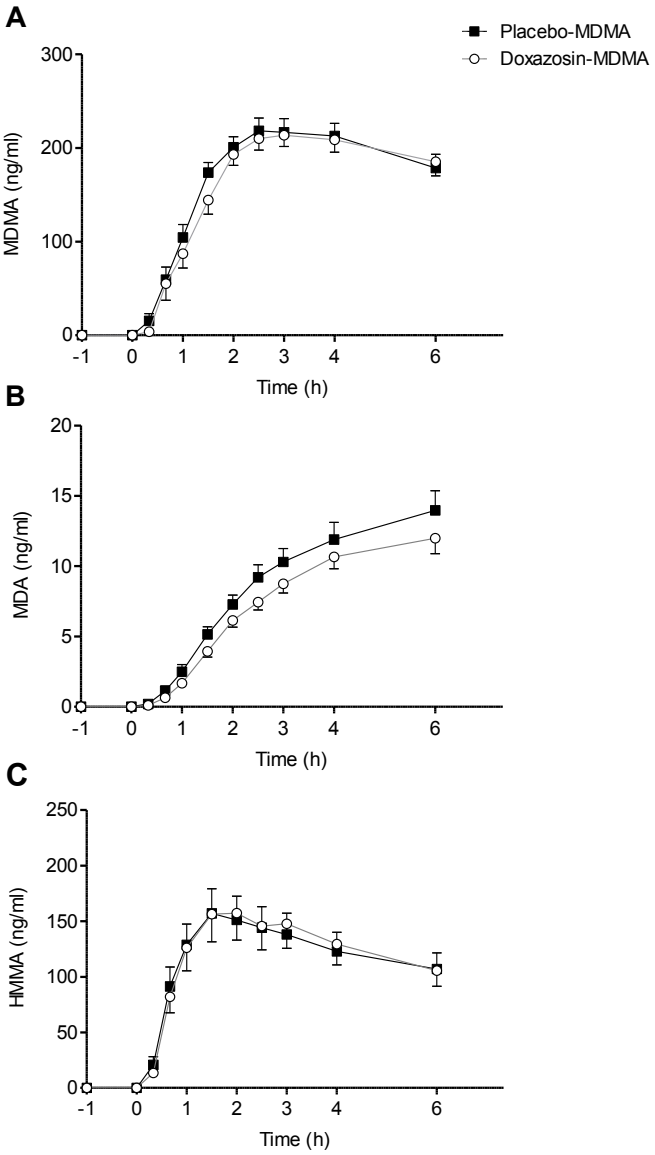

FIGURE 4. Plasma concentration time profiles for MDMA (A) and its metabolites MDA (B) and HMMA (C). Doxazosin slightly reduced the exposure to MDA (B) but had no effect on MDMA (A) or HMMA (C) plasma concentrations. Data are expressed as mean±SEM (n=16).

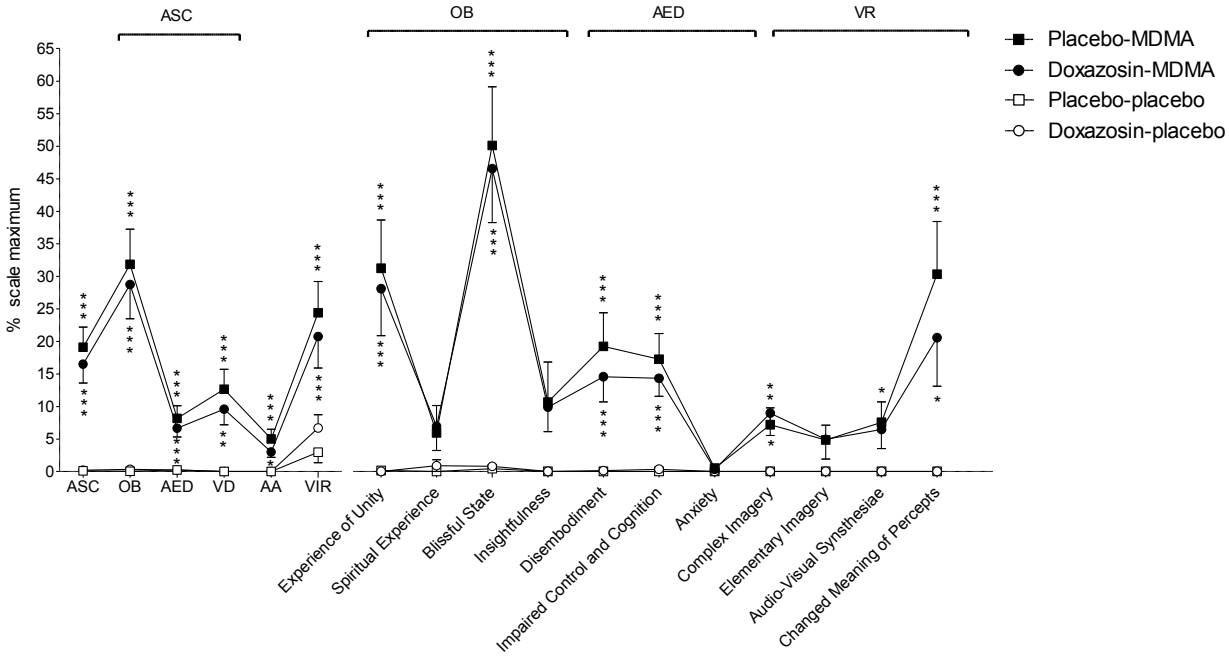

FIGURE S1. Drug effects in the 5-Dimensions Altered States of Consciousness (ASC) Scale. MDMA significantly increased the ASC sum score, Oceanic Boundlessness (OB), Anxious Ego Dissolution (AED), Visionary Restructuralization (VR), Audiory Alterations (AA) and Vigilanze Reduction (VIR) and most of the subsale scores (*p<0.05, **p<0.01,***p<0.001, placebo-placebo vs. placebo-MDMA or doxazosin-MDMA. Doxazosin had no effect on the response to MDMA in any of the scales or subscales. Values are expressed as mean±SEM (n=16).

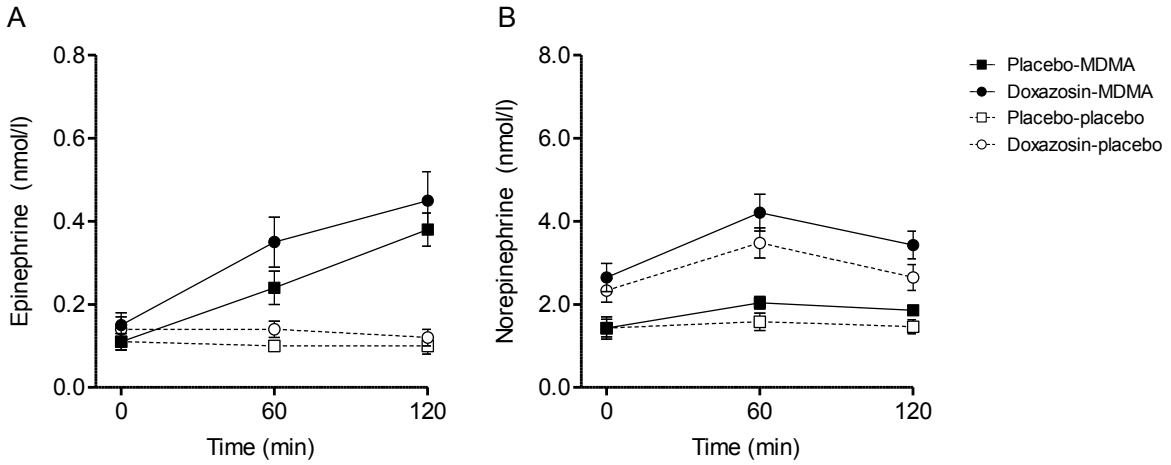

FIGURE S2. Drug effects on circulating catecholamines. MDMA increased plasma levels of epinephrine and this effect was not altered by doxazosin (A). MDMA alone produced a weak non-significant increase in norepinephrine plasma levels compared to placebo (B). Doxazosin significantly increased levels of norepinephrine and effects with MDMA were additive (B). Values are mean±SEM (n=16).

Paper Six: Role of Combined Carrier-mediated NE and 5-HT Release

Duloxetine Inhibits Effects of MDMA ('Ecstasy') *In Vitro* and in Humans in a Randomized Placebo Controlled Laboratory Study

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Duloxetine Inhibits Effects of MDMA (''Ecstasy'') *In Vitro* and in Humans in a Randomized Placebo-Controlled Laboratory Study

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Abstract

This study assessed the effects of the serotonin (5-HT) and norepinephrine (NE) transporter inhibitor duloxetine on the effects of 3,4–methylenedioxy-methamphetamine (MDMA, ecstasy) *in vitro* and in 16 healthy subjects. The clinical study used a double-blind, randomized, placebo-controlled, four-session, crossover design. *In vitro,* duloxetine blocked the release of both 5-HT and NE by MDMA or by its metabolite 3,4-methylenedioxyamphetamine from transmitter-loaded human cells expressing the 5-HT or NE transporter. In humans, duloxetine inhibited the effects of MDMA including elevations in circulating NE, increases in blood pressure and heart rate, and the subjective drug effects. Duloxetine inhibited the pharmacodynamic response to MDMA despite an increase in duloxetine-associated elevations in plasma MDMA levels. The findings confirm the important role of MDMA-induced 5-HT and NE release in the psychotropic effects of MDMA. Duloxetine may be useful in the treatment of psychostimulant dependence.

Trial Registration: Clinicaltrials.gov NCT00990067

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Introduction

Amphetamine derivatives, including 3,4-methylenedioxy-methamphetamine (MDMA, ''ecstasy'') bind to monoamine transporters and potently release serotonin (5-hydroxytryptamine [5-HT]), norepinephrine (NE), and dopamine (DA) through the 5-HT (SERT), NE (NET), and DA (DAT) transporters, respectively [1,2,3,4]. The pharmacological effect of MDMA can be blocked by monoamine transporter inhibitors. *In vitro*, the MDMA-induced release of NE, DA, or 5-HT from rat brain synaptosomes preloaded with monoamines is competitively inhibited by the monoamine transporter inhibitor indatraline [5,6]. In humans, SERT inhibition reduced the psychotropic response to MDMA [7,8,9]. NET inhibition also attenuated the acute effects of MDMA [10] and amphetamine [11] in humans. In contrast, clonidine, which inhibits the vesicular release of NE, did not inhibit the effects of MDMA in humans [12]. Thus, the available evidence indicates that the MDMA-induced transporter-mediated release of 5-HT and NE appears to be involved in aspects of the acute subjective and cardiovascular responses to psychostimulants [2,7,10,11]. However, the response to MDMA in humans was only moderately affected when either the SERT or NET was pharmacologically blocked [7,10]. Therefore, we evaluated the effects of dual SERT and NET inhibition with duloxetine on the pharmacokinetics (PK) and pharmacodynamics (PD) of MDMA in humans. Duloxetine was used because it is the most potent and selective dual SERT and NET inhibitor, although it also inhibits the DAT with 10- to 100-fold lower potency compared with the SERT and NET [13,14]. MDMA is mainly metabolized to 3,4-dihydroxymethamphetamine (HHMA) by cytochrome P450 (CYP) 2D6-mediated *O*-demethylation, followed by catechol-*O*-methyltransferase-catalyzed methylation to 4-hydroxy-3-methoxymethamphetamine (HMMA) [15]. Because duloxetine inhibits CYP 2D6 [16], we expected an increase in plasma MDMA concentrations after duloxetine pretreatment. MDMA is also *N*-demethylated to the active metabolite 3,4-mehthylenedioxyamphetamine (MDA). Whether the effects of MDA on 5-HT and NE release are inhibited by transporter inhibitors is unknown. Additionally, the inhibition of MDMA's effect on 5-HT and NE release by duloxetine has not been studied. Therefore, we also assessed the effects of duloxetine on 5-HT and NE release induced by MDMA or MDA *in vitro* using cells that express the respective human transporters. We also sought to link the *in vitro* and *in vivo* data to provide additional insights into the differential modulatory

role of 5-HT and NE in the effects of MDMA in humans. Because the data on monoamine transporter affinity and inhibition have mostly been derived from studies that used rat transporters [17], we investigated the binding and inhibition characteristics of the human monoamine transporters for MDMA, MDA, and duloxetine and the transporter inhibitors used in previous clinical studies [7,8,9,10] and *in vitro* studies [5,6]. Finally, we used an *ex vivo* binding assay to assess whether plasma samples taken from the drug-treated participants in the clinical study exhibit SERT, NET, and DAT-binding properties *ex vivo*.

The overall hypothesis of the present study was that duloxetine would potently bind to SERT and NET and block the MDMA- and MDA-induced transporter-mediated release of 5-HT and NE *in vitro* and markedly reduce the acute effects of MDMA *in vivo* in humans.

Methods

Clinical Study

The protocol for the clinical trial, the CONSORT checklist, and the CONSORT flowchart are available as supporting information; see Protocol S1, Checklist S1, and Figure 1. There were no changes to the protocol during the study.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Canton of Basel, Switzerland. All of the subjects provided written informed consent before participating in the study, and they were paid for their participation.

Design

We used a double-blind, placebo-controlled, randomized, crossover design with four experiential conditions (placebo-placebo, duloxetine-placebo, placebo-MDMA, and duloxetine-MDMA) in a balanced order. The washout periods between the sessions were at least 10 days long.

Participants

Sixteen healthy subjects (eight men, eight women) with a mean±SD age of 26.1±6.0 years participated in the study. The allocation to treatment order was performed by drawing from blocks of eight different balanced drug treatment sequences by a pharmacist not involved in the study. Each code was stored in a sealed envelope until the termination of the study. Data from all 16 subjects were available for the final analysis (Figure 1). The sample-size estimation showed that 13 subjects would be needed to detect a meaningful reduction of 20% of the MDMA drug effect by duloxetine with more than 80% power using a within-subjects study design. The exclusion criteria included the following; *(i)* age <18 or >45 years, *(ii)* pregnancy determined by a urine test before each session, *(iii)* body mass index <18.5 kg/m^2 or >25 kg/m^2, *(iv)* personal or family (first-degree relative) history of psychiatric disorder (determined by the structured clinical interview of Axis I and Axis II disorders according the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition [18] supplemented by the SCL-90-R Symptom Checklist [19,20] *(v)* regular use of medications, *(vi)* chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematological, and chemical blood analyses, *(vii)* smoking more than 10 cigarettes per day, *(viii)* a lifetime history of using illicit drugs more than five times with the exception of cannabis, *(ix)* illicit drug use within the last 2 months, and *(x)* illicit drug use during the study determined by urine tests conducted before the test sessions. None of the 16 subjects had used ecstasy previously. The subjects were asked to abstain from excessive alcohol consumption between the test sessions and limit their alcohol use to one glass on the day before the test session. All of the subjects were phenotyped for cytochrome P450 (CYP) 2D6

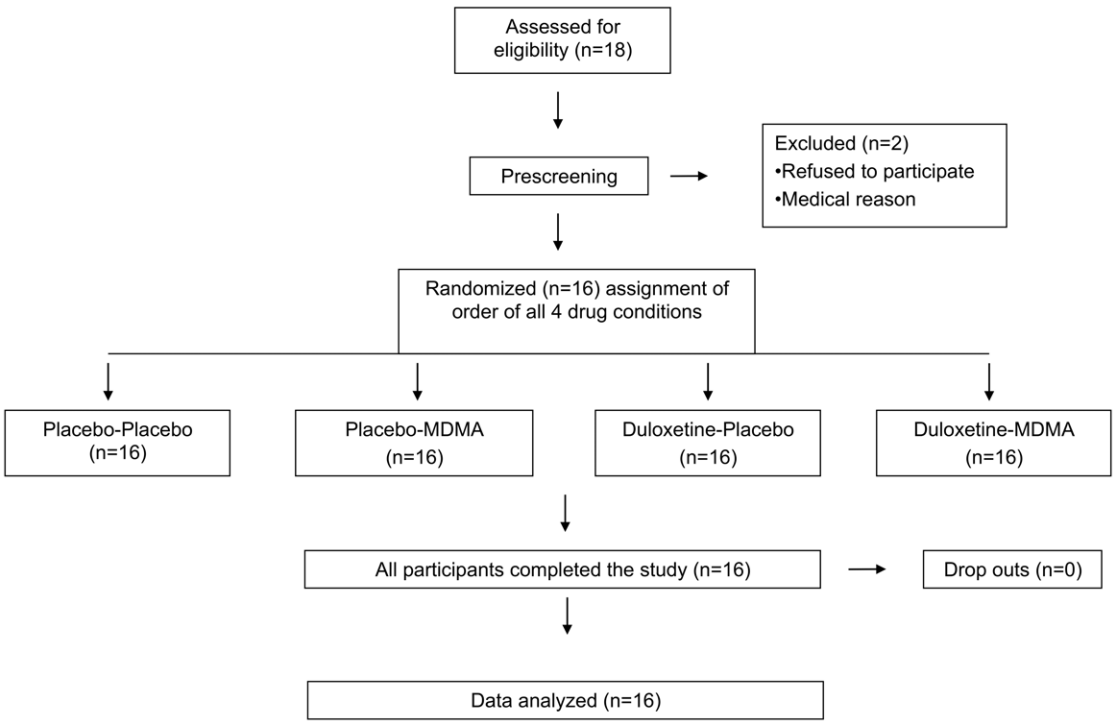

Figure 1. CONSORT flowchart.
doi:10.1371/journal.pone.0036476.g001

activity using dextromethorphan. Thirteen extensive, two intermediate, and one poor CYP 2D6 metabolizer were identified in the study. The female subjects were investigated during the follicular phase (day 2–14) of their menstrual cycle.

Drugs

(±)MDMA hydrochloride ($C_{11}H_{15}NO_2$, Lipomed, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules (100 mg and 25 mg). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute dose of 125 mg that corresponded to an average dose of 1.87±0.36 mg/kg body weight. This dose of MDMA corresponds to a typical recreational dose of ecstasy, and comparable doses of MDMA have previously been used in controlled settings. Duloxetine (Cymbalta, Eli Lilly, Vernier, Switzerland) was prepared as 60 mg gelatine capsules, and identically looking placebo (lactose) capsules were similarly prepared. Duloxetine (120 mg) or placebo was administered twice 16 and 4 h before MDMA or placebo administration, respectively. The dose of the two administrations of duloxetine (120 mg/day on two separate days) was in the upper range of the chronic doses used clinically (60–120 mg/day). This dosing schedule was used to obtain high plasma concentrations of duloxetine similar to those reached with chronic administration of 60 mg/day. Drugs were administered without food.

Assessments

Psychometric measures. The psychometric measures included Visual Analog Scales (VAS) [8,10], the Adjective Mood Rating Scale (AMRS) [21], and 5-Dimensions of Altered States of Consciousness (5D-ASC) [22,23]. The VASs included "any drug effect," "good drug effect," "bad drug effect," "drug liking," "drug high," "stimulated," "fear," "closeness to others," "talkative," and "open" [8,10,12,24,25]. The VASs were presented as 100 mm horizontal lines marked "not at all" on the left and "extremely" on the right. The VASs for "closeness to others," "open," and "talkative" were bidirectional (±50 mm). The VASs were administered 4 h before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, and 5 h after MDMA or placebo administration. The 60-item Likert-type scale of the short version of the AMRS [21] was administered 4 h before and 1.25, 2, and 5 h after MDMA or placebo administration. The AMRS contains subscales for activity, extroversion and introversion, well-being, emotional excitation, anxiety-depression, and dreaminess. The 5D-ASC rating scale measures alterations in mood, perception, experience of self in relation to the environment, and thought disorder. The 5D-ASC rating scale comprises five subscales or dimensions [22] and 11 lower-order scales [23]. The 5D-ASC dimension "oceanic boundlessness" (OB, 27 items) measures derealization and depersonalization associated with positive emotional states, ranging from heightened mood to euphoric exaltation. The corresponding lower-order scales include "experience of unity," "spiritual experience," "blissful state," and "insightfulness." The 5D-ASC dimension "anxious ego dissolution" (AED, 21 items) summarizes ego disintegration and loss of self-control phenomena, two phenomena associated with anxiety. The corresponding lower-order scales include "disembodiment," "impaired control of cognition," and "anxiety." The dimension "visionary restructuralization" (VR, 18 items) consists of the lower-order scales "complex imagery," "elementary imagery," "audiovisual synesthesia," and "changed meaning of percepts." Two other dimensions of the scale were not used in our study. The global ASC score was determined by adding the OB, AED, and VR scores. The 5D-ASC scale was administered 4 h after MDMA or placebo administration.

Physiologic measures. Physiologic measures were assessed repeatedly 4, 3, 2, and 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Heart rate, systolic blood pressure, and diastolic blood pressure were measured using an OMRON M7 blood pressure monitor (OMRON Healthcare Europe, Hoofddorp, The Netherlands). Measures were taken twice per time point with an interval of 1 min, and the average was used for the analysis. Core (tympanic) temperature was assessed using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY). The temperature of the room was maintained at 23.2±0.5°C. Adverse effects were assessed using the List of Complaints (LC) [26], which consists of 66 items that yield a total adverse effects score and reliably measure physical and general discomfort.

Plasma catecholamines and Pharmacokinetics (PK). Blood samples to determine the concentrations of NE and epinephrine were collected 4 h before and 1 and 2 h after MDMA or placebo administration. The levels of free catecholamines (NE and epinephrine) were determined using high-performance liquid chromatography (HPLC) with an electrochemical detector as described previously [10]. Plasma concentrations of copeptin were also determined in this study as reported elsewhere [27]. Samples of whole blood for the determination of MDMA, MDA, HMMA, and duloxetine were collected into lithium heparin monovettes -4, 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, and 6 h after administration of MDMA or placebo. Plasma concentrations of MDMA, MDA, HMMA, and duloxetine were analyzed by HPLC coupled to a tandem mass spectrometer as described previously [12]. The assays were linear in the concentration ranges of 1–1000 ng/ml for MDMA and MDA, 1–500 ng/ml for HMMA, and 2.5–1000 ng/ml for duloxetine. The performance of the method was monitored using quality control (QC) samples at the lower limit of quantification (LLOQ) and at two or three QC concentrations. The interassay accuracy values for the QC samples ranged from 97.5% to 100% for MDMA, from 95.3% to 103% for MDA, from 91.1% to 106% for HMMA, and from 93.2% to 96.4% for duloxetine. The interassay precision values ranged from 2.8% to 8.0% for MDMA, from 3.8% to 10.5% for MDA, from 3.1% to 8.8% for HMMA, and from 4.7% to 9.3% for duloxetine. No hydrolysis was performed. Thus, the values for HMMA represent the drug concentrations of the non-conjugated metabolite. All blood samples were collected on ice and centrifuged within 10 min at 4°C. The plasma was then stored at –20°C until the analysis.

In vitro Studies

Binding to monoamine transporters in vitro. Human embryonic kidney (HEK) 293 cells (Invitrogen, Zug, Switzerland) stably transfected with the human NET, SERT, or DAT as previously described [28] were cultured. The cells were collected and washed three times with phosphate-buffered saline (PBS). The pellets were frozen at –80°C. The pellets were then resuspended in 400 ml of 20 mM HEPES-NaOH, pH 7.4, that contained 10 mM EDTA at 4°C. After homogenization with a Polytron (Kinematica, Lucerne, Switzerland) at 10000 rotations per minute (rpm) for 15 s, the homogenates were centrifuged at 48000× g for 30 min at 4°C. Aliquots of the membrane stocks were frozen at –80°C. All assays were performed at least three times. The test compounds were diluted in 20 µl of binding buffer (252 mM NaCl, 5.4 mM KCl, 20 mM Na_2HPO_4, 3.52 mM KH_2PO_4, pH 7.4) and 10 point dilution curves were made and transferred to 96-well white polystyrene assay plates (Sigma-Aldrich, Buchs, Switzerland). *N*-

methyl-^{3}H-nisoxetine (~87 C_i/mmol, Perkin-Elmer) was the radioligand for the NET assay and had a dissociation constant (K_d) of 9 nM. Fifty microliters of 12 nM [^{3}H]-nisoxetine was added to each well of the assay plates, targeting a final [^{3}H]-nisoxetine concentration of 3 nM. [^{3}H]-citalopram (~72 C_i/mmol; Perkin-Elmer) was the radioligand for the SERT assay and had a K_d of 2.2 nM. Fifty microliters of 8 nM [^{3}H]-citalopram was added to each well of the SERT assay plates, targeting a final [^{3}H]-citalopram concentration of 2 nM. [^{3}H]-WIN35,428 (~86 C_i/mmol; Perkin-Elmer) was the radioligand for the DAT assay and had a K_d of 12 nM. Fifty microliters of [^{3}H]-WIN35,428 (~40 nM concentration) was added to each well of the hDAT assay plates, targeting a final [^{3}H]-WIN35428 concentration of 10 nM. Twenty microliters of binding buffer alone in the assay plate defined the total binding, whereas binding in the presence of 10 µM indatraline defined nonspecific binding. Frozen NET, SERT, or DAT membrane stocks were thawed and resuspended to a concentration of approximately 0.04 mg protein/ml binding buffer (1:1 diluted in H_2O) using a polytron tissue homogenizer. The membrane homogenates (40 µg/ml) were then lightly mixed for 5–30 min with polyvinyl toluene (PCT) wheat germ agglutinin-coated scintillation proximity assay (WGA-SPA; Amersham Biosciences) beads at 7.7 mg beads/ml homogenate. One hundred thirty microliters of the membrane/bead mixture were added to each well of the assay plate that contained radioligand and test compounds (final volume in each well, 200 µl) to start the assay, which was incubated for approximately 2 h at room temperature with agitation. The assay plates were then counted in the PVT SPA counting mode of a Packard Topcount. Fifty microliters of the [^{3}H]-nisoxetine, [^{3}H]-citalopram, or [^{3}H]-WIN35428 stocks were counted in 5 ml of ReadySafe scintillation cocktail (Beckman Industries) on a Packard 1900CA liquid scintillation counter to determine the total counts added to the respective assays. Non-linear regression was used to fit the data to sigmoid curves and determine IC_{50} values for binding and uptake. K_i values for binding and uptake were calculated using the following Cheng-Prusoff equation: $K_i = IC_{50}/(1+ [S]/K_m)$.[29].

Monoamine uptake in vitro. Two different methodological approaches were used to assess the effects of the drug on monoamine uptake. Method A used centrifugation through silicon oil, and method B used buffer to stop the reaction and wash the cells. *Method A:* The SERT, NET, and DAT functions were evaluated in human HEK 293 cells that stably expressed human SERT, NET, and DAT. The cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Zug, Switzerland) with 10% fetal bovine serum and 250 µg/ml geneticine. The cells (100 µl, 4×10^6 cells/ml) were incubated for 10 min with 25 µl uptake buffer (9.99 mM L-glucose, 0.492 mM $MgCl_2$, 4.56 mM KCl, 119.7 mM NaCl, 0.7 mM NaH_2PO_4, 1.295 mM NaH_2PO_4, 0.015 mM sodium bicarbonate, and 1 mg/ml ascorbic acid for [^{3}H]-DA uptake) that contained various concentrations of inhibitor at 25°C. Fifty microliters of 5 nM (final concentration) [^{3}H]-5-HT (80 C_i/mmol; Anawa), [^{3}H]-NE (14.8 C_i/mmol; Perkin-Elmer), or [^{3}H]-DA (13.8 C_i/mmol; Perkin-Elmer) was added to start uptake. Uptake was stopped after 10 min, and radioactivity was measured as described below for 5-HT and NE release. Cell integrity after MDMA treatment was confirmed by the Toxilight toxicity assay (Lonza, Basel, Switzerland). The data were fit by non-linear regression, and K_m, EC_{50}, and E_{max} values were calculated using Prism (GraphPad, San Diego, CA). Preliminary experiments showed that the accumulation of 5-HT and NE by the cells was time-dependent and complete after 5 min for both 5-HT and NE, respectively. The 5-HT and NE transport velocity was concentration-dependent and could be described by Michaelis-Menten kinetics. The K_m values were 489±147 nM, 450±125 nM, and 1707±297 nM for 5-HT, NE, and DA, respectively. Nonspecific uptake was determined for each experiment in the presence of 10 µM fluoxetine for SERT cells, 10 µM nisoxetine for NET cells, and 10 µM mazindol for DAT cells and subtracted from the total counts to yield specific uptake. Nonspecific uptake was <10% of total uptake. *Method B:* Ligand potencies to inhibit [^{3}H]-DA, [^{3}H]-5-HT, and [^{3}H]-NE uptake via the human DAT, SERT and NET recombinantly expressed in HEK 293 cells were determined. The cells were grown in Dulbecco's modified Eagle's medium (Invitrogen, Zug, Switzerland) with 10% fetal bovine serum and 250 µg/ml geneticine in cell culture flasks. One day before the experiment, the cells were seeded in a volume of 110 µl at a density of 0.3 million cells/ml in 96-well plates (Packard) and incubated at 37°C and 5% CO_2 overnight. On the day of the uptake experiment, the 96-well plates that contained the cells were washed with Krebs Ringer bicarbonate buffer (Sigma-Aldrich, Buchs, Switzerland). Test compounds (100 µl, diluted in Krebs Ringer bicarbonate buffer) were added to the microtiter plates and incubated at 37°C for 30 min. Afterward, 50 µl [^{3}H]-DA (35–54 C_i/mmol; Perkin-Elmer; final concentration, 100 nM), [^{3}H]-5-HT (28–100 C_i/mmol; Perkin-Elmer; final concentration, 10 nM), or [^{3}H]-NE (5.3–14 C_i/mmol; Perkin-Elmer; final concentration, 100 nM) were added to DAT-, SERT-, and NET-containing cells, respectively, and incubated for 10 min at 37°C. Extracellular [^{3}H]-DA, [^{3}H]-5-HT, and [^{3}H]-NE were removed, and the plates were washed twice with Krebs Ringer bicarbonate buffer. Nonspecific uptake was determined in the presence of 10 µM indatraline. Scintillant (Microscint 40, 250 µl) was dispensed to every well, and radioactivity was determined at least 1 h later on the Packard Topcount plate reader. The data were fit by non-linear regression, and the IC_{50} was calculated using Excel (Microsoft, Redmont, CA, USA). The compounds were tested at least three times. The K_m values were 1082 nM for [^{3}H]-5-HT and >10000 nM for [^{3}H]-DA and [^{3}H]-NE.

5-HT and NE release in vitro. Transporter-mediated MDMA- and MDA-induced 5-HT and NE release was evaluated using [^{3}H]-5-HT- and [^{3}H]-NE-preloaded HEK 293 cells that stably expressed human SERT and NET, respectively. The procedures were adapted from previous studies [2,3]. SERT- or NET-expressing cells (100 µl, 4×10^6 cells/ml) were incubated at 25°C for 10 min with 50 µl of 5 nM (final concentration) [^{3}H]-5-HT or 10 nM [^{3}H]-NE solutions, respectively. Steady-state load with radiolabeled substrate was reached within 5 min and remained stable for 60 min for both cell lines. Duloxetine or other transporter inhibitors (5 µl) were added after 10 min, and the release of [^{3}H]-5-HT and [^{3}H]-NE was then initiated after another 2 min by the addition of MDMA, MDA, or buffer (25 µl). The release reaction was stopped after 10 and 30 min for [^{3}H]-5-HT and [^{3}H]-NE, respectively. The release times were based on the evaluation of the release-over-time curves for MDMA and MDA. The release of [^{3}H]-5-HT and [^{3}H]-NE was complete within 5 and 25 min, respectively, when a new steady state was reached and maintained for 30 min. To stop the release reaction and wash the cells, 100 µl of the cell suspension was transferred to 0.5 ml microcentrifuge tubes that contained 50 µl of 3 M KOH and 200 µl silicon oil (1:1 mixture of silicon oil types Ar20 and Ar200; Wacker Chemie, Munich, Germany) and centrifuged in a tabletop microfuge (Eppendorf, Basel, Switzerland) for 3 min at 13,200 rpm. This transports the cells through the silicon oil layer to the KOH layer, thereby separating the cells from the buffer, which remains on top of the silicon oil layer [30]. The centrifuge tubes were then transferred to liquid nitrogen. The amount of

tracer that remained in the cells was quantified by cutting the frozen centrifuge tube above the KOH/oil interface and putting the tip of the tube with the cell pellet in a scintillation vial that contained 500 µl lysis buffer (0.05 M TRIS-HCl, 50 mM NaCl, 5 mM EDTA, and 1% Nonidet P-40 substitute in water). The samples were then shaken for 1 h on a rotary shaker, and 7 ml of scintillation fluid (Ultimagold, Perkin Elmer, Schwerzenbach, Switzerland) was added. Cell-associated radioactivity was then counted. The silicon oil assay allowed for the precise termination of the transport/release process and an effective cell wash. The experimental control condition (100% retained) was defined as the [^{3}H]-5HT or [^{3}H]-NE that remained in the cells when buffer and duloxetine were added without MDMA or MDA. A second control condition (100% release) was defined as the [^{3}H]-5-HT or [^{3}H]-NE released by 100 µM tyramine [6]. Data analysis using either of the two control conditions yielded similar results, and the data are presented as release expressed as the percentage of monoamine retained. Dose-response curves were generated using 9–11 concentrations of MDMA/MDA. Nonspecific binding/uptake was determined using preincubation with 10 µM fluoxetine for SERT cells and 10 µM nisoxetine for NET cells before incubation with radioligands and was <3% of total activity. All data points were derived from at least three independent experiments, each assayed in triplicate. The data were fit by non-linear regression, and EC_{50} and E_{max} values were calculated using Prism (GraphPad, San Diego, CA).

Ex vivo Binding to Monoamine Transporters

Plasma samples for assessing *ex vivo* binding to monoamine transporters were collected 120 min after MDMA/placebo administration. We determined the potencies of the plasma to inhibit [^{3}H]-nisoxetine, [^{3}H]-citalopram, and [^{3}H]-WIN35,428 binding to NET, SERT, and DAT, respectively, according to the method described previously [10]. IC_{50} values were calculated as a percentage of the plasma sample dilutions required to obtain 50% of the maximum effect. Indatraline (10 µM) in human plasma was used to achieve 100% inhibition. Undiluted plasma samples were set at 100%. Thus, an IC_{50} of 10% indicates that a 10-fold diluted plasma sample displaced 50% of the radioligand.

Statistical Analyses

Pharmacodynamics. Clinical data values were transformed to differences from baseline. Peak effects (E_{max}) were determined for repeated measures. E_{max} values were compared using General Linear Models repeated-measures analysis of variance, with drug as within-subject factor, using Statistica 6.0 software (StatSoft, Tulsa, OK). Tukey *post hoc* comparisons were performed based on significant main effects of treatment. Additional analyses of variance were performed, with period as factor to exclude period effects. Correlation analyses were performed using Pearson's correlations. The criterion for significance was $p<0.05$. Mean arterial pressure (MAP) was calculated from diastolic blood pressure and systolic blood pressure using the following formula: $MAP = DBP + (SBP - DBP)/3$.

Pharmacokinetics. The plasma concentration data for MDMA, MDA, HMMA, and duloxetine were analyzed using non-compartmental methods. C_{max} and t_{max} were obtained directly from the observed concentration-time curves. The terminal elimination rate constant (λ_z) was estimated by log-linear regression after semilogarithmic transformation of the data, using the last two to three data points of the terminal linear phase of the concentration-time curve of MDMA or duloxetine. Terminal elimination half-life ($t_{1/2}$) was calculated using λ_z and the equation $t_{1/2} = ln_2/\lambda_z$. The area under the plasma concentration-time curve up to 6 h ($AUC_{0\text{-}6h}$) was calculated using the linear trapezoidal rule. The $AUC_{0-\infty}$ was determined by extrapolation of AUC_{0-6h} using λ_z. The PK parameters were determined using the PK functions for Excel (Microsoft, Redmont, CA, USA). Plasma concentrations were only determined up to 6 h after MDMA administration because the aim of the study was to assess potential changes in MDMA plasma levels while relevant pharmacodynamic effects or MDMA were present. It was therefore not possible to determine $t_{1/2}$ for HMMA and MDA because of their long $t_{1/2}$, which would require sampling for an extended time.

PK-PD modeling: First, a soft-link PK-PD model was used to evaluate the *in vivo* relationship between the concentration of MDMA and subjective effect of the drug. The change in the VAS for any drug effect was used as the pharmacodynamic measure in each individual. Because we observed clockwise hysteresis in the effect-concentration relationship over time, we used PK-PD data pairs within the ascending part of the individual curves up to E_{max} or C_{max}. Our estimate of E_{max}, which should represent the maximal response portion of the dose-response curve, may already have been affected by tolerance. However, E_{max} values of 100% (scale maximum) or stable high values were reached by most subjects, indicating that tolerance was not an issue early in the effect-time curve. Based on the good brain penetration of MDMA and absence of a time lag, we assumed rapid equilibration between plasma and the central compartment (brain). A sigmoid E_{max} model was then fitted to the pooled data of all individuals: $E = E_{max} \times C_p^{h/}(EC_{50}^{h}+C_p^{h})$, in which E is the observed effect, C_p indicates the MDMA plasma concentration, EC_{50} indicates the plasma concentration at which 50% of the maximal effect is reached, E_{max} is the maximal effect, and h is the Hill slope. The sigmoid E_{max} model provided a better fit than a simple E_{max} or linear model. Data pooling was used because only few data pairs were available per subject. Non-linear regression was used to obtain parameter estimates. Second, we also used a hard-link PK-PD model to predict *in vivo* PD effects based on the *in vitro* concentration-response data linked to the observed individual *in vivo* PK. The *in vitro* concentration-response relationship was described by a sigmoidal dose-response variable slope model fitted to the effects of MDMA on 5-HT or NE release using non-linear regression (Prism, GraphPad, San Diego, CA). The equation was the following: $E = E_{max}/(1+10^{(LogEC50-C)\times h})$, in which C denotes the concentration of MDMA in the assay, and h denotes the Hill slope. The *in vitro* effect-concentration relationship was determined for MDMA-induced 5-HT and NE release separately, and separate PD predictions were derived for each model. Similar to the soft-link PK-PD model, a single compartment PK model (plasma = brain concentration) was used, and only ascending PK or PD values were included. The *in vivo* data were linked to the PK of each individual, and a mean predicted effect-time curve was established.

Results

Pharmacodynamics (PD)

Duloxetine markedly reduced the psychotropic and cardiostimulant responses to MDMA in humans. Duloxetine decreased all aspects of MDMA's subjective effects in the VASs [8,10], including psychostimulant effects such as feelings of "good drug effects," "drug liking," "drug high," and "stimulation" (Table 1; Fig. 2b-d) but also so-called "entactogenic" or "empathogenic" MDMA-typical effects [31,32] such as feelings of being "open," "closer to others," and more "talkative" (Table 1; Fig. 2e and f). In the AMRS [21], duloxetine prevented MDMA-induced increases in "well-being," "emotional excitation," and "extroversion"

Table 1. Pharmacodynamic peak drug effects.

		Placebo-placebo	Duloxetine-placebo	Placebo-MDMA	Duloxetine-MDMA	$F_{3,45}$ =	$p<$
Visual Analog Scales							
Any drug effect	E_{max}	3.81±3.62	6.00±2.52$^{\#\#\#}$	86.69±3.57***	33.19±7.74*** $^{\#\#\#}$	74.47	0.001
Good drug effect	E_{max}	4.56±4.37	8.75±5.01$^{\#\#\#}$	89.38±4.67***	40.56±9.50*** $^{\#\#\#}$	42.89	0.001
Drug liking	E_{max}	4.13±4.06	7.56±4.43$^{\#\#\#}$	90.69±4.82***	38.38±8.91*** $^{\#\#\#}$	52.60	0.001
Drug high	E_{max}	1.94±1.94	4.81±2.93$^{\#\#\#}$	87.81±4.85***	28.94±9.35** $^{\#\#\#}$	55.45	0.001
Stimulated	E_{max}	4.13±1.94	5.13±2.45$^{\#\#\#}$	76.31±6.84***	22.25±7.65$^{\#\#\#}$	46.25	0.001
Open	E_{max}	1.38±0.94	0.38±0.38$^{\#\#\#}$	32.16±4.29***	6.00±3.26$^{\#\#\#}$	36.88	0.001
Closeness	E_{max}	0.00±0.00	0.00±0.00$^{\#\#\#}$	27.31±3.87***	4.63±2.49$^{\#\#\#}$	37.32	0.001
Talkative	E_{max}	1.19±0.81	0.31±0.31$^{\#\#\#}$	28.81±5.12***	10.69±3.73$^{\#\#\#}$	21.13	0.001
Adjective Mood Rating Scale							
Well-being	E_{max}	1.66±0.49	0.38±0.16$^{\#\#\#}$	7.06±1.01***	3.56±1.08$^{\#\#}$	18.0	0.001
Emotional excitation	E_{max}	0.69±0.35	0.69±0.27$^{\#\#\#}$	4.94±0.97***	1.31±0.37$^{\#\#\#}$	14.7	0.001
Extroversion	E_{max}	0.63±0.24	0.38±0.16$^{\#\#\#}$	3.50±0.61***	1.44±0.43$^{\#\#\#}$	17.5	0.001
Introversion	E_{max}	0.38±1.56	1.13±0.30	2.62±0.65**	1.69±0.59	5.4	0.01
Dreaminess	E_{max}	0.63±0.33	1.35±0.35	2.94±0.66**	1.81±0.48	4.1	0.05
Activity	E_{min}	−1,88±0.50	−2.69±0.69	−4.69±1.04*	−2.81±0.78	2.6	0.06
Circulating catecholamines							
Epinephrine (nM)	E_{max}	0.42±0.12	0.46±0.10	0.50±0.12	0.26±0.10		ns
Norepinephrine (nM)	E_{max}	−0.22±0.13	−0.18±0.07$^{\#\#\#}$	0.44±0.12***	−0.19±0.10$^{\#\#\#}$	14.7	0.001
Physiologic effect							
SBP (mm Hg)	E_{max}	8.56±1.75	6.19±1.42$^{\#\#\#}$	29.94±3.41***	10.94±1.58$^{\#\#\#}$	24.6	0.001
DPB (mm Hg)	E_{max}	6.25±1.25	6.00±0.97$^{\#\#\#}$	22.13±2.08***	9.22±1.57$^{\#\#\#}$	23.3	0.001
MAP (mm Hg)	E_{max}	5.80±1.27	5.11±1.01$^{\#\#\#}$	21.76±2.73***	8.54±1.46$^{\#\#\#}$	20.3	0.001
Heart rate (beats/min)	E_{max}	9.19±1.29	5.06±1.27$^{\#\#\#}$	26.06±2.77***	11.09±1.55$^{\#\#\#}$	25.5	0.001
Body temperature (°C)	E_{max}	0.23±0.04	0.19±0.04$^{\#\#\#}$	0.54±0.07**	0.39±0.08	7.3	0.001
List of Complaints (total score)							
Acute adverse effects	at 3 h	−0.06±0.52	−1.81±1.09$^{\#\#\#}$	5.56±1.72**	−1.25±1.49$^{\#\#}$	29.5	0.001
Sub-acute adverse effects	at 24 h	−1.00±0.58	−2.88±1.35$^{\#\#}$	3.88±1.09*	−0.38±1.32$^{\#}$	24.6	0.001
Ex vivo binding ($IC_{50\%}$)							
NET		>25	14.3±0.6*** $^{\#\#}$	23.4±0.7	13.7±0.7*** $^{\#\#\#}$	20.4	0.001
SERT		>25	1.5±0.2 *** $^{\#\#\#}$	>25	1.4±0.2 *** $^{\#\#\#}$	243.1	0.001
DAT		>25	>25	>25	>25		

Values are mean±SEM of changes from baseline of 16 subjects. *p<.05, **p<.01, and ***p<.001 vs. Placebo-placebo. $^{\#}$p<.05, $^{\#\#}$p<.01, $^{\#\#\#}$p<.001 vs. Placebo-MDMA. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. IC50%, inhibition constant calculated as % of plasma sample dilution with undiluted plasma set as 100%; NET, norepinephrine transporter; SERT, SERT, serotonin transporter; DAT, dopamine transporter; ns, nonsignificant.

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(Fig. 3). In the 5D-ASC [22,23], duloxetine robustly reduced MDMA's effects on the total ASC score ($p<0.001$) and in all three main dimensions of the scale (main effect of drug: $F_{3,45} = 26.2$, 32.6, 5.67, and 26.6 for ASC, OB, AED, and VR, respectively; all $p<0.001$; Fig. 4). Duloxetine prevented the MDMA-induced increase in circulating plasma NE levels, an endocrine marker for sympathetic system activation (Table 1), and reduced the blood pressure and heart rate response to MDMA (Table 1; Fig. 5). MDMA-induced increases in plasma NE at 60 min correlated with elevations in MAP ($r = 0.57$, $p<0.05$) and increases in VAS scores for "good drug effects," "liking," "open" ($r = 0.65$, 0.69, 0.77 and 0.63, respectively; all $p<0.01$), supporting the modulatory role of NE in these effects of MDMA. ANOVAs with period as factor showed no effect of treatment order, confirming the absence of period effects.

Pharmacokinetics

The robust decrease in the PD response to MDMA after duloxetine was not the result of a pharmacokinetic interaction between duloxetine and MDMA because duloxetine increased exposure to MDMA. MDMA and duloxetine are both substrates and inhibitors of CYP 2D6 [16]. The moderate CYP 2D6 inhibitor duloxetine increased both the C_{max} and $AUC_{0\text{-}6h}$ of the CYP 2D6 substrate MDMA by 16±4% (mean ± SEM; $F_{1,15} = 12.64$, $p<0.01$) and 18±5% ($F_{1,15} = 8.95$, $p<0.01$), respectively (Fig. 6 and Table 2). Duloxetine had no effect on exposure to MDA, the active metabolite of MDMA. Duloxetine decreased the C_{max} and $AUC_{0\text{-}6h}$ of the inactive CYP 2D6-formed MDMA metabolite HMMA by 46±6% ($F_{1,15} = 70.03$, $p<0.001$) and 48±6% ($F_{1,15} = 166.10$, $p<.001$), respectively. Plasma duloxetine concentrations nonsignificantly increased beginning 1 h after

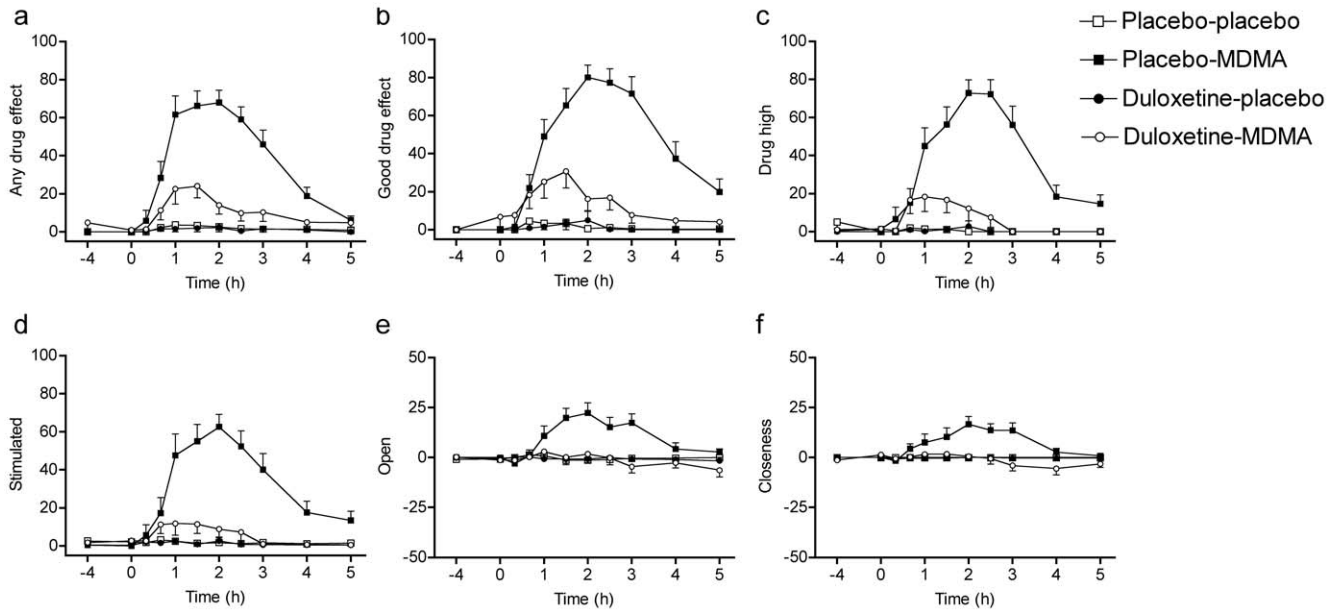

Figure 2. Duloxetine inhibited the psychotropic effects of MDMA. MDMA produced stimulant-like (**b–d**) and ''entactogenic'' (**e, f**) effects compared with placebo ($p<0.001$ for all scales). Duloxetine significantly inhibited MDMA-induced elevations in all of these subjective effects (**a–f**) ($p<0.001$ for all scales). Values are expressed as mean+SEM ($n = 16$).
doi:10.1371/journal.pone.0036476.g002

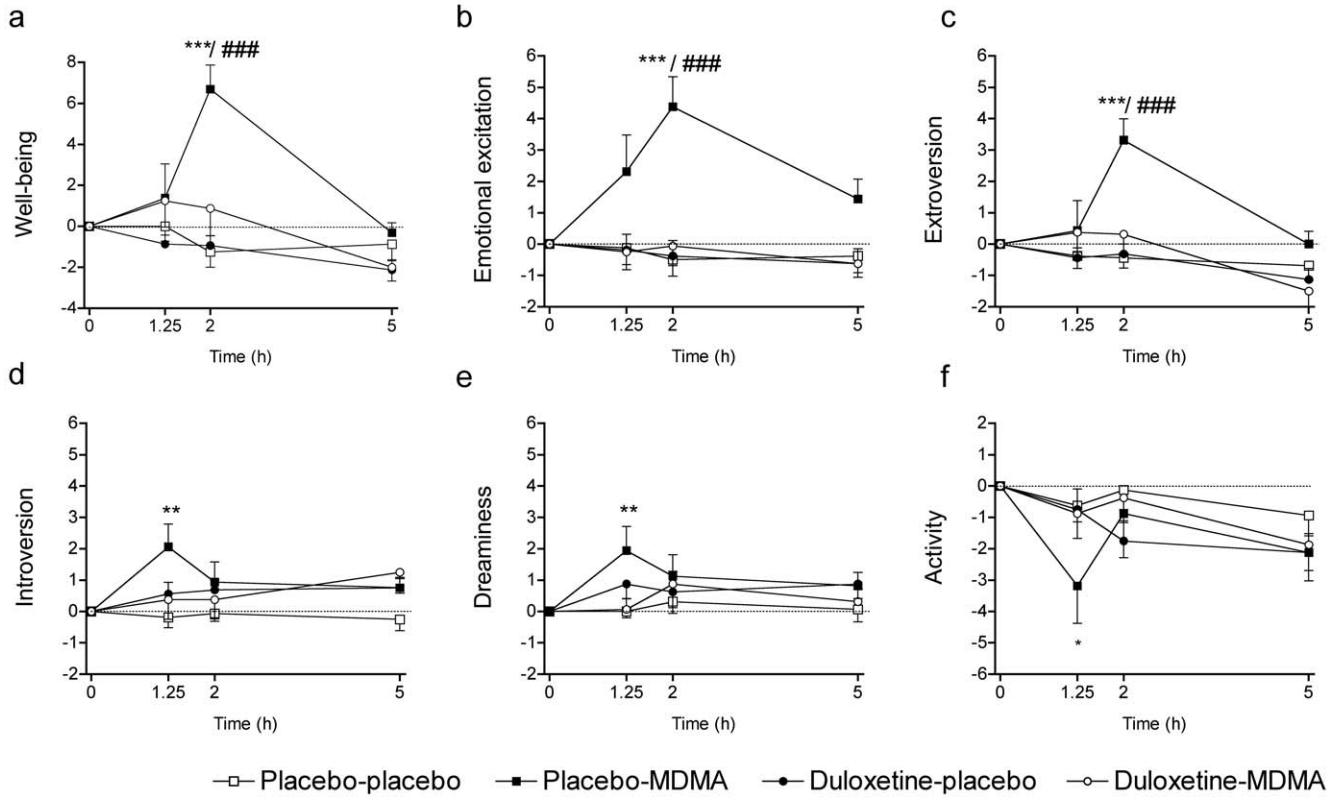

Figure 3. Duloxetine prevented the acute emotional effects of MDMA in the Adjective Mood Rating Scale. MDMA produced a state of well-being (a), emotional excitation (b), increased introversion at drug onset at 1.25 h (d), increased extroversion at 2 h (c), increased dreaminess (e), and decreased performance-oriented activity (f) (*$p<0.05$, **$p<0.01$, ***$p<0.001$, *vs*. placebo-placebo). Duloxetine prevented MDMA-induced elevations in well-being, emotional excitation, and extroversion (a-c) ($^{###}p<0.001$, placebo-MDMA *vs*. duloxetine-MDMA). Values are expressed as mean+SEM ($n = 16$).
doi:10.1371/journal.pone.0036476.g003

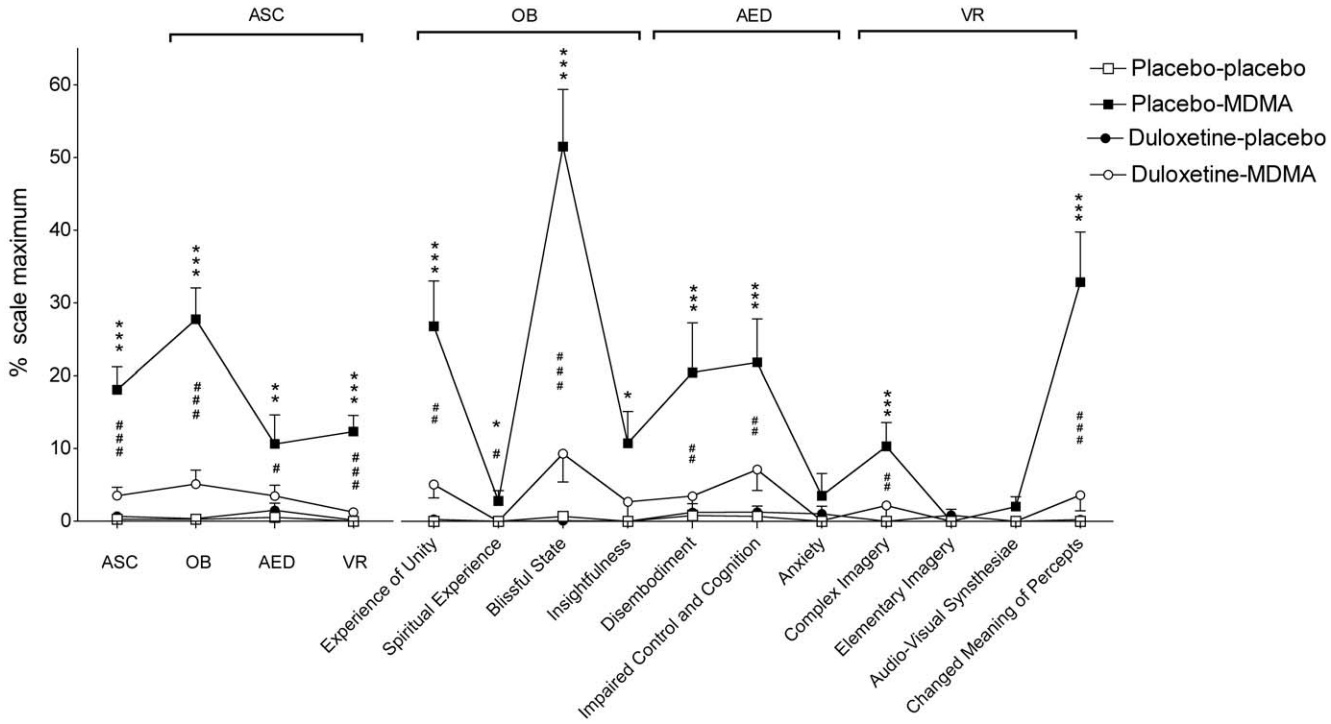

Figure 4. Duloxetine prevented the acute effects of MDMA in the Altered States of Consciousness (ASC) scale. MDMA significantly increased the ASC sum score, Oceanic Boundlessness (OB), Anxious Ego Dissolution (AED), and Visionary Restructuralization (VR) dimensions, and most of the subscales (*$p<0.05$, **$p<0.01$, ***$p<0.001$, placebo-placebo vs. placebo-MDMA). Duloxetine significantly reduced the effect of MDMA in all dimensions and subscales ($^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, placebo-MDMA vs. duloxetine-MDMA). Values are expressed as mean+SEM (n = 16).
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MDMA administration (Fig. 5), consistent with the inhibitory effect of MDMA on duloxetine metabolism via CYP 2D6. Interindividual differences in CYP 2D6 activity also affected the PK of MDMA. Lower CYP 2D6 function (i.e., a lower dextromethorphan:dextrorphan urine concentration ratio) was associated with a longer $t_{1/2}$ of MDMA ($r = 0.65$, $p<0.01$).

PK-PD Relationship

Fig. 7 shows the mean PD effects of MDMA plotted against simultaneous plasma concentrations at the different time points (hysteresis loops). The increases in "any drug effect" (Fig. 7a) and MAP (Fig. 7b) returned to baseline within 6 h when MDMA concentrations were still high. This clockwise hysteresis indicates that a smaller MDMA effect was seen at a given plasma concentration later in time, indicating rapid acute pharmacodynamic tolerance, which was similarly described for cocaine [33]. Duloxetine robustly reduced the physical and subjective response to MDMA, but it increased exposure to MDMA, illustrated by the downward and rightward shift of the MDMA hysteresis loops (Fig. 7).

Adverse Effects

MDMA produced adverse effects, such as sweating, difficulty concentrating, thirst, and lack of appetite, resulting in an increase in total LC scores at both 3 and 24 h after drug administration (Table 1). Duloxetine produced daytime somnolence and moderate insomnia. No severe adverse events were observed.

In vitro Studies

MDMA-induced 5-HT and NE release studies *in vitro*. MDMA was nonsignificantly more potent in releasing NE via NET than 5-HT via SERT ($IC_{50} = 0.55$ and 1.69 μM, respectively; Fig. 8; Table 3), consistent with earlier work that used human [3,34] and rat [2] transporters. MDA similarly released monoamines with EC_{50} values of 0.85 and 2.77 μM for NE and 5-HT, respectively (Fig. 8; Table 3). Thus, both amphetamines were active transporter-mediated monoamine releasers and exhibited slightly higher potency at NET than SERT. Duloxetine potently inhibited the ability of MDMA and MDA to induce 5-HT release from SERT and NE release from NET cells (Fig. 8). Duloxetine (0.1 μM) decreased the E_{max} by approximately 50% and shifted the concentration-effect curves to the right, consistent with a mixed competitive and noncompetitive mode of inhibition. A high concentration of duloxetine (10 μM) completely blocked the effects of MDMA and MDA (Fig. 8). We then compared the inhibitory effect of duloxetine on MDMA-induced monoamine release to the inhibitory effects of the selective SERT inhibitor citalopram and selective NET inhibitor reboxetine, each of which have been shown to attenuate some of the effects of MDMA in humans [7,10]. The potencies of duloxetine and citalopram to inhibit MDA- and MDMA-induced 5-HT release were similar (Fig. S1; Table 3). The potencies of duloxetine and reboxetine to block MDMA-induced NE release were also similar (Fig. S1; Table 3). These *in vitro* data indicate that duloxetine inhibited both SERT and NET similarly to citalopram and reboxetine, respectively.

PK-PD and *in vitro-in vivo* relationship. Duloxetine mainly affected the E_{max} of MDMA in the *in vivo* PK-PD relationship of MDMA (Fig. 9a) consistent with a primarily

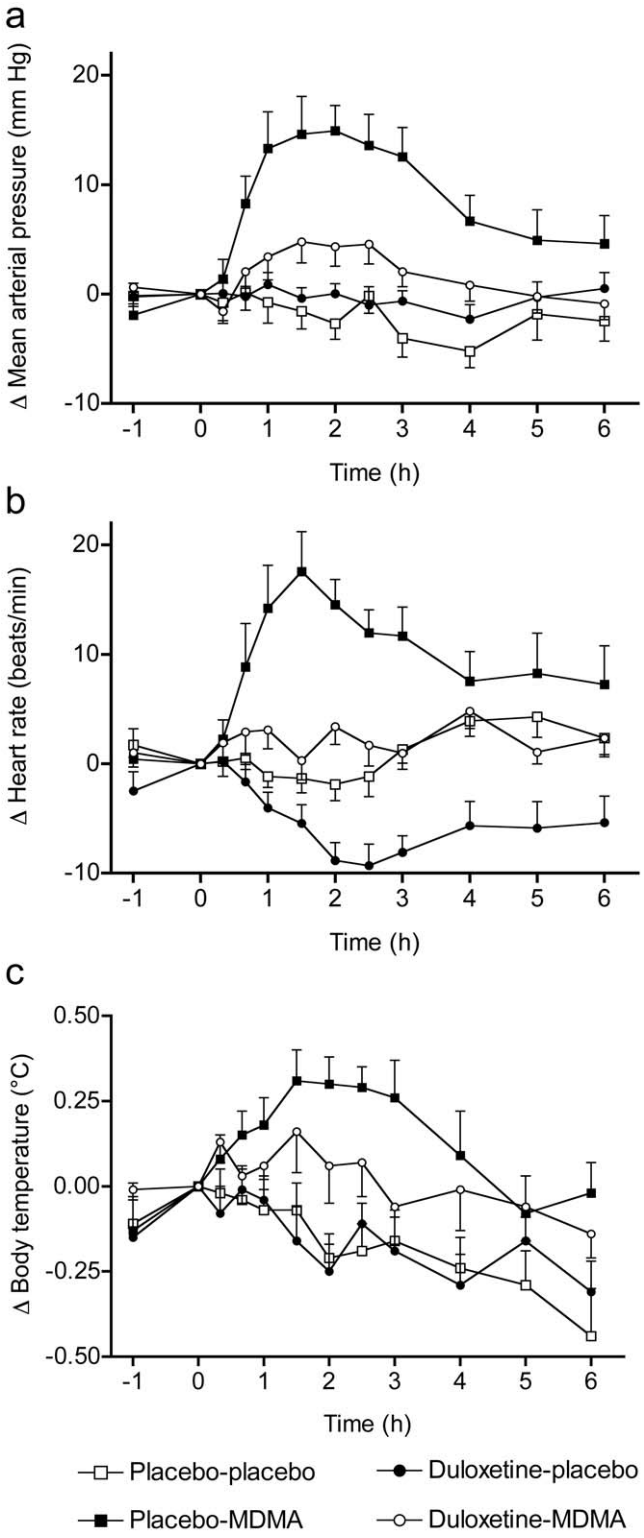

Figure 5. Duloxetine reduced the cardiostimulant response to MDMA. Duloxetine reduced the elevations in mean arterial blood pressure (**a**) and heart rate (**b**) in response to MDMA. Duloxetine also nonsignificantly lowered the MDMA-induced increase in body temperature (**c**). Values are expressed as mean+SEM of 16 subjects.
doi:10.1371/journal.pone.0036476.g005

noncompetitive mode of inhibition and similar to the effect of duloxetine on monoamine release produced by MDMA *in vitro*. Duloxetine decreased the E_{max} from 93.8±7.3% to 20.8±4% for placebo-MDMA compared with duloxetine-MDMA, respectively. The EC_{50} values were 92.5±7.6 ng/mL (0.48 μM) and 83.8±25 ng/mL (0.43 μM) for placebo-MDMA and duloxetine-MDMA, respectively. The EC_{50} of the PK-PD curve of placebo-MDMA in humans was 74 ng/ml (0.38 μM), similar to the EC_{50} values of MDMA to release 5-HT and NE *in vitro*. The plasma concentrations of duloxetine (C_{max} = 112 ng/ml or 0.38 μM) were also in the range of the concentrations that reduced MDMA-induced 5-HT and NE release *in vitro*. To relate our *in vitro* data to the PD of MDMA in humans, we linked the concentration-effect relationship of the *in vitro* effect of MDMA on 5-HT and NE release to the individual concentration-time curves of our subjects (Fig. 9b). The observed effect-time curve for MDMA in humans was predicted well by the *in vitro* NE release model, assuming similar concentrations in plasma and brain and no time lag. The 5-HT release model fitted, but 2- to 10-fold higher MDMA concentrations in the brain than in plasma would be needed to obtain similar pharmacodynamic effects as NE. The higher potency of MDMA to release NE *vs.* 5-HT *in vitro* also predicted that NE release occurred at lower MDMA plasma and brain concentrations and therefore sooner after MDMA administration, playing a predominant role during the initial drug effect (i.e., rush, stimulant effect). 5-HT release becomes relatively more important later in time and predominantly mediates "entactogenic" effects, including feelings of being open and closer to others, that prevail later. The model predicted that the half-maximal effects would be reached at 40±2 min and 70±14 min for NE and 5-HT release, respectively (Fig. 9b). The observed half-maximal subjective drug effect of MDMA was reached 44±4 min after drug administration. At that time, the models predicted 4 (3–6)-fold higher NE release compared with 5-HT release, consistent with the view of a primary role for NE in the early effects of MDMA.

Monoamine transporter binding *in vitro*. The binding of MDMA and MDA to monoamine transporters was weak (Table 4) compared with the high potency of MDMA to release 5-HT and NE. The binding profile of MDMA was consistent with other binding studies that used human transporters [3] but different from studies that used rat transporters [17]. Duloxetine showed more than 100-fold higher affinity for both SERT and NET compared with the affinity of MDMA for these transporters in the same assay, supporting our approach of using duloxetine to prevent MDMA from interacting with SERT and NET (Table 4).

Monoamine uptake inhibition *in vitro*. MDMA inhibited NET three-fold more potently than SERT, consistent with previous studies that used human transporters [3,35] but in contrast to data derived from mouse and rat transporters [17,35,36] (Table 5). MDA was equally potent to MDMA in inhibiting NET and SERT. Both MDMA and MDA showed low potency to inhibit DAT. Duloxetine was more potent in inhibiting SERT than NET (Table 5), which was expected [13]. Because the selective SERT inhibitor citalopram and selective NET inhibitor reboxetine have previously been shown to attenuate the psychological effects of MDMA [7,10], we compared duloxetine with these inhibitors. Duloxetine exhibited similar potency as citalopram to inhibit SERT but 2- to 5-fold lower potency as reboxetine to inhibit NET (Table 5).

Ex vivo Binding Studies

The ability of duloxetine to block monoamine transporters in our study was confirmed with an *ex vivo* assay, in which plasma from duloxetine-treated subjects inhibited *ex vivo* radioligand binding to SERT and NET but not DAT (Table 1). We also found a 10-fold higher affinity for SERT compared with NET,

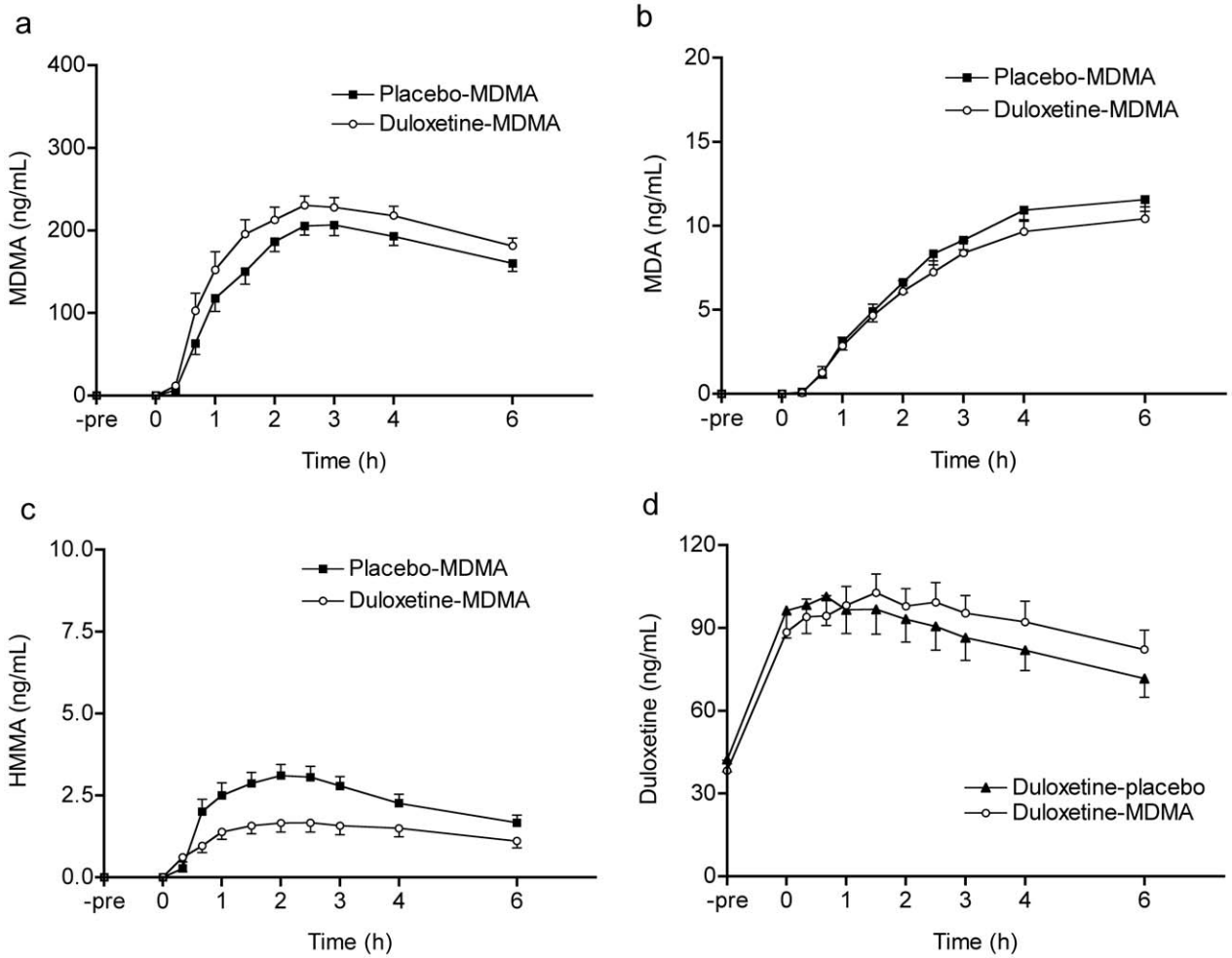

Figure 6. Duloxetine increased MDMA exposure. Pharmacokinetics of MDMA, MDA, HMMA, and duloxetine (**a–d**). Duloxetine was administered 16 h and 4 h before MDMA, which was administered at the 0 h time point. Duloxetine increased the C_{max} and AUC_{0-6} of MDMA (**a**), had no significant effect on MDA exposure (**b**), and decreased the C_{max} and AUC_{0-6} of HMMA (**c**). Plasma duloxetine concentrations were similar in the duloxetine-placebo and duloxetine-MDMA groups before MDMA administration (at –4 h and 0 h). Duloxetine concentrations increased 1 h after MDMA administration in the duloxetine-MDMA *vs*. duloxetine-placebo group (**d**). Values are expressed as mean±SEM of 16 subjects. MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine.
doi:10.1371/journal.pone.0036476.g006

Table 2. Pharmacokinetic parameters of MDMA, MDA, HMMA, and duloxetine.

	C_{max} (ng/ml)	T_{max} (h)	$T_{1/2}$ (h)	AUC_{0-6} (ng/ml h)	$AUC_{(0-\infty)}$ (ng/ml h)
MDMA					
Placebo-MDMA	221.31±11.63	2.34±0.19	8.17±0.74	952.75±45.89	2908.55±275.64
Duloxetine-MDMA	253.63±13.60**	2.66±0.29	7.14±0.40	1106.87±57.22**	2915.28±154.27
MDA					
Placebo-MDMA	11.75±0.70	5.50±0.22	–	46.60±3.02	–
Duloxetine-MDMA	10.67±0.72	5.25±0.30	–	41.95±3.38	–
HMMA					
Placebo-MDMA	3.36±0.34	1.84±0.17	–	13.57±1.58	–
Duloxetine-MDMA	2.00±0.38***	1.89±0.25	–	8.14±1.45***	–
Duloxetine					
Duloxetine-placebo	106.77±10.25	5.14±0.29	10.97±1.04	799.88±74.40	1960.18±229.54
Duloxetine-MDMA	111.69±7.06	5.95±0.39	11.37±1.43	814.31±52.73	2189.45±297.99

C_{max}, maximum plasma concentration; T_{max}, time from drug administration to maximum plasma concentration; $AUC_{0-\infty}$, area under concentration-time curve extrapolated to infinity. HMMA, 4-hydroxy-3-methoxymethamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; MDA, 3,4-methylenedioxyamphetamine.
**p<.01, ***p<.001, vs. Placebo-MDMA. Values are mean±SEM (n =16).
doi:10.1371/journal.pone.0036476.t002

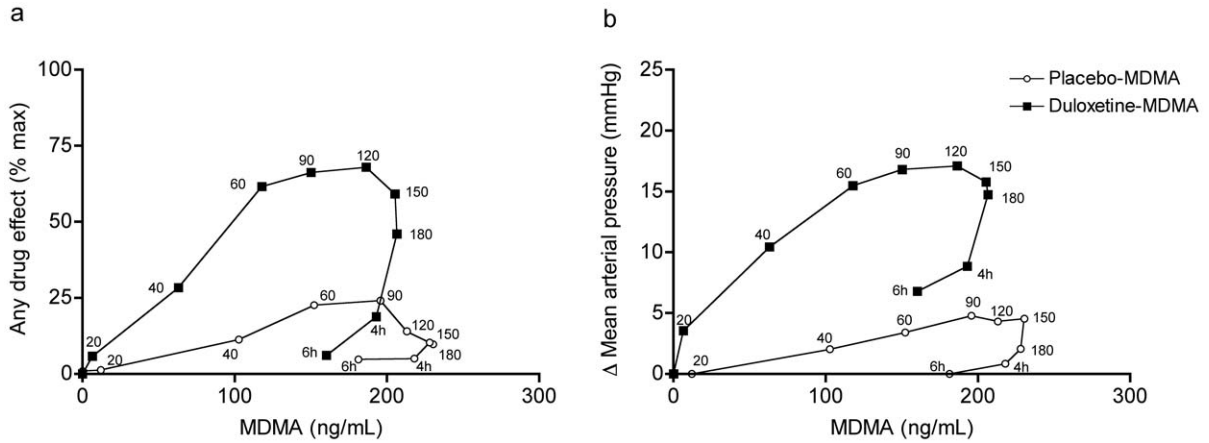

Figure 7. Pharmacokinetic-pharmacodynamic (PK-PD) relationship. MDMA effects are plotted against simultaneous MDMA plasma concentrations (**a, b**). The time of sampling is noted next to each point in minutes or hours after MDMA administration. The clockwise hysteresis indicates acute tolerance to the effects of MDMA. Duloxetine pretreatment markedly reduced physical and subjective responses to MDMA in the hysteresis loops (**a, b**).
doi:10.1371/journal.pone.0036476.g007

which was previously shown [13] and consistent with the *in vitro* profile of duloxetine. We calculated the duloxetine concentration in the plasma samples using the K_i values of duloxetine for SERT and NET binding (Table 2) and the IC_{50} values derived from the *ex vivo* binding in the duloxetine-placebo group (Table 1). The values (mean ± SE) obtained were 388±36 nM and 576±44 nM duloxetine using SERT and NET binding, respectively, which was well in agreement with the duloxetine plasma concentrations determined by LC-MS/MS (314±2.5 nM). Plasma from MDMA-treated subjects did not differ from placebo-treated subjects with regard to *ex vivo* radioligand binding to monoamine transporters (Table 1). This finding is consistent with the relatively low *in vitro* binding affinity of MDMA, which does not reflect the high pharmacological activity of the drug. Our assay assessed binding to

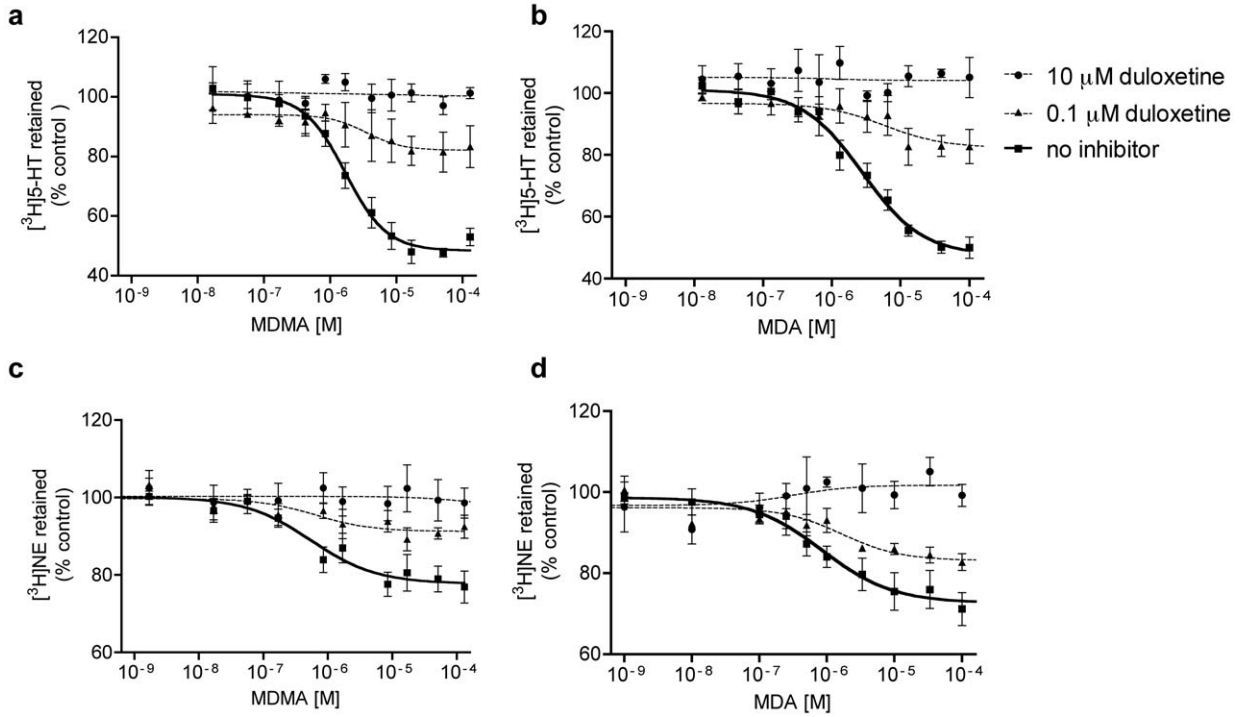

Figure 8. Duloxetine blocked MDMA- and MDA-induced 5-HT and NE efflux. Duloxetine inhibited SERT-mediated 5-HT release by MDMA (**a**) and MDA (**b**). Duloxetine also inhibited NET-mediated NE release by MDMA (**c**) and MDA (**d**). Values are expressed as mean ± SEM (n = 3–6) of retained radiolabeled substrate following incubation with various concentrations of MDMA and MDA.
doi:10.1371/journal.pone.0036476.g008

Table 3. Inhibition of MDMA-induced 5-HT or NE release by different inhibitors.

	SERT		NET	
	EC_{50} (μM) (95% CI)	E_{max}, % retained, (95% CI)	EC_{50} (μM) (95% CI)	E_{max}, % retained, (95% CI)
MDMA alone	1.69 (1.07–2.66)	48 (42–55)	0.55 (0.17–1.81)	78 (73–82)
MDMA plus 0.1 μM duloxetine	3.51 (0.46–27)	82 (75–90)	0.59 (0.02–19)	90 (84–97)
MDMA plus 0.1 μM citalopram	3.17 (1.89–5.31)	72 (68–77)	na	na
MDMA plus 0.1 μM reboxetine	na	Na	3.35 (0.63–179)	78 (56–102)
MDA alone	2.77 (1.78–4.30)	48 (41–54)	0.85 (0.29–2.55)	73 (67–79)
MDA plus 0.1 μM duloxetine	6.86 (0.5–100)	83 (77–89)	2.06 (0.35–12.12)	80 (73–87)
MDA plus 0.1 μM citalopram	5.0 (1.28–19.6)	59 (44–75)	na	na

95% CI, 95% confidence interval; na, not assessed.
doi:10.1371/journal.pone.0036476.t003

the SERT and NET binding site for [^{3}H]-citalopram and [^{3}H]-nisoxetine, respectively. A possible explanation for the low affinity of MDMA in this assay could be a binding site for MDMA that is different from citalopram and nisoxetine at SERT and NET, respectively, consistent with the noncompetitive mode of inhibition of the MDMA-induced 5-HT and NE release by duloxetine.

Discussion

The present study showed that the dual SERT and NET inhibitor duloxetine markedly decreased the psychotropic and cardiovascular responses to MDMA in human subjects, confirming and extending previous work with selective SERT [7,8,9] and NET [10] inhibitors. The inhibition of the effect of MDMA by duloxetine in humans was pronounced and primarily noncompetitive. *In vitro*, duloxetine similarly blocked the interactive effects of MDMA with SERT and NET to release 5-HT and NE. The present findings provide further support for a central role of SERT and NET as targets of MDMA with regard to its acute effects in humans. Previous clinical data indicated that 5-HT release primarily mediates the MDMA-typical "empathogenic" mood effects of MDMA [7], whereas NE release may be responsible for the stimulant and cardiovascular effects of the drug [10]. In the present study, dual inhibition of 5-HT and NE release robustly blocked both aspects of the MDMA effect, consistent with the role of both 5-HT and NE. The precise mode of interaction of amphetamine derivatives, including MDMA, with monoamine transporters remains to be elucidated and may involve the exchange of amphetamine with the transmitter, channel-like conformational changes of the transporter [37], or transporter internalization [38,39,40], MDMA is structurally similar to 5-HT, and a common binding site has been proposed in transmembrane domain 6 of SERT [41]. A distinct binding site was found for SERT inhibitors, including citalopram and fluoxetine, proximal to the 5-HT binding site [42]. Some SERT inhibitors may therefore allosterically inhibit the interaction between MDMA and SERT to release 5-HT. Consistent with these molecular data, our study showed that duloxetine inhibited MDMA-induced 5-HT release, NE release, and the response to MDMA in humans possibly according to a noncompetitive inhibition mode. Both our *in vitro* and *in vivo* findings may indicate acute allosteric inhibition of the effects of MDMA by duloxetine. Prior work with rat brain synaptosomes showed that indatraline competitively inhibited MDMA-induced 5-HT release [5]. However, later studies indicated that many SERT inhibitors also decreased the E_{max} for different monoamine releasers, suggesting unique transporter

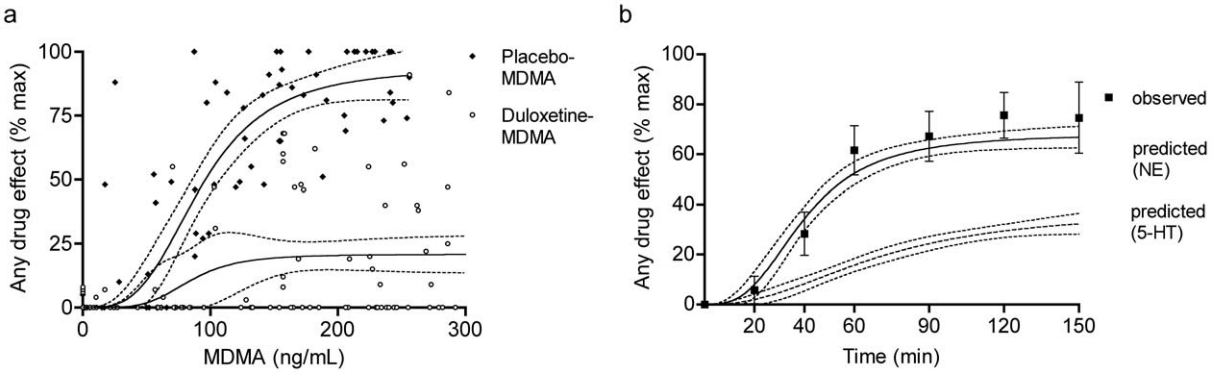

Figure 9. Pharmacokinetic-pharmacodynamic modeling. Duloxetine lowered E_{max} in the MDMA concentration-effect curve (**a**) with little effect on EC_{50}, similar to the effect of MDMA on monoamine release *in vitro*. Diamonds and circles represent concentration-effect data pairs for ascending concentrations for placebo-MDMA and duloxetine-MDMA, respectively (**a**). The solid lines show the fit of a sigmoid E_{max} PD model to the observed PK data (**a**). Dashed lines indicate the 95% confidence interval (CI) of the estimation error (**a**). NE release predicted the observed subjective effect of MDMA *in vivo* (**b**). Predicted effects are shown as curves (mean ±95% CI) that represent the fit of the *in vitro* concentration-effect data to the 16 individual plasma concentration-time curves (**b**). Observed values are expressed as mean±SEM of 16 subjects (**b**). MDMA, 3,4-methylenedioxymethamphetamine; NE, norepinephrine; 5-HT, serotonin.
doi:10.1371/journal.pone.0036476.g009

Table 4. Binding affinities to human monoamine transporters.

	SERT	NET	DAT
MDMA	13.3±0.47	22.4±14.6	6.52±2.24
MDA	18.7±2.76	17.8±4.06	26.4±4.24
Duloxetine	0.005±0.002	0.07±0.05	0.70±0.07
Reboxetine	0.24±0.02	0.015±0.01	16.2±4.91
Citalopram	0.005±0.001	5.06±3.00	21.4±10.5
Indatraline	0.02±0.008	0.03±0.02	0.01±0.01
Paroxetine	0.004±0.001	0.42±0.17	0.77±0.18

Values are mean±SD of K_i (μM) (n≥3). Radioligands were ^{3}[H]citalopram, ^{3}[H]nisoxetine, and ^{3}[H]-WIN35,428 for SERT, NET, and DAT, respectively.
doi:10.1371/journal.pone.0036476.t004

interactions for different inhibitor-releaser combinations [6]. This indicates that different SERT inhibitors may also more or less effectively reduce the effects of psychostimulants in humans. Nevertheless, several of the present findings indicate that the effect of duloxetine on the MDMA response was likely attributable to the dual inhibition of SERT and NET and not only the result of potent SERT inhibition alone. First, duloxetine blocked MDMA-induced NE release *in vitro* and MDMA-induced increases in plasma NE *in vivo*, similar to the selective NET inhibitor reboxetine [10]. Second, we documented, *ex vivo*, NET binding in plasma from duloxetine-treated subjects, and duloxetine has previously been shown to effectively inhibit NET in humans [13]. Third, potent and selective inhibition of SERT alone using citalopram in a single high dose [7], fluoxetine for 5 days [8], or paroxetine for 3 days [9] failed to block the effects of MDMA in humans to the extent seen here with dual SERT and NET inhibition. Conversely, selectively blocking NET alone also did not as effectively reduce the effects of MDMA in humans [10] as blocking both SERT and NET. The importance of NE as a modulator of the acute effects of MDMA is also supported by the fact that NE plasma levels after MDMA treatment in the present study correlated with the subjective effects and increases in blood pressure. Furthermore, we compared our *in vitro* 5-HT and NE release data to clinical data in humans and showed that the NE release link model better predicted the ascending subjective effects of MDMA in humans than the 5-HT release link model. A full assessment of the relative efficacy of SERT and NET inhibitors to prevent the effects of MDMA would require administration of SERT and NET inhibitors alone and in combination and dose-response studies. However, such studies were not ethically feasible because we did not want to expose our MDMA-naive subjects to more than two doses of MDMA in a crossover design.

The role of DA in the reinforcing effects of psychostimulants is well established, but unknown is whether DA is critical for the acute effects of MDMA. We found that MDMA exhibited higher affinity for DAT than NET or SERT *in vitro*. However, MDMA functionally exhibited significantly higher inhibition potency of the SERT and NET compared with DAT, respectively. MDMA is also more potent in releasing 5-HT and NE compared with DA *in vitro* [3], and the magnitude of 5-HT release exceeded DA release in the nucleus accumbens, striatum, and prefrontal cortex, assessed with *in vivo* microdialysis in rats [43]. DAT inhibition did not affect the acute response to MDMA in rhesus monkeys [44]. Additionally, the D_2 dopamine receptor antagonist haloperidol only weakly attenuated MDMA-induced euphoria in humans and only at doses that produced significant dysphoria [45]. Whether DAT (NET) inhibitors, such as bupropion or methylphenidate, inhibit the effects of MDMA in humans remains to be tested. Duloxetine is a potent SERT and NET inhibitor but also weak DAT inhibitor [13,46], which was confirmed in the present *in vitro* study. We cannot exclude the possibility that the relatively high dose of duloxetine used in the present study also inhibited MDMA-induced DA release. Notably, the present *ex vivo* binding studies further showed that the plasma from the subjects treated with duloxetine exhibited binding to SERT and NET but not DAT.

The transporter-independent vesicular release of monoamines could theoretically contribute to the mechanism of action of MDMA. We recently showed that this is not the case for NE because clonidine, which blocks transporter-independent vesicular NE release, did not alter the effects of MDMA in humans [12]. Additionally, MDMA did not directly stimulate the Ca^{2+}-dependent vesicular release of DA [47]. Nevertheless, MDMA may indirectly stimulate the DA system and induce the vesicular release of DA by downstream 5-HT-DA or NE-DA system interactions. For example, 5-HT release by MDMA stimulates DA release via 5-HT_2 receptor activation [48], and this indirect effect on the DA system is also prevented by SERT inhibition [49]. Thus, downstream DA system activation may be a contributing factor to MDMA-induced euphoria and the mechanism of action of psychostimulants in general, even when SERT and NET may be considered the primary pharmacological targets.

Finally, it is also possible that duloxetine induced adaptive effects on monoamine systems that reduced the response to MDMA in vivo. For example, decreases in SERT but not in NET

Table 5. Monoamine transport inhibition.

	SERT	NET	DAT
	K_i (μM) (95% CI)	K_i (μM) (95% CI)	K_i (μM) (95% CI)
MDMA*	1.40 (1.00–1.96)	0.470 (0.334–0.598)	16.7 (11.5–24)
MDA*	2.41 (1.49–3.92)	0.341 (0.253–0.461)	11 (7.5–17)
Duloxetine	0.050 (0.04–0.07)*	0.126 (0.099–0.161)*	2.26 (0.7–3.8)#
Reboxetine	2.07 (1.4–2.6)#	0.036 (0.030–0.044)*	16.4 (11.5–25.2)#
Citalopram*	0.045 (0.037–0.057)	>20	>20
Indatraline#	0.09 (0.06–0.12)	0.043 (0.03–0.06)	0.025 (0.01–0.04)
Paroxetine#	0.014 (0.01–0.02)	1.12 (0.03–1.7)	4.83 (2.4–7.3)

*method A; #method B; 95% CI, 95% confidence interval; values are significantly different ($p<0.05$) if 95% CI do not operlap.
doi:10.1371/journal.pone.0036476.t005

binding sites were documented following chronic administration of duloxetine in rats [50].

In conclusion, the present study adds to a better understanding of the mechanism of action of MDMA in humans. The data support the roles of both NE and 5-HT in the acute effects of MDMA. The robust and almost complete prevention of the effects of MDMA by duloxetine suggests that dual transporter inhibitors may be useful in the prevention of the acute and long-term consequences of MDMA and potentially other psychostimulants in addicted subjects.

Supporting Information

Figure S1 Potency and efficacy of MDMA- and MDA-induced 5-HT and NE release inhibition by duloxetine, citalopram, and reboxetine. Both duloxetine and citalopram inhibited MDMA-induced (a, c) and MDA-induced (b, d) 5-HT release *in vitro* with approximately similar potency and efficacy. The potency of duloxetine to block MDMA-induced NE release was also similar to the selective NET inhibitor reboxetine (e, f). EC_{50} and E_{max} values are shown in Table 3. Data points represent mean ± SEM.
(TIF)

Protocol S1 Trial Protocol.
(DOC)

Checklist S1 CONSORT Checklist.
(DOC)

Acknowledgments

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Author Contributions

Conceived and designed the experiments: CMH LDS MEL. Performed the experiments: CMH LDS VGN NV MD SK EG JH MCH MEL. Analyzed the data: CMH LDS MCH MEL. Wrote the paper: CMH LDS MCH MEL.

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Paper Seven: Effects of MDMA on Vasopressin

Sex Differences in the Effects of MDMA (Ecstasy) on Plasma Copeptin in Healthy Subjects

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Sex Differences in the Effects of MDMA (Ecstasy) on Plasma Copeptin in Healthy Subjects

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Background: 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) misuse is associated with hyponatremia particularly in women. Hyponatremia is possibly due to inappropriate secretion of plasma arginine vasopressin (AVP).

Objective: To assess whether MDMA increases plasma AVP and copeptin in healthy male and female subjects and whether effects depend on MDMA-induced release of serotonin and norepinephrine. Copeptin, the C-terminal part of the AVP precursor preprovasopressin, is cosecreted with AVP and can be determined more reliably.

Methods: We used a randomized placebo-controlled crossover design. Plasma and urine osmolalities as well as AVP and copeptin levels were measured in 16 healthy subjects (eight female, eight male) at baseline and after MDMA (125 mg) administration. In addition, we tested whether effects of MDMA on AVP and copeptin secretion can be prevented by pretreatment with the serotonin and norepinephrine transporter inhibitor duloxetine (120 mg), which blocks MDMA-induced transporter-mediated release of serotonin and norepinephrine.

Results: MDMA significantly elevated plasma copeptin levels at 60 min and at 120 min compared with placebo in women but not in men. The copeptin response to MDMA in women was prevented by duloxetine. MDMA also nonsignificantly increased plasma AVP levels in women, and the effect was prevented by duloxetine. Although subjects drank more water after MDMA compared with placebo administration, MDMA tended to increase urine sodium levels and urine osmolality compared with placebo, indicating increased renal water retention.

Conclusion: MDMA increased plasma copeptin, a marker for AVP secretion, in women but not in men. This sex difference in MDMA-induced AVP secretion may explain why hyponatremia is typically reported in female ecstasy users. The copeptin response to MDMA is likely mediated via MDMA-induced release of serotonin and/or norepinephrine because it was prevented by duloxetine, which blocks the interaction of MDMA with the serotonergic and noradrenergic system. **(*J Clin Endocrinol Metab* 96: 0000–0000, 2011)**

Abuse of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) has been associated with the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (1, 2) and symptomatic hyponatremia particularly in women (3, 4). Specifically, a case series of ecstasy-associated hyponatremia included 18 cases, of which 17 were women (4). Another larger retrospective series of ecstasy exposures reported to a poison center found hyponatremia (Na <130 mmol/liter) in 73 (38.8%) of 188 cases (3). Of the 73 cases with hyponatremia, 55 (75.3%) were women and 18 (24.7%) men (3). Thus, female sex was significantly associated with increased odds of hyponatremia and increased odds of associated coma among these cases (3). A small nonblinded laboratory study showed

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Abbreviations: AVP, Arginine vasopressin; MDMA, 3,4-methylenedioxymethamphetamine; SIADH, syndrome of inappropriate secretion of antidiuretic hormone.

that MDMA significantly increased plasma concentrations of arginine vasopressin (AVP) at 1–4 h after controlled MDMA administration in eight healthy male volunteers (5, 6). This study provides evidence for a stimulatory effect of MDMA on AVP secretion. However, no female subjects were included. We assessed MDMA effects on AVP system activation and associated changes in plasma and urine osmolality as well as sodium levels in resting healthy subjects with *ad libitum* water intake in a controlled laboratory setting.

MDMA is a substrate of both the serotonin and norepinephrine transporter (7). It enters presynaptic nerve terminals and potently releases serotonin and norepinephrine through the transporter (7). AVP secretion is thought to be regulated by serotonergic (8) and noradrenergic (9) pathways, and these monoamines could act as mediators for the effects of MDMA on the AVP system. The MDMA-induced carrier-mediated release of serotonin and norepinephrine can be reduced by serotonin and norepinephrine transporter inhibitors, respectively (10, 11). We therefore assessed whether blockade of both the serotonin and norepinephrine transporter with duloxetine would prevent potential effects of MDMA on AVP secretion in the present study.

The reliable determination of plasma AVP is problematic. We therefore measured copeptin in addition to AVP levels. Copeptin is the C-terminal part of the AVP precursor preprovasopressin. Copeptin is produced together with AVP in equimolar ratio and exhibits similar kinetics in response to osmotic changes (12–14). In contrast to AVP, copeptin levels remain stable in serum or plasma samples and can easily and reliably be measured (12).

We hypothesized that MDMA would increase AVP and copeptin levels, particularly in women, and that pretreatment with the serotonin-norepinephrine transport inhibitor duloxetine would prevent this effect.

Subjects and Methods

Study subjects

The study was performed in 16 healthy subjects (eight women, eight men). Women were (mean ± SD) 29.0 ± 7.1 yr old. Body weight was 59.0 ± 6.9 kg. Men were 23.3 ± 3.1 yr old. Body weight was 79.5 ± 9.8 kg. Exclusion criteria included age under 18 or over 45 yr, pregnancy (urine pregnancy test before each test session), body mass index below 18.5 or over 25 kg/m^2, personal or family (first-degree relatives) history of psychiatric disorder, regular use of medications, chronic or acute physical illness (normal physical exam, normal electrocardiogram, and standard hematological and chemical blood analyses), smoking, lifetime prevalence of illicit drug use over five times (except for tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study (urine tests before test sessions). Subjects were asked to abstain from excessive alcohol consumption between test sessions and in particular to limit their use to one glass on the day before the test sessions. Subjects abstained from caffeinated beverages on the test days. Female subjects were investigated during the follicular phase (d 2–14) of their menstrual cycle when the reactivity to amphetamines (15) and osmotic sensitivity (16) are expected to be similar to men. All subjects gave their written informed consent before participating in the study, and subjects were paid for participation.

Study procedures

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The study was registered at www.clinicaltrials.gov (number NCT00990067) with neuroendocrine measures as a secondary outcome. We used a randomized placebo-controlled crossover design with four conditions (placebo-MDMA, placebo-duloxetine, duloxetine-MDMA, and placebo-placebo) in balanced order. Washout periods between sessions lasted 10–14 d. Duloxetine (120 mg) or placebo was administered twice 16 and 4 h before MDMA (125 mg) or placebo, respectively. We assessed plasma and urine osmolality as well as plasma and urine sodium 4 h before and 120 min after MDMA/placebo administration. Plasma levels of copeptin were assessed 4 h before and at 60 and 120 min after MDMA/placebo. Plasma levels of AVP were assessed 4 h before and 120 min after MDMA/placebo. Subjects were not engaged in any physical activity and were resting in hospital beds during the test session. Subjects had a small standardized breakfast at the beginning of each test session. Fluid consumption was not restricted up to a total intake of 2000 ml water during the session and was recorded from 4 h before to 120 min after MDMA/placebo administration when the last hormone measurement was performed. In addition, saline was administered via an iv catheter to keep catheters open for blood sampling at a rate of 100 ml/h from 0–120 min after MDMA/placebo administration. The study design also included additional assessments of subjective and cardiovascular effects, blood drawings for pharmacokinetics, and monitoring of adverse events for 6 h after MDMA/placebo administration as will be described elsewhere (Simmler, L. D., C. M. Hysek, J. Huwyler, M. E. Liechti, unpublished data).

Measurements

Measurements were done in duplicates in a blinded fashion in a single batch. AVP was assessed in EDTA plasma using a RIA (Direct Vasopressin RIA; Bühlmann Laboratories AG, Schönenbuch/Basel, Switzerland). The lower detection limit was 0.82 pmol/liter, and the intraassay precision was 6.0%. Copeptin levels were assessed using an immunoassay (LIA CT-proAVP; B.R.A.H.M.S./ThermoFisher Scientific, Hennigsdorf/Berlin, Germany) as described previously (12) and modified as described previously (14). The lower detection limit was 0.4 pmol/liter, and the intraassay coefficient of variation was less than 5%. Sodium concentrations were measured by indirect potentiometry (Hitachi 917; Roche Diagnostics, Rotkreuz, Switzerland). Osmolality was measured by cryoscopy (Micro Osmometer; Advances Instruments for Switzerland Instruments, Zurich, Switzerland).

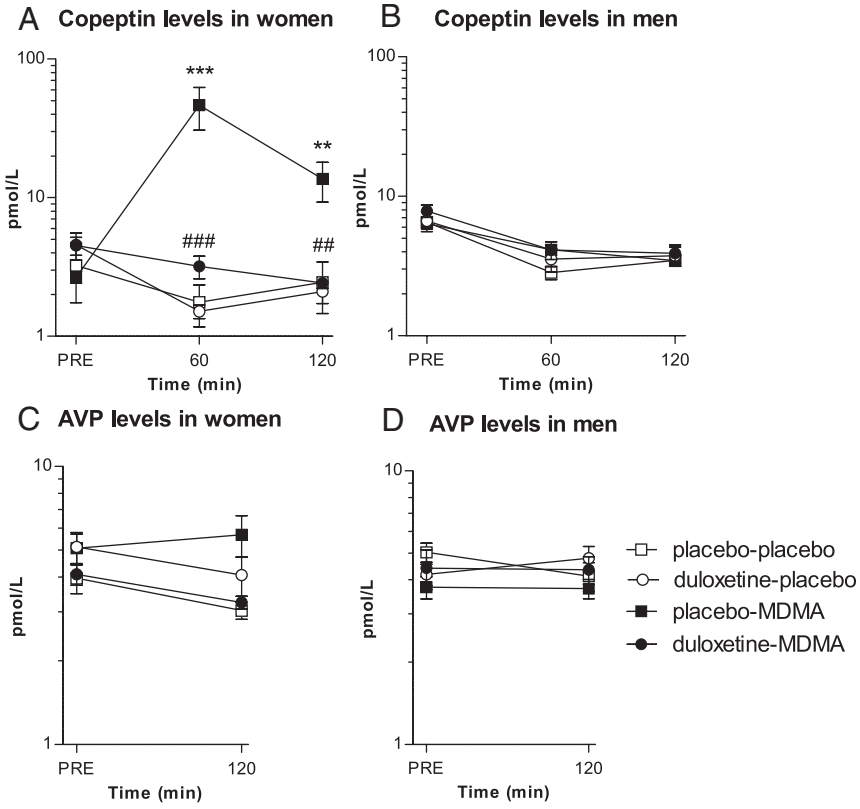

FIG. 1. Mean values ± SEM for plasma levels of copeptin and AVP in eight female and eight male healthy subjects 4 h before (PRE) and 60 and 120 min after MDMA (125 mg) or placebo. A, MDMA significantly increased copeptin levels in women at 60 and 120 min after drug administration compared with placebo. Duloxetine pretreatment prevented the MDMA-induced elevation in circulating copeptin in women. B, MDMA did not alter copeptin levels in men. C, Similar to its effects on MDMA-induced copeptin increases, duloxetine also prevented the nonsignificant increase in AVP at 120 min after MDMA administration in women. D, There were no drug effects on AVP levels in men. **, $P < 0.01$; ***, $P < 0.001$ *vs.* placebo-placebo; ##, $P < 0.01$; ###, $P < 0.001$ *vs.* placebo-MDMA.

Study drugs

(±)MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules (100 and 25 mg). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute dose of 125 mg. This dose of MDMA corresponds to a typical recreational dose of ecstasy, and comparable doses of MDMA have previously been used in controlled settings. Because MDMA was dosed in an absolute dose of 125 mg, differences in body weight resulted in different weight-adjusted relative MDMA doses of 1.6 ± 0.23 mg/kg (range, 1.4–2.1 mg/kg) in men and 2.1 ± 0.25 mg/kg (range, 1.8–2.5 mg/kg) in women. Duloxetine (Cymbalta; Eli Lilly SA, Vernier, Switzerland) was prepared as 60-mg gelatin capsules, and identically looking placebo (lactose) capsules were similarly prepared.

Statistical analysis

Repeated-measures ANOVA with the factors drug (placebo-placebo, duloxetine-placebo, placebo-MDMA, and duloxetine-MDMA) and time (baseline, 60 min, and 120 min) stratified for sex and followed by pairwise Tukey *post hoc* tests was used to assess differences in the effects of the different drugs. Nonnormally distributed variables were log normalized before the ANOVA. Correlation analyses were performed using Spearman's rank correlations using the total of all values (n = 128). All tests were two tailed, and the significance level was set to $P = 0.05$.

Results

ANOVA on plasma copeptin levels yielded a significant drug × time × sex interaction [$F_{(6,84)} = 3.93$; $P = 0.0017$]. MDMA significantly elevated plasma copeptin levels at 60 min ($P < 0.001$) and at 120 min ($P < 0.01$) compared with placebo in women (Fig. 1A) but not in men (Fig. 1B). The MDMA-induced increase in plasma copeptin in women was prevented by duloxetine pretreatment both at 60 min ($P < 0.001$) and 120 min ($P < 0.01$) (Fig. 1A). A similar trend was observed for AVP levels but drug effects did not reach significance (Fig. 1, C and D). Oral liquid intake varied across drug treatments, but there were no sex differences [main effect of drug: $F_{(3,42)} = 8.62$; $P < 0.001$, no drug × sex interaction]. Oral liquid intake (mean ± SEM) was 612 ± 50 ml after placebo-placebo, 1267 ± 118 ml after duloxetine-placebo ($P < 0.001$ *vs.* placebo-placebo), 1198 ± 130 ml after placebo-MDMA ($P = 0.001$ *vs.* placebo-placebo), and 807 ± 83 ml after duloxetine-MDMA ($P = 0.02$ *vs.* duloxetine-placebo, and $P = 0.051$ *vs.* placebo-MDMA). Urine osmolality decreased significantly over time [main effect of time: $F_{(1,14)} = 62.69$; $P < 0.001$]. Urine osmolality tended to be higher after placebo-MDMA or duloxetine-MDMA compared with placebo-placebo or duloxetine-placebo as evidenced by a near-significant drug × time interaction in the ANOVA [$F_{(3,42)} = 2.70$; $P = 0.058$] (Fig. 2, A and B). A similar trend was observed for urine sodium levels [drug × time interaction: $F_{(3,42)} = 2.33$; $P = 0.088$] (Fig. 2, C and D). There were no significant drug effects on plasma sodium levels or plasma osmolality (Fig. 2, E–H). Circulating copeptin levels correlated with AVP levels (all: $r_s = 0.34$, $P < 0.001$; women: $r_s = 0.53$, $P < 0.001$; men: $r_s = 0.28$, $P < 0.05$]. Copeptin levels were also correlated with plasma and urine osmolality [$r_s = 0.22$; $P < 0.05$ and $r_s = 0.68$; $P < 0.001$, respectively] as well as with plasma and urine sodium [$r_s = 0.18$; $P <$

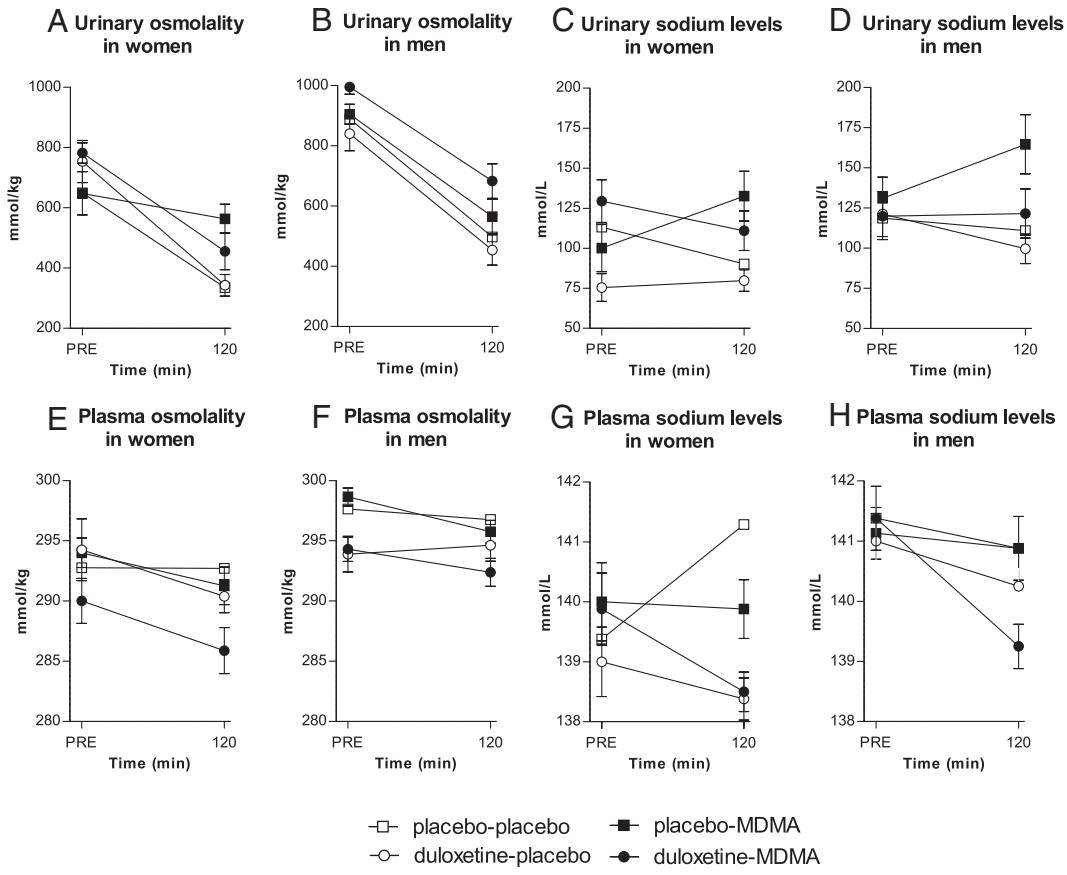

FIG. 2. Mean values ± SEM for sodium and osmolality in urine and plasma in eight female and eight male healthy subjects 4 h before (PRE) and 120 min after MDMA (125 mg) or placebo. The two treatment conditions including MDMA (placebo-MDMA and duloxetine-MDMA) tended to increase both urine osmolality (A and B) and urine sodium levels (C and D) in both sexes. There were no treatment effects on plasma osmolality or plasma sodium levels (E–H).

0.05 and $r_s = 0.28$; $P < 0.01$, respectively]. Baseline copeptin levels were significantly lower in women than men [$F_{(1,14)} = 8.38$; $P = 0.012$]. The relative dose of MDMA (in milligrams per kilogram body weight) did not correlate with the MDMA-induced increase in plasma copeptin within the two sex groups. In the present study, MDMA also produced marked subjective and cardiovascular stimulant effects as will be reported separately elsewhere (Simmler, L. D., C. M. Hysek, J. Huwyler, M. E. Liechti, unpublished data).

Discussion

We found that MDMA increased circulating copeptin, a marker for AVP secretion, in women but not in men. This sex difference in MDMA-induced AVP secretion is in line with the clinical observation that ecstasy-associated hyponatremia is typically reported in female users (3, 4). Other sex differences in the response to MDMA or ecstasy have previously been reported and include increased subjective effects in women compared with men to equal weight-adjusted doses of MDMA (18), more pronounced depression after ecstasy use (19), and a potential increase in serotonergic neurotoxicty in association with long-term use of ecstasy in women (20). The present findings indicate that women may be at increased risk for developing hyponatremia and associated neurotoxicity due to their sex-specific stronger AVP response to MDMA. In addition, the threshold levels of plasma sodium at which neurological complications occur appear to be higher in women than men (21, 22), and woman are more likely than men to die from hyponatremic encephalopathy after surgery (21, 23). Seizures and coma were also more frequently reported in female cases of ecstasy-associated hyponatremia compared with men (3). However, ecstasy-associated hyponatremia may have multiple causes, and MDMA-induced AVP secretion may be only one of several contributing factors. Dry mouth and physical exertion with sweating followed by hyperhydration with electrolyte-free water may all contribute to the development of hyponatremic states in recreational ecstasy users. Even loss of sodium into the gastrointestinal tract has been discussed (24).

The AVP system is activated by factors typically associated with MDMA consumption in a party setting in-

cluding dehydration (12–14), heat (25), and physical activity (12, 26), all of which are potentially increasing the risk of SIADH. Our results indicate that direct activation of the AVP system by MDMA may play a crucial facilitating role in the development of ecstasy-associated SIADH, in particular in women, because we controlled carefully for confounding factors that may increase AVP. Subjects were well hydrated orally and iv and resting comfortably in hospital beds in a temperature-controlled research environment. Of note, our subjects drank more water after MDMA than after placebo administration possibly due to a dry mouth and increased thirst after MDMA administration (18). Fluid consumption would be expected to decrease copeptin secretion (13), counteracting the effects of MDMA. However, copeptin levels were actually increased during the MDMA condition, which further supports the concept that MDMA activated the AVP system via pharmacological stimulation, although we cannot exclude an indirect effect via increased thirst perception (14). Furthermore, urine osmolality and urine sodium levels tended to be higher after MDMA compared with placebo administration despite the increase in oral fluid intake. This finding indicates that MDMA increased renal fluid retention, which is consistent with an elevated secretion of AVP.

The AVP response to MDMA in women was blocked by duloxetine pretreatment. Duloxetine prevents the transporter-mediated release of serotonin and norepinephrine by MDMA. Thus, MDMA-induced AVP secretion appears to be mediated by serotonin and norepinephrine. This clinical finding is in line with preclinical studies indicating a role for central serotonin (8) and norepinephrine (9) systems in AVP secretion. The mediating role of the serotonin system in AVP regulation is also supported by the fact that several serotonergic medications are typically associated with an increased risk of SIADH (22). The precise mechanism of the serotonin/norepinephrine-AVP system interaction is not known. AVP and copeptin are also hypothalamic stress hormones (27, 28), and MDMA is a pharmacological stressor. MDMA activates the hypothalamo-pituitary-adrenal axis and increases plasma corticotropin and cortisol (29, 30). In addition, MDMA increases aldosterone secretion in rats. Cortisol and mineralocorticoids also influence the electrolyte and body fluid balance. We did not assess the role of steroids in the present study. However, steroids increase renal sodium reabsorption and would thereby antagonize AVP effects on plasma osmolality.

In our study, MDMA (125 mg) had no effect on AVP or copeptin plasma levels in male subjects, whereas an earlier study showed an increase in AVP after a lower dose of MDMA (47.5 mg) in eight healthy men (5, 6). This discrepancy is likely due to differences in the study design and setting. Importantly, subjects were free to drink as much as they wanted in our study, and fluid consumption was higher after MDMA than after placebo which could have counteracted any MDMA effects on AVP secretion and even abolished any MDMA effects in men. In addition, our subjects were resting in hospital beds, eliminating any contributing effects of physical activity on AVP secretion. Nevertheless, it is surprising that our comparatively high dose of MDMA did not affect AVP or copeptin secretion despite pronounced subjective and cardiovascular stimulant effects of MDMA in the same subjects (Simmler, L. D., C. M. Hysek, J. Huwyler, M. E. Liechti, unpublished data). Interestingly, similar inconsistencies are seen in the clinical reports on ecstasy-associated hyponatremia. Hyponatremia was found in 55 (52.4%) of 105 women and 18 (21.7%) of 83 men in ecstasy exposures reported to the California Poison Control System (3). However, other reports indicate that hyponatremia is a relatively rare complication of ecstasy use. Ecstasy-associated hyponatremia was observed in only two (5%) of 40 monointoxications (31) or was not reported (32) according to other poison center studies. Hyponatremia was also a rare medical complication according to a series of intoxication cases presenting to emergency rooms (17, 33, 34). Taken together, the available data point toward an important role of additional contributing personal (sex, menstrual phase, and genetic factors) and/or environmental (heat and hydration) factors that may contribute and modulate the effects of MDMA on AVP secretion and osmotic regulation.

Our study has several limitations. The study sample size is relatively small. Only single doses of MDMA and duloxetine were used. However, the doses were selected in the upper dose range and produced pronounced effects on a variety of outcomes. Importantly, the absolute dose of MDMA was the same in both sexes and was not adjusted for body weight, resulting in higher relative doses of MDMA per kilogram of body weight in women compared with men. Thus, we cannot exclude that the observed sex difference was in fact a dose effect with women receiving higher relative doses of MDMA than men. However, relative MDMA doses did not correlate with MDMA-induced changes in copeptin levels within the male and female groups, supporting the view that our finding represents a true sex difference and not a dose effect. Furthermore, fluid consumption was different across treatment conditions, which may have counteracted effects of MDMA on AVP secretion because subjects consumed more liquids after MDMA than after placebo administration. Finally, urine osmolality and associated AVP system activation was higher in men than women at the beginning

of the study, which may have differentially affected the response to MDMA.

With regard to the validity of the outcome measures, we documented a correlation of plasma AVP and copeptin, confirming previous studies (12, 14). In addition, copeptin plasma concentrations also weakly correlated with plasma and urine osmolalities as expected based on osmoregulation and as previously documented in hypo-, iso-, and hyperosmolar states in healthy subjects (14). We also confirmed the previously reported sex differences in basal plasma copeptin concentration (12, 13).

In conclusion, we found that MDMA increased copeptin plasma levels reflecting AVP system stimulation in women but not in men. The finding is consistent with an increased risk for the development of hyponatremia and associated complications after recreational ecstasy use in women compared with men. AVP system activation by MDMA is likely due to the serotonin- and norepinephrine-releasing properties of MDMA.

Acknowledgments

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Paper Eight: Effects of MDMA on Emotion Recognition (Mind Reading)

MDMA Enhances "Mind Reading" of Positive Emotions and Impairs "Mind Reading" of Negative Emotions

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ORIGINAL INVESTIGATION

MDMA enhances "mind reading" of positive emotions and impairs "mind reading" of negative emotions

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Abstract
Rationale 3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) increases sociability. The prosocial effects of MDMA may result from the release of the "social hormone" oxytocin and associated alterations in the processing of socioemotional stimuli.
Materials and methods We investigated the effects of MDMA (125 mg) on the ability to infer the mental states of others from social cues of the eye region in the Reading the Mind in the Eyes Test. The study included 48 healthy volunteers (24 men, 24 women) and used a double-blind, placebo-controlled, within-subjects design. A choice reaction time test was used to exclude impairments in psychomotor function. We also measured circulating oxytocin and cortisol levels and subjective drug effects.
Results MDMA differentially affected mind reading depending on the emotional valence of the stimuli. MDMA enhanced the accuracy of mental state decoding for positive stimuli (e.g., friendly), impaired mind reading for negative stimuli (e.g., hostile), and had no effect on mind reading for neutral stimuli (e.g., reflective). MDMA did not affect psychomotor performance, increased circulating oxytocin and cortisol levels, and produced subjective prosocial effects, including feelings of being more open, talkative, and closer to others.
Conclusions The shift in the ability to correctly read socioemotional information toward stimuli associated with positive emotional valence, together with the prosocial feelings elicited by MDMA, may enhance social approach behavior and sociability when MDMA is used recreationally and facilitate therapeutic relationships in MDMA-assisted psychotherapeutic settings.

Keywords Emotion · MDMA · Oxytocin · Cortisol · Social cognition · Face recognition

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) increases empathic feelings and sociability (Bedi et al. 2009, 2010; Dumont et al. 2009). The prosocial effects of MDMA could result from the emotional interoceptive effects of the drug but also from the altered perception or processing of social signals. For example, acute administration of MDMA in ecstasy users decreased the accuracy of facial fear recognition (Bedi et al. 2010), attenuated responses to threatening faces in the amygdala (Bedi et al. 2009), and enhanced responses to happy expressions in the ventral striatum (Bedi et al. 2009). Thus, MDMA may increase sociability by reducing recognition and responses to threatening social stimuli and enhancing responses to rewarding stimuli. Here, we evaluated whether MDMA also alters the ability to identify more complex emotions assessed with the Reading the Mind in the Eyes Test (RMET).

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The neurochemical mechanisms that underlie the social effects of MDMA are largely unexplored. The social neuropeptide oxytocin is a key regulator of emotional and social behavior (Meyer-Lindenberg et al. 2011; Neumann 2008) and may mediate the social effects of MDMA. In fact, in rats, MDMA has been shown to activate oxytocin-containing neurons in the hypothalamus (Thompson et al. 2007), release oxytocin from the hypothalamus (Forsling et al. 2002), and increase plasma levels of oxytocin (Thompson et al. 2007). MDMA increased social interaction in male rats (Thompson et al. 2007, 2009), an effect blocked by intraventricular administration of an oxytocin receptor antagonist (Thompson et al. 2007). MDMA also elevated plasma concentrations of oxytocin in humans (Dumont et al. 2009; Wolff et al. 2006).

Oxytocin has been shown to improve mind reading in the RMET (Domes et al. 2007b; Guastella et al. 2010). MDMA releases oxytocin and may similarly improve performance in the RMET. However, in other tests, oxytocin selectively improved the recognition of happy facial expressions but impaired the decoding of negative facial expressions (Di Simplicio et al. 2009; Marsh et al. 2010). We therefore explored whether MDMA differentially interferes with the ability to decode complex emotions in the RMET depending on the emotional valence of the stimuli.

MDMA releases norepinephrine, serotonin, and dopamine from nerve terminals via their corresponding monoamine transporter (Rothman et al. 2001). To explore the mechanism of action of MDMA, we investigated the effects of three pretreatments on the response to MDMA. We used the norepinephrine transporter inhibitor reboxetine to block the MDMA-induced release of norepinephrine (Hysek et al. 2011). The dual serotonin and norepinephrine transporter inhibitor duloxetine was used to block the MDMA-induced release of both serotonin and norepinephrine (Simmler et al. 2011a). Clonidine was used to block any MDMA-induced transporter-independent vesicular release of norepinephrine (Hysek et al. 2012).

Materials and methods

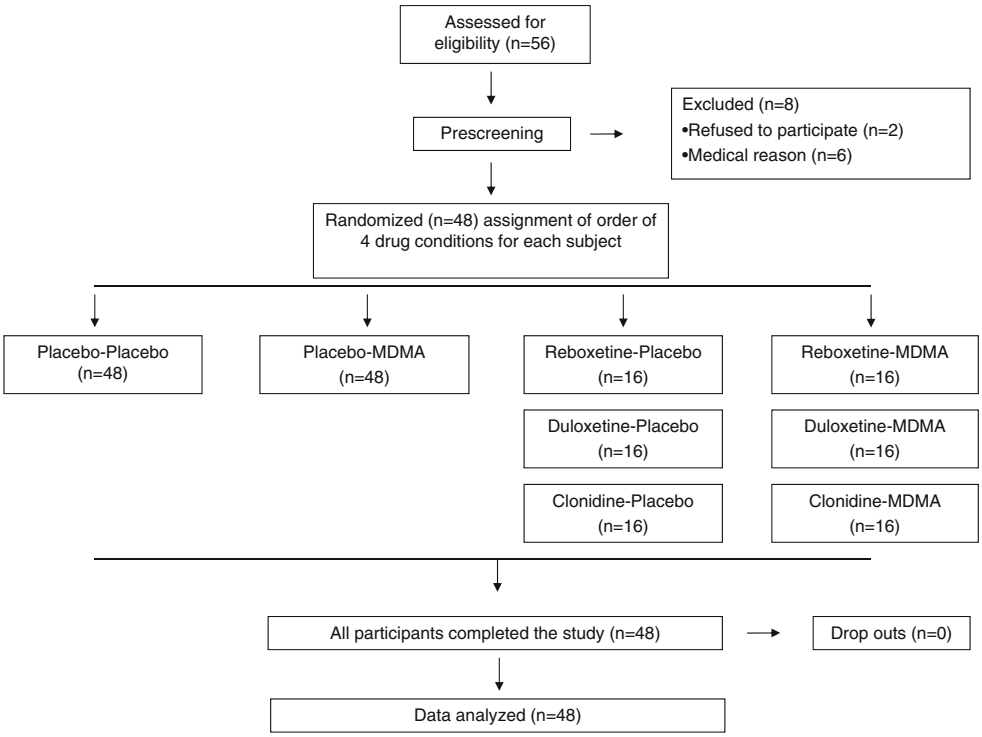
Study design

This was a prospectively designed pooled analysis of three double-blind, placebo-controlled, randomized, within-subjects studies (Hysek et al. 2011, 2012; Simmler et al. 2011a, b). The pre-specified primary endpoint of the pooled analysis was to demonstrate an effect of MDMA on RMET performance compared with placebo in 48 subjects. All subjects included in the three studies received MDMA, placebo, one of three different pretreatments prior to MDMA, or the pretreatment alone (Fig. 1). Thus, the four experiential conditions for all subjects were placebo-placebo, pretreatment-placebo, placebo-MDMA, and pretreatment-MDMA in balanced order. Of the 48 subjects, 16 (eight male, eight female) received the serotonin-norepinephrine transport inhibitor duloxetine as pretreatment, 16 (eight male, eight female) received the norepinephrine transport inhibitor reboxetine as pretreatment, and 16 (eight male, eight female) received the α_2 adrenergic receptor agonist clonidine as pretreatment. The random allocation sequence was developed by a clinical pharmacist and concealed from all individuals involved in study management. The washout periods between sessions were ≥10 days. The studies were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, and NCT01136278). Target sample size of the pooled study was based on the effects of oxytocin in the RMET in previous studies (Domes et al. 2007b; Guastella et al. 2010). The sample size of the individual studies was based on power analyses indicating that 13 subjects would be needed to detect a reduction of 20% in the subjective effects of MDMA (the primary outcome) by the pretreatments with more than 80% power using a within-subjects study design. Test sessions took place in a quiet hospital research ward with no more than two research subjects present per session.

Volunteers

Forty-eight healthy subjects (24 men, 24 women) aged 18 to 44 years (mean ± SD, 26 ± 5 years) and with a body weight of 68 ± 11 kg were recruited on the university campus. The exclusion criteria included the following: (1) age <18 or >45 years, pregnancy determined by a urine test before each test session; (2) body mass index <18.5 or >25 kg/m^2; (3) personal or family (first-degree relative) history of psychiatric disorder (determined by the structured clinical interview for axis I and axis II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Wittchen et al. 1997) supplemented by the SCL-90-R Symptom Checklist (Derogatis et al. 1976; Schmitz et al. 2000), Freiburg Personality Inventory (Fahrenberg et al. 1984), and Trait Scale of the State-Trait Anxiety Inventory (Spielberger et al. 1970); (4) the regular use of medications; (5) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematology, and chemical blood analyses; (6) smoking more than 10 cigarettes per day; (7) a lifetime history of using illicit drugs more than five times, with the exception of cannabis; (8) illicit drug use within the last 2 months; and (9) illicit drug use during the study determined by urine tests conducted before the test sessions using TRIAGE 8 (Biosite, San Diego, CA, USA). The subjects were asked to abstain from

Fig. 1 Study diagram



excessive alcohol consumption between test sessions and limit alcohol use to one glass on the day before each test session. All of the subjects were nonsmokers. Thirty-six subjects had previously used cannabis. Fourteen subjects reported using illicit drugs (one to four times). Four subjects had tried ecstasy, two had tried lysergic acid diethylamide, seven had tried psilocybin, four had tried cocaine, and one had tried amphetamine. Importantly, 44 subjects were MDMA-naive. Female subjects were investigated during the follicular phase (days 2–14) of their menstrual cycle to account for the potential confounding effects of sex hormones and cyclic changes in the reactivity to amphetamines (White et al. 2002). All of the subjects provided their written informed consent before participating in the study, and they were paid for their participation.

Measures

Reading the Mind in the Eyes Test (RMET)

The RMET (Baron-Cohen et al. 2001) was used to assess the identification of complex emotions 90 min after the administration of 125 mg MDMA or identical placebo. The RMET was originally developed to assess the social cognitive abilities of high functioning individuals with autism spectrum disorder (Baron-Cohen et al. 2001). In the RMET, 36 pictures of the eye region of faces are presented on a computer screen, and participants are asked to decide which of four words best describes what the person in the picture is thinking or feeling (Baron-Cohen et al. 2001). RMET scores are calculated as the total number of correct discriminations of all 36 items. Additionally, subscores in the present study were computed for positive (eight items), negative (12 items), and neutral (16 items) emotional valence as previously described (Harkness et al. 2005) and used by others (Fertuck et al. 2009).

Choice reaction time task (CRTT)

We used an adaptive five CRTT to assess potential drug effects on sustained attention and executive motor function (Schachinger et al. 2003). In this test, the subjects had to respond to the presentation of five different colored lights by pressing the button with the corresponding color as quickly and accurately as possible (Schachinger et al. 2003). A training run was performed before the first baseline assessment and data were analyzed as drug-induced changes from baseline to correct for training effects (Haschke et al. 2010). The CRTT was performed before and 120 min after administration of MDMA or placebo in 32 subjects. The task is sensitive to benzodiazepine administration (Haschke et al. 2010).

Endocrine measures

Blood samples for the determination of plasma oxytocin and cortisol levels were collected in 32 and 48 subjects before and 120 min after drug administration, respectively. Plasma oxytocin concentrations were determined using a radioimmunoassay

in the Neurobiology Department (Inga D. Neumann), University of Regensburg, Germany, as previously described (Landgraf et al. 1995). Plasma cortisol concentrations were determined using an automated solid-phase chemiluminescence immunoassay (Immulite 2000 Cortisol, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA).

Subjective effects

Subjective effects were assessed using the Addiction Research Center Inventory (ARCI) (Martin et al. 1971) and visual analog scales (VASs) (Hysek et al. 2011). The ARCI is a true–false questionnaire with five empirically derived scales (Martin et al. 1971). The Amphetamine scale is sensitive to the effects of d-amphetamine, the Benzedrine Group scale is a stimulant scale consisting mainly of items relating to intellectual efficiency and energy, the Morphine-Benzedrine Group scale is a measure of euphoria, the Pentobarbital-Chlorpromazine-Alcohol Group scale is a measure of sedation, and the Lysergic Acid Diethylamine Group scale is a measure of dysphoria and somatic symptoms. The ARCI has previously been shown to be sensitive to the effects of MDMA (Farre et al. 2007; Tancer and Johanson 2007). The ARCI was used in its validated German version (Bopp et al. 2005) before and 2.5 and 5 h after drug administration. Visual analog scores were used to assess "any drug effects" and prosocial effects, including "closeness to others," "open," and "talkative." VASs were presented as 100 mm horizontal lines marked from "not at all" on the left to "extremely" on the right. VASs assessing prosocial feelings were bidirectional (±50 mm). VAS scores were assessed before and 0, 0.33, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, and 6 h after drug administration. The study included additional pharmacodynamic and pharmacokinetic outcomes as reported elsewhere (Hysek et al. 2011, 2012; Simmler et al. 2011a, b). All outcome measures were assessed identically and at the same time points following MDMA or placebo administration across all three studies.

Drugs

(±) MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was obtained from the Swiss Federal Office of Public Health and prepared as gelatin capsules (100 and 25 mg). Identical placebo (lactose) capsules were prepared. MDMA was administered in a single absolute oral dose of 125 mg. This dose of MDMA corresponds to a typical recreational dose or the dose of MDMA used as an adjunct to psychotherapy (Mithoefer et al. 2010). In the reboxetine-MDMA study, reboxetine (8 mg, Edronax; Pfizer, Zurich, Switzerland) or identical placebo (lactose) was administered at 20:00 hours the day before the test session and again at 7:00 hours on the test day. MDMA or placebo was administered at 8:00 hours, 1 and 12 h after reboxetine. In the duloxetine-MDMA study, duloxetine (120 mg, Cymbalta, Eli Lilly, Vernier, Switzerland) or identical placebo (lactose) was administered at 20:00 hours the day before the test session and again at 8:00 hours on the test day. MDMA or placebo was administered at 12:00 hours, 4 and 16 h after duloxetine. Reboxetine and duloxetine were administered twice in high doses to obtain plasma concentrations similar to those reached with chronic daily administrations of the drugs and as previously used to manipulate the norepinephrine function in healthy subjects (Roelands et al. 2008). In the clonidine-MDMA study, clonidine (150 μg, Catapresan; Boeringer Ingelheim, Basel, Switzerland) or identical placebo (lactose) was administered at 8:00 hours, 1 h before MDMA or placebo (9:00 hours). Clonidine has previously been shown to produce sympatholytic effects in this dose in healthy subjects (Anavekar et al. 1982; Nieuwenhuis et al. 2007). The pretreatment times used for the three drugs resulted in maximal plasma concentrations of the pretreatments at the time of the maximal effect of MDMA (Hysek et al. 2011, 2012). On the test days, oral drug administration was supervised by study personnel. Compliance with the first administration of reboxetine and duloxetine in the evening prior to the test day was confirmed analytically in plasma (Hysek et al. 2011; Simmler et al. 2011a).

Statistical analyses

For the statistical analyses, data from the three studies were pooled, and endocrine measures and reaction times were transformed to differences from baseline. Peak effects (E_{max}) were determined for repeated measures. E_{max} values and RMET scores were compared using one-way General Linear Model repeated-measures analysis of variance (ANOVA) with drug (MDMA vs. placebo) as a factor using STATISTICA 6.0 (StatSoft, Tulsa, OK, USA). Data from the three substudies on all four treatment conditions were assessed using ANOVAs, with drug (placebo-placebo, placebo-MDMA, pretreatment-placebo, and pretreatment-MDMA) as a factor, followed by the Tukey post hoc test. Sequence effects were tested by including treatment order as a factor. Potential associations between MDMA-induced endocrine changes and subjective effects or RMET accuracy were assessed using Spearman's rank correlations. The criterion for significance was $p<0.05$.

Results

RMET

As shown in Fig. 2, MDMA improved mind reading performance in the RMET for stimuli with a positive emotional

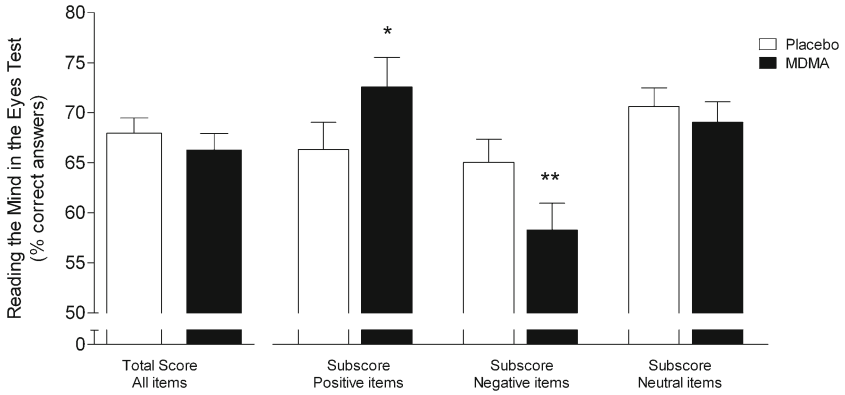


Fig. 2 MDMA had differential effects on performance in the Reading the Mind in the Eyes Test (RMET) depending on the emotional valence of the stimuli. MDMA increased the ability in affective mind reading for expressions with a positive emotional valence (positive items, *$p<0.05$) and impaired mind reading for negative items (**$p<0.01$) compared to placebo. MDMA did not alter performance for neutral items or the total score (all items). Values are mean ± SEM accuracy (percentage of correct items) in 48 subjects

valence ($F_{1,\ 47}=5.13$, $p<0.05$) and impaired performance for stimuli with a negative emotional valence ($F_{1,\ 47}=7.05$, $p<0.01$). Improvements in reading positive emotions were seen in 40 of the 48 participants, and impairments in reading negative emotions were seen in 38 of the 48 participants. MDMA had no effect on the accuracy of mind reading for emotionally neutral stimuli or the total performance score. There were no sex differences. No statistically significant main effects of sequence and no sequence×drug interaction were found, excluding sequence effects of treatment on test performance. Drug effects on the RMET in each of the three studies are shown in Tables 2, 3, and 4. MDMA consistently exerted similar effects on mind reading in each of the three studies as in the pooled analysis, but the effects did not reach statistical significance. Duloxetine nonsignificantly attenuated the effects of MDMA on RMET performance. Similar weak and nonsignificant reductions of the MDMA effect were also observed for reboxetine and clonidine.

CRTT

MDMA did not alter reaction time in the CRTT (Table 1) or the RMET ($F_{1,\ 47}=1.8$, p=NS). In the individual studies, none of the drugs altered reaction time in the CRTT (Tables 2, 3 and 4).

Endocrine effects

MDMA increased plasma levels of oxytocin ($F_{1,\ 31}=8.00$, $p<0.01$) and cortisol ($F_{1,\ 47}=110$, $p<0.001$) compared with placebo (Table 1). In the duloxetine-MDMA study sample, duloxetine reduced the MDMA-induced increase in plasma levels of oxytocin and cortisol (Table 3). Neither reboxetine nor clonidine significantly affected the endocrine effects of MDMA (Tables 2 and 4).

Subjective effects

MDMA increased scores on the Amphetamine Group, Benzedrine Group, Morphine-Benzedrine Group, Pentobarbital-Chlorpromazine-Alcohol Group, and LSD Group scales of the ARCI compared with placebo ($F_{1,\ 47}=36.4$, 5.1, 44.7, 36.4, and 15.2, respectively; all $p<0.001$, with the exception of the Benzedrine Group [$p<0.05$]; Table 1). MDMA also increased VAS scores for "any drug effect," "closeness," "open," and "talkative" ($F_{1,\ 47}=1183$, 98.0, 105, and 105, respectively; all $p<0.001$; Table 1). The endocrine effects of MDMA were not associated with the subjective effects of MDMA or performance on the RMET (all $r_s<0.28$, all $p>0.1$). Duloxetine reduced MDMA-induced increases in all VAS scores (Table 3). Duloxetine also reduced the effect of MDMA on the Amphetamine and Morphine-Benzedrine Group scales, which were the only scales that showed significant effects of MDMA in the ARCI in the duloxetine-MDMA study (Table 3). In the reboxetine-MDMA study, reboxetine lowered MDMA-induced increases in the VAS scores for "any drug effects" and "closeness" (Table 2). The effects of MDMA on all subscales of the ARCI were nonsignificantly lower after reboxetine administration. In contrast, clonidine had no effect on the subjective response to MDMA in the clonidine-MDMA study (Table 4). No severe adverse effects were reported.

Discussion

The main finding of the present study was that MDMA improved performance on the RMET for positive stimuli and impaired performance for negative stimuli, indicating that MDMA differentially affected the ability to correctly decode social facial stimuli depending on the emotional valence of the

Table 1 Mean ± SEM values for endocrine, psychomotor, and subjective effects of MDMA (n=48)

	Placebo	MDMA
Hormones		
Oxytocin (Δ pg/mL)	5.0±3.9	28.1±4.0**
Cortisol (Δ nmol/L)	−262.0±27.1	174.5±29.8***
Choice reaction time test (CRTT)		
Reaction time (Δ ms)	4.6±3.7	−1.7±6.1
Addiction Research Inventory (ARCI)		
Amphetamine	−0.1±0.1	2.4±0.4***
Benzedrine Group	0.6±0.18	1.3±0.3*
Morphine-Benzedrine Group	0.2±0.2	5.5±0.8***
Pentobarbital-Chlorpromazine-Alcohol Group	0.2±0.2	3.0±0.5***
LSD Group	0.5±0.2	2.1±0.4***
Visual analog scales		
Any drug effect	2.2±1.3	87.2±2.4***
Closeness	0.2±0.2	28.2±2.8***
Open	0.8±0.4	30.9±2.4***
Talkative	0.6±0.3	26.7±2.6***

*p<0.05, **p<0.01, ***p<0.001 compared to placebo

stimulus. In a party setting, the use of MDMA may therefore improve the correct reading of positive facial expressions and, combined with elevated mood and extroversion, may lead to higher approach behavior and sociability. In contrast, the misreading of negative social information as being more neutral or positive may result in higher social risk behavior. When

Table 2 Mean ± SEM values and statistics for the reboxetine-MDMA study (n=16)

	Placebo-Placebo	Reboxetine-Placebo	Placebo-MDMA	Reboxetine-MDMA	$F_{3,\ 45}$ =	p <
Hormones						
Oxytocin (Δ pg/mL)	NA	NA	NA	NA		
Cortisol (Δ nmol/L)	−203±46	−230±60$^{\#\#\#}$	245±47***	144±60***	21.86	0.001
Reading the Mind in the Eyes Test						
Total score	0.679±0.03	0.679±0.03	0.649±0.03	0.684±0.03	0.99	NS
Positive items	0.719±0.04	0.688±0.04	0.734±0.06	0.727±0.04	0.46	NS
Negative items	0.630±0.06	0.599±0.05	0.552±0.05	0.615±0.04	1.44	NS
Neutral items	0.695±0.04	0.734±0.04	0.680±0.04	0.715±0.04	1.19	NS
Choice reaction time task						
Mean reaction time (ms)	4.5±5.7	16.1±8.5	0.42±13.3	21.9±10.1	1.23	NS
Addiction Research Center Inventory						
Amphetamine	0.2±0.1	0.4±0.3$^{\#\#}$	4.1±0.9***	3.3±0.6**	12.04	0.001
Benzedrine Group	1.1±0.4	0.7±0.3$^{\#}$	2.5±0.4	1.7±0.5	5.74	0.05
Morphine-Benzedrine Group	0.4±0.2	0.7±0.5$^{\#\#\#}$	8.4±1.3***	5.4±1.1**	19.33	0.001
Pentobarbital-Chlorpromazine-Alcohol Group	0.6±0.2	1.4±0.6$^{\#\#}$	4.4±0.9***	3.1±0.7**	9.18	0.001
LSD Group	0.7±0.2	0.9±0.3$^{\#\#\#}$	4.3±0.8***	2.8±0.6*	10.4	0.001
Visual analog scales						
Any drug effect	1.9±1.3	8.0±3.4	85±4.8***	68±6.2***$^{,\#\#}$	120.40	0.001
Closeness	0.3±0.2	0.0±0.0$^{\#\#\#}$	34±5.9***	21±4.5***$^{,\#}$	22.66	0.001
Open	1.0±0.8	4.2±2.2$^{\#\#\#}$	30±3.1***	23±4.9	22.73	0.001
Talkative	0.5±0.4	2.2±1.3$^{\#\#\#}$	26±4.3***	20±5.1	18.56	0.001

NA not assessed, *NS* not significant

*p<0.05, **p<0.01, ***p<0.001, compared with Placebo-Placebo; $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001, compared with Placebo-MDMA

Table 3 Mean ± SEM values and statistics for the duloxetine-MDMA study (n=16)

	Placebo-Placebo	Duloxetine-Placebo	Placebo-MDMA	Duloxetine-MDMA	$F_{3, 45}$ =	p <
Hormones						
Oxytocin (Δ pg/mL)	1.4±7.1	1.6±4.7	22.2±9.1*	1.8±5.9$^{\#}$	4.56	0.01
Cortisol (Δ nmol/L)	−354±46	−241±30$^{\#\#\#}$	157±62***	−181±29*,$^{\#\#\#}$	25.65	0.001
Reading the Mind in the Eyes Test						
Total score	0.665±0.02	0.661±0.02	0.656±0.02	0.689±0.02	1.28	NS
Positive items	0.656±0.05	0.680±0.04	0.750±0.05	0.711±0.04	1.71	NS
Negative items	0.630±0.02	0.661±0.03	0.578±0.04	0.667±0.03	2.26	NS
Neutral items	0.695±0.02	0.641±0.04	0.668±0.05	0.695±0.04	0.70	NS
Choice reaction time task						
Mean reaction time (ms)	4.7±7.1	3.4±32	−3.8±30	4.5±30	0.28	NS
Addiction Research Center Inventory						
Amphetamine	−0.1±0.2	−0.3±0.2	4.6±0.5***	0.9±0.5$^{\#\#\#}$	35.13	0.001
Benzedrine Group	0.6±0.2	−0.1±0.3	1.4±0.4	−0.2±0.5$^{\#}$	4.07	0.05
Morphine-Benzedrine Group	0.5±0.5	0.6±0.2	8.4±0.9***	2.3±0.8$^{\#\#\#}$	41.74	0.001
Pentobarbital-Chlorpromazine-Alcohol Group	0.1±0.4	1.9±0.5	3.1±0.8	2.3±0.9	3.92	0.05
LSD Group	0.8±0.2	0.1±0.3	0.6±0.5	0.9±0.5	0.84	NS
Visual analog scales						
Any drug effect	3.8±3.6	6.0±2.5	86.7±3.6***	33±8***,$^{\#\#\#}$	74.47	0.001
Closeness	0.0±0.0	0.0±0.0	27.3±3.9***	4.6±2.5$^{\#\#\#}$	37.32	0.001
Open	1.4±0.9	0.4±0.4	32.2±4.3***	6.0±3.3$^{\#\#\#}$	36.88	0.001
Talkative	1.2±0.8	0.3±0.3	28.8±5.1***	10.7±3.7$^{\#\#\#}$	21.13	0.001

NA not assessed, *NS* not significant

*p<0.05, **p<0.01, ***p<0.001, compared with Placebo-Placebo; $^{\#}$$p$<0.05, $^{\#\#}$$p$<0.01, $^{\#\#\#}$$p$<0.001, compared with Placebo-MDMA

MDMA is administered during psychotherapy to treat post-traumatic stress disorder (Mithoefer et al. 2010), the MDMA-induced shift in accuracy toward a better perception of positive emotional stimuli may facilitate the therapeutic alliance (Johansen and Krebs 2009).

MDMA did not affect total RMET score or the decoding of stimuli with neutral emotional valence. Thus, MDMA did not improve mind reading overall. Our finding in mostly non-ecstasy-experienced volunteers is consistent with a previous work, in which MDMA did not alter performance on the RMET in 21 ecstasy users (Bedi et al. 2010). The latter study did not evaluate whether emotional valence modulates the effect of MDMA on the RMET. However, in another test in the same study, MDMA differentially reduced the accurate identification of negative, threat-related facial signals but did not affect the identification of neutral or positive emotions (Bedi et al. 2010). The emotion-specific effect of MDMA on the decoding of facial expressions suggests that MDMA may differentially affect brain areas involved in the processing of emotional information. Indeed, functional magnetic resonance imaging showed that MDMA attenuated the response to angry faces in the amygdala, a structure activated by negative social signals and fear (Zald 2003), and enhanced the response to happy faces in the ventral striatum (Bedi et al. 2009), a structure activated by reward expectation (Knutson and Cooper 2005). Altogether, the data indicate that MDMA lowers reactivity to negative social stimuli, such as threat, and enhances responding to positive social stimuli, such as a smile.

We found that MDMA increased plasma levels of oxytocin, confirming a placebo-controlled MDMA study (Dumont et al. 2009) and observations in clubbers following the use of ecstasy pills (Wolff et al. 2006). Oxytocin is a candidate for the mediation of the empathic and social effects of MDMA (Thompson et al. 2007). For example, MDMA increased social interaction in rats that interacted for the first time, predominantly reflected by an increase in adjacent lying behavior. This effect of MDMA was reduced by pretreatment with an oxytocin antagonist (Thompson et al. 2007). Similar to MDMA, oxytocin also reduced activation of the amygdala in response to threatening social stimuli (Kirsch et al. 2005), although other work showed that oxytocin reduced amygdala responses regardless of the emotional valence of the facial stimuli (Domes et al. 2007a) in men and enhanced amygdala responses to fearful stimuli in women (Domes et al. 2010), suggesting both sex differences and more complex effects of oxytocin on emotion processing. Particularly relevant for the present study, intranasal oxytocin administration improved performance on the RMET in healthy male subjects (Domes

Table 4 Mean ± SEM values and statistics for the clonidine-MDMA study (n=16)

	Placebo-Placebo	Clonidine-Placebo	Placebo-MDMA	Clonidine-MDMA	$F_{3, 45}$ =	p <
Hormones						
Oxytocin (Δ pg/mL)	9.2±6.3	12.1±12.4	33.9±3.4*	20.2±5.5	3.32	0.05
Cortisol (Δ nmol/L)	−229±42	−241±43	122±42	190±36***	37.02	0.001
Reading the Mind in the Eyes Test						
Total score	0.69±0.03	0.698±0.02	0.684±0.03	0.698±0.03	0.16	NS
Positive items	0.62±0.05	0.641±0.04	0.695±0.05	0.656±0.05	0.88	NS
Negative items	0.69±0.04	0.641±0.04	0.620±0.05	0.661±0.04	1.12	NS
Neutral items	0.73±0.04	0.770±0.02	0.727±0.04	0.746±0.03	0.54	NS
Choice reaction time task						
Mean reaction time (ms)	NA	NA	NA	NA		
Addiction Research Center Inventory						
Amphetamine	−0.4±0.2	−0.5±0.3$^{###}$	3.3±0.4***	3.3±0.5***	37.11	0.001
Benzedrine Group	0.1±0.2	−1.5±0.5$^{##}$	0.7±0.6	0.5±0.4	5.36	0.01
Morphine-Benzedrine Group	−0.4±0.4	−0.9±0.5$^{###}$	7.7±1.0***	7.4±1.0***	46.12	0.001
Pentobarbital-Chlorpromazine-Alcohol Group	0.1±0.4	2.7±0.7	4.6±0.7***	4.0±0.8***	11.08	0.001
LSD Group	0.1±0.2	0.6±0.2	1.4±0.7	1.7±0.4*	3.84	0.05
Visual analog scales						
Any drug effect	0.9±0.9	16.8±6.2$^{###}$	89.6±4.0***	81.6±6.9***	87.55	0.001
Closeness	0.4±0.4	0.0±0.0$^{###}$	23.4±4.6***	24.1±4.7***	20.35	0.001
Open	0.0±0.0	0.0±0.0$^{###}$	30.3±4.4***	30.2±5.0***	33.71	0.001
Talkative	0.0±0.0	0.0±0.0$^{###}$	24.8±4.4***	24.7±4.5***	24.26	0.001

NA not assessed, *NS* not significant

*p<0.05, **p<0.01, ***p<0.001, compared with Placebo-Placebo; $^{#}$$p$<0.05, $^{##}$$p$<0.01, $^{###}$$p$<0.001, compared with Placebo-MDMA

et al. 2007b) or male subjects with autism spectrum disorders (Guastella et al. 2010). The effect of MDMA on the decoding of positive emotional information in the present study might therefore be explained by the oxytocinergic properties of MDMA. Notably, oxytocin selectively improved the recognition of specific emotions in previous studies, similar to MDMA in the present study. Specifically, oxytocin selectively enhanced the recognition of happy facial expressions (Marsh et al. 2010; Schulze et al. 2011), reduced misclassifications of positive or ambiguous emotions as negative emotions (Di Simplicio et al. 2009), increased the memory for positive faces (Guastella et al. 2008), and slowed reaction times during the recognition of negative fearful facial expressions (Di Simplicio et al. 2009). Altogether, these data support the hypothesis that the effects of MDMA on mind reading are very similar to those of oxytocin and are potentially mediated by this neuropeptide. MDMA-induced increases in the plasma concentration of oxytocin were not correlated with RMET performance in our study. However, plasma samples were not available for all subjects of the study and it is also unclear whether plasma concentrations of oxytocin reflect brain concentrations of this neuropeptide.

The administration of oxytocin in humans does not produce subjective mood effects. However, a drug discrimination study showed that rats trained to respond for MDMA also responded if MDMA was substituted by the oxytocin receptor agonist carbetocin, and responding for MDMA was reduced by administration of the oxytocin receptor antagonist atosiban (Broadbear et al. 2011). Oxytocin may therefore contribute to the interoceptive subjective effects of MDMA. Whether the subjective state of positive feelings and closeness to others elicited by MDMA in humans is also associated with increased emotional empathy (i.e., the sharing of experiences of emotional states perceived in others) remains to be tested. The finding that MDMA did not improve overall performance on the RMET in the present study and a previous study (Bedi et al. 2010) and the lack of improved face or vocal affect recognition (Bedi et al. 2010) suggest that MDMA does not improve cognitive empathy overall (i.e., the recognition of emotional states in others). Oxytocin has recently been shown to increase emotional but not cognitive empathy in healthy male volunteers (Hurlemann et al. 2010). We did not assess the effects of MDMA on emotional empathy. Studies on the effects of MDMA on different measures of emotional and cognitive empathy are needed.

In the present study, MDMA also increased plasma levels of cortisol, consistent with previous studies (Harris et al.

2002; Mas et al. 1999). We did not observe any association between cortisol levels and RMET performance, and high stress- compared with low stress-induced cortisol elevations in healthy subjects did not alter RMET scores in another study (Smeets et al. 2009).

In the ARCI, MDMA produced moderate amphetamine-type effects with only slight stimulation, pronounced euphoria, as well as moderate alcohol-like and moderate hallucinogen-like effects similar to earlier works (Farre et al. 2007; Tancer and Johanson 2007). In the VAS, MDMA produced its MDMA-typical "entactogenic" effects including closeness to others, openness, and talkativeness as described earlier (Hysek et al. 2011; Liechti et al. 2001). We also assessed the effects of different pretreatments on the response to MDMA. Duloxetine, which inhibits MDMA-induced monoamine transporter-dependent serotonin and norepinephrine release (Simmler et al. 2011a, b), reduced all the amphetamine-type and euphorigenic psychotropic effects of MDMA in the ARCI, the entactogen-like aspects of the MDMA response in all the VAS, and also endocrine effects of MDMA. Duloxetine also tended to attenuate the effects of MDMA on RMET, although these trends were not statistically significant. Reboxetine, which inhibits MDMA-induced norepinephrine release (Hysek et al. 2011), reduced some of the psychotropic effects in the VAS but not the endocrine effects of MDMA. Clonidine, which inhibits any MDMA-induced transporter-independent vesicular release of norepinephrine (Hysek et al. 2012), had no effect on either the subjective or endocrine response to MDMA. The finding that inhibition of the MDMA-induced serotonin and norepinephrine release by duloxetine was more effective in reducing the acute MDMA effects in humans than inhibition of the release of norepinephrine alone by reboxetine or clonidine suggests that serotonin may be primarily responsible for the acute effects of MDMA in humans. This view is also consistent with earlier mechanistic studies in humans (Farre et al. 2007; Liechti et al. 2000; Liechti and Vollenweider 2000; Tancer and Johanson 2007). The data also indicate a primary role for serotonin in the effects of MDMA on oxytocin release, emotion identification, and MDMA's potential prosocial effects.

In conclusion, the MDMA-induced shift in the ability to detect socioemotional information, together with the prosocial feelings elicited by MDMA, is likely to enhance social approach behavior and sociability when MDMA is used recreationally. The change in the processing of emotional information may also facilitate therapeutic relationships in MDMA-assisted psychotherapy.

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Paper Nine: Effects of MDMA on Pupillary Function

Effects of MDMA on the pupillary light reflex alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin

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ORIGINAL INVESTIGATION

Effects of MDMA alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex

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Abstract
Rationale Pupillometry can be used to characterize autonomic drug effects.
Objective This study was conducted to determine the autonomic effects of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), administered alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin, on pupillary function.
Methods Infrared pupillometry was performed in five placebo-controlled randomized studies. Each study included 16 healthy subjects (eight men, eight women) who received placebo–MDMA (125 mg), placebo–placebo, pretreatment–placebo, or pretreatment–MDMA using a crossover design.
Results MDMA produced mydriasis, prolonged the latency, reduced the response to light, and shortened the recovery time. The impaired reflex response was associated with subjective, cardiostimulant, and hyperthermic drug effects and returned to normal within 6 h after MDMA administration when plasma MDMA levels were still high. Mydriasis was associated with changes in plasma MDMA concentration over time and longer-lasting. Both reboxetine and duloxetine interacted with the effects of MDMA on pupillary function. Clonidine did not significantly reduce the mydriatic effects of MDMA, although it produced miosis when administered alone. Carvedilol and doxazosin did not alter the effects of MDMA on pupillary function.
Conclusions The MDMA-induced prolongation of the latency to and reduction of light-induced miosis indicate indirect central parasympathetic inhibition, and the faster recovery time reflects an increased sympathomimetic action. Both norepinephrine and serotonin mediate the effects of MDMA on pupillary function. Although mydriasis is lasting and mirrors the plasma concentration–time curve of MDMA, the impairment in the reaction to light is associated with the subjective and other autonomic effects of MDMA and exhibits acute tolerance.

Keywords Pupil · Pupillary reflex · Pupillometry · MDMA · Norepinephrine · Serotonin

Introduction

3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) induces the transporter-mediated release of serotonin and norepinephrine (Liechti and Vollenweider 2001; Rothman et al. 2001; Verrico et al. 2007) and produces cardiostimulant and psychostimulant effects in humans (Hysek et al. 2011). The autonomic sympathomimetic effects of MDMA in humans include increases in blood pressure, heart rate, body temperature, and pupil diameter (Farre et al. 2004, 2007; Hysek et al. 2012c; Kolbrich et al. 2008; Liechti et al. 2001; Mas et al. 1999). Pupil size and the response to a flashlight stimulus are typically assessed in the evaluation of intoxicated patients. Mydriasis is a clinical hallmark of sympathomimetic toxicity in cases of ecstasy or cocaine use. Laboratory studies have also shown an increase in pupil diameter after MDMA administration (Farre et al. 2004, 2007; Kolbrich et al. 2008; Mas et al. 1999). However, whether MDMA alters the pupillary light reflex response and how pupillary changes are linked to MDMA exposure and other pharmacodynamic effects of the drug are unknown. Additionally, the pharmacological mechanism by which

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MDMA produces mydriasis and the potential changes in pupillary function are unclear. Mydriasis and alterations in the pupillary light reflex may result from increased sympathetic activity, the release of norepinephrine, and α_1-adrenergic receptor stimulation directly in the iris or from a decrease in parasympathetic activity (Loewenfeld 1999). At the level of the iris, the latency to the light reflex and miotic response to light are thought to reflect parasympathetic activation (Heller et al. 1990; Loewenfeld 1999), whereas redilation is considered to mainly reflect sympathetic activation (Loewenfeld 1999; Morley et al. 1991). Notably, the parasympathetic input to the pupil may also be inhibited centrally via α_2-adrenergic receptors in the Edinger–Westphal nucleus by an increase in sympathetic activity (Phillips et al. 2000a; Siepmann et al. 2007; Szabadi and Bradshaw 1996). Furthermore, the serotonin system has been shown to indirectly influence pupillary function, possibly by enhancing sympathetic activity (Prow et al. 1996). Therefore, the MDMA-induced release of norepinephrine in the periphery may stimulate α_1-adrenergic receptors in the iris or inhibit parasympathetic activity via central α_2-adrenergic receptors in the Edinger–Westphal nucleus. The adrenergic mechanisms may be further enhanced by the potent MDMA-induced release of serotonin. To explore the mechanism of action of MDMA on pupillary function, we investigated the effects of five pretreatments on the response to MDMA. We used the norepinephrine transporter inhibitor reboxetine to block the transporter-mediated, MDMA-induced release of norepinephrine (Hysek et al. 2011; i.e., the indirect sympathomimetic effect of MDMA). The serotonin and norepinephrine transporter inhibitor duloxetine was similarly used to block the MDMA-induced, transporter-mediated release of both serotonin and norepinephrine (Simmler et al. 2011). The α_2-adrenergic agonist clonidine was used as a sympathicolytic to inhibit the transporter-independent vesicular release of norepinephrine (Hysek et al. 2012a). Carvedilol and doxazosin were used to block postsynaptic $\alpha_1\beta_{1-3}$- and α_1-adrenergic receptors, respectively (Hysek et al. 2012c; i.e., to directly antagonize the effects of norepinephrine in the iris, on the cardiovascular system, and on body temperature). The series of studies included additional outcome measures presented elsewhere (Hysek et al. 2011, 2012a, b, d; Simmler et al. 2011).

Material and methods

Study design

This was a pooled analysis of five double-blind, double-dummy, placebo-controlled, randomized, crossover studies (Hysek et al. 2011, 2012a, b, d; Simmler et al. 2011). The primary aim of the pooled analysis was to assess the effects of MDMA on pupil size and pupillary light reflex compared with placebo in all 80 subjects and to explore associations with the pharmacokinetics of MDMA and other pharmacodynamic measures. All of the subjects included in the five studies received MDMA, placebo, one of five different pretreatments prior to MDMA, or the pretreatment alone (Fig. 1). Thus, the four experiential conditions for all of the subjects were placebo–placebo, pretreatment–placebo, placebo–MDMA, and pretreatment–MDMA in a balanced order. Each of the five studies included 16 subjects (eight male, eight female). The pretreatments used in the five studies were reboxetine, duloxetine, clonidine, carvedilol, and doxazosin. The random allocation sequence was developed by a clinical pharmacist and concealed from all of the individuals involved in the study management. The washout periods between sessions were ≥10 days. The studies were conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines on Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland. The use of MDMA in healthy subjects was authorized by the Swiss Federal Office of Public Health, Bern, Switzerland. The studies were registered at ClinicalTrials.gov (NCT00886886, NCT00990067, NCT01136278, NCT01270672, and NCT01386177).

Participants

Eighty healthy subjects (40 men and 40 women) aged 18 to 44 years (mean ± SD, 25±5 years) were recruited on the university campus. The exclusion criteria included the following: (1) age <18 or >45 years, (2) pregnancy determined by a urine test before each test session, (3) body mass index <18.5 or >25 kg/m^2, (4) personal or family (first-degree relative) history of psychiatric disorder [determined by the structured clinical interview for axis I and axis II disorders according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (Wittchen et al. 1997), supplemented by the SCL-90-R Symptom Checklist (Derogatis et al. 1976; Schmitz et al. 2000)], (5) the regular use of medications, (6) chronic or acute physical illness assessed by physical examination, electrocardiogram, standard hematology, and chemical blood analyses, (7) smoking more than 10 cigarettes per day, (8) a lifetime history of using illicit drugs more than five times, with the exception of cannabis, (9) illicit drug use within the last 2 months, and (10) illicit drug use during the study determined by urine tests conducted before the test sessions using TRIAGE 8 (Biosite, San Diego, CA, USA). The subjects were asked to abstain from excessive alcohol consumption between test sessions and limit alcohol use to one drink on the day before each test session. Eight of the 80 subjects had previously tried ecstasy (one to two times). Female subjects were investigated during the follicular phase (day

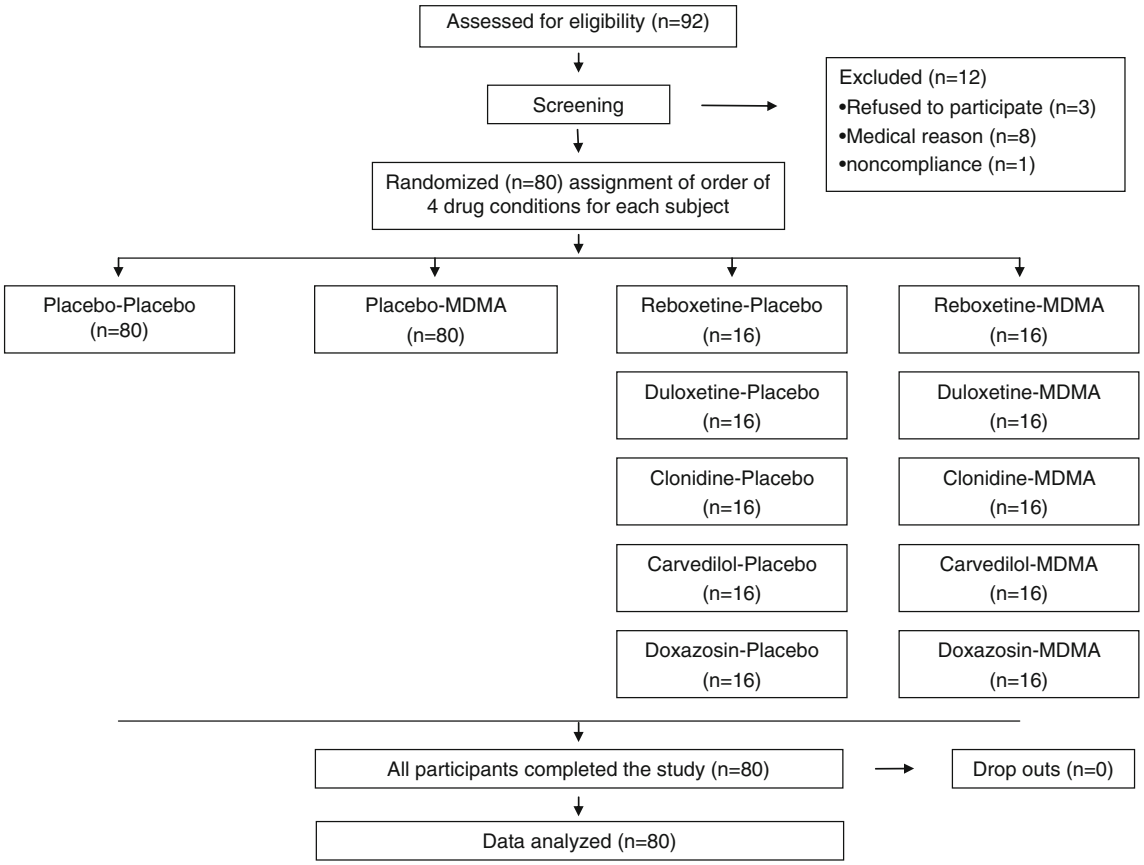

Fig. 1 Study diagram

2–14) of their menstrual cycle to account for the potential confounding effects of sex hormones and cyclic changes in the reactivity to amphetamines (White et al. 2002). All of the subjects provided their written informed consent before participating in the study, and they were paid for their participation.

Measures

Pupillometry

Pupillometry was performed 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. Pupil function was measured under standardized dark–light conditions of 5.7±0.8 lx assessed by a Voltcraft MS-1300 lux meter (Voltcraft, Hirschau, Germany) following a dark adaption time of 1 min. Pupillometry was performed using a handheld PRL-200 infrared pupillometer (NeurOptics, Irvine, CA, USA; Taylor et al. 2003). The subjects were instructed to focus on a black dot on a white wall at a distance of 4 m. After a 10-s focusing period, measurements were taken for 5 s. During this time frame, the following parameters were assessed: dark-adapted pupil diameter (MAX), minimal pupil diameter after a light stimulus (MIN), and latency to the pupillary light reflex (Fig. 2). The constriction amplitude was calculated as MAX−MIN. The time taken by the pupil to recover 75 % of the initial resting pupil size after it reached constriction

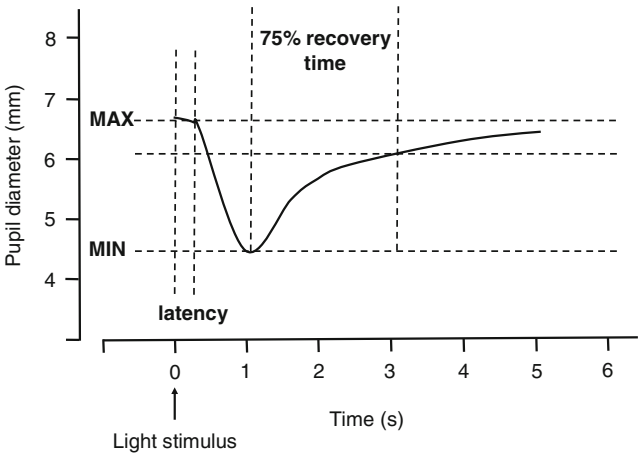

Fig. 2 Schematic drawing of the light reflex response. *MAX* represents the dark-adapted resting pupil size before the light stimulus. *Latency* represents the time of the onset of constriction. *MIN* represents the minimal pupil size after the light stimulus. The constriction amplitude was calculated as MAX−MIN. The *75 % recovery time* is the time to recover 75 % of the initial resting pupil size after reaching MIN

was also assessed. The dynamic pupil measurements were triggered by a light impulse of 180 μW intensity and duration of 167 ms. Measurements were performed on both eyes, and the average values were used for further analyses.

Subjective drug effect

Subjective drug effects were assessed using visual analog scales (VAS) reported in detail elsewhere (Hysek et al. 2011, 2012a). In the present report, we included only the VAS rating of "any subjective drug effects," measured using a 100=mm horizontal line marked "not at all" on the left and "extremely" on the right. The VAS was repeatedly administered 1 h before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration. The scale is very sensitive to the overall psychotropic effects of MDMA (Farre et al. 2007; Hysek et al. 2011). The comprehensive assessments of different aspects of the psychotropic response to MDMA have been presented in the reports of the individual studies (Hysek et al. 2011, 2012a, b, d).

Blood pressure, heart rate, and body temperature

Blood pressure and heart rate were assessed repeatedly before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h after MDMA or placebo administration using an OMRON M7 monitor (Omron Healthcare Europe, Hoofddorp, The Netherlands) in the dominant arm and after a resting time of 5 min. Measures were taken twice per time point with an interval of 1 min, and the average was used for analysis. Mean arterial pressure (MAP) was calculated from diastolic and systolic blood pressure using the formula MAP = diastolic blood pressure + (systolic blood pressure−diastolic blood pressure)/3. Core (tympanic) temperature was assessed using a GENIUS 2 ear thermometer (Tyco Healthcare Group, Watertown, NY, USA).

Pharmacokinetics of MDMA

Blood samples were collected before and 0, 0.33, 0.66, 1, 1.5, 2, 2.5, 3, 4, and 6 h after MDMA or placebo administration, and plasma MDMA levels were determined as previously described (Hysek et al. 2012a). The data for the plasma concentrations of MDMA were analyzed using noncompartmental methods. Maximal plasma concentration and the time to maximal plasma concentration were obtained directly from the concentration–time curves of the observed values. Plasma concentrations were only determined up to 6 h after MDMA administration because the aim of the study was to assess plasma exposure only during the time of the pharmacodynamic effects of MDMA.

Drugs

(±)-MDMA hydrochloride (Lipomed AG, Arlesheim, Switzerland) was prepared as gelatin capsules (100 and 25 mg). Identical placebo (mannitol) capsules were prepared. MDMA was administered in a single absolute oral dose of 125 mg. This dose of MDMA corresponds to a typical recreational dose or the dose of MDMA used as an adjunct to psychotherapy (Mithoefer et al. 2010). In the reboxetine–MDMA study, reboxetine (Edronax; 8 mg; Pfizer, Zurich, Switzerland) or identical placebo was administered at 8:00 p.m. on the day before the test session and again at 7:00 a.m. on the test day. MDMA or placebo was administered at 8:00 a.m., 1 and 12 h after reboxetine. In the duloxetine–MDMA study, duloxetine (Cymbalta; 120 mg; Eli Lilly, Vernier, Switzerland) or identical placebo was administered at 8:00 p.m. on the day before the test session and again at 8:00 a.m. on the test day. MDMA or placebo was administered at 12:00 p.m., 4 and 16 h after duloxetine. Reboxetine and duloxetine were administered twice at high doses to obtain peak plasma concentrations of (mean ± SD) 372±34 and 107±10 ng/ml, respectively, similar to the concentrations reached with chronic daily administration of 4 and 60 mg of the drugs, respectively (Hysek et al. 2011; Simmler et al. 2011), and as previously used to manipulate noradrenergic function in healthy subjects (Roelands et al. 2008). Compliance with the first administration of reboxetine and duloxetine on the evening prior to the test day was confirmed analytically in plasma (Hysek et al. 2011, 2012d). In the clonidine–MDMA study, clonidine (Catapresan; 150 μg; Boehringer Ingelheim, Basel, Switzerland) or identical placebo was administered at 8:00 a.m., 1 h before MDMA or placebo (9:00 a.m.; Hysek et al. 2012a). Clonidine has previously been shown to produce sympatholytic effects at this dose in healthy subjects (Anavekar et al. 1982; Bitsios et al. 1996; Nieuwenhuis et al. 2007) and was expected to produce peak plasma concentrations in the range of 0.6–0.7 ng/ml (Anavekar et al. 1982; Keranen et al. 1978). In the carvedilol–MDMA study, carvedilol (Dilatrend; 50 mg; Roche, Basel, Switzerland) or identical placebo was administered at 8:00 a.m., 1 h before MDMA or placebo (9:00 a.m.; Hysek et al. 2012c). The same dose of carvedilol has previously been shown to attenuate the smoked cocaine-induced increases in heart rate and blood pressure in humans (Sofuoglu et al. 2000) and was expected to produce peak plasma concentrations in the range of 120–180 mg/ml (Henderson et al. 2006; Morgan 1994). At this dose, carvedilol is expected to inhibit both α_1- and β-adrenergic receptors (Sofuoglu et al. 2000; Tham et al. 1995), with fivefold to tenfold higher activity at β receptors (Tomlinson et al. 1988, 1992). In the doxazosin–MDMA study, continued-release doxazosin (Cardura; 4 mg; Pfizer, Zurich, Switzerland) or identical placebo was used. A first dose of 4 mg of doxazosin was administered 3 days before MDMA or placebo (−64 h) at 5:00 p.m., a

second dose of 8 mg was administered 2 days before MDMA or placebo (−40 h) at 5:00 p.m., and a third dose of 8 mg was administered the day before MDMA or placebo administration (−16 h) at 5:00 p.m. The subjects were reminded by a phone call or phone text message to ingest the capsules, and medication containers were checked to confirm that the first two doses of doxazosin were administered. The last administration was supervised by study personnel at the research facility. This administration schedule accounted for the long t_{max} of 8–10 h of the continuous-release formulation of doxazosin and reduced the risk of hypotension (Chung et al. 1999). Based on similar dosing regimes in healthy subjects (Chung et al. 1999; Shirai et al. 2010), the mean estimated peak plasma concentration of doxazosin was 30±5 ng/ml, similar to the concentration with steady-state dosing of 4 mg (Chung et al. 1999). The pretreatment times for the administration of the five pretreatments resulted in maximal plasma concentrations of the pretreatments at the time of or shortly before the maximal effect of MDMA, based on our analytical results (Hysek et al. 2011, 2012a, c, d) or published data (Anavekar et al. 1982; Chung et al. 1999; Henderson et al. 2006; Keranen et al. 1978; Morgan 1994; Shirai et al. 2010). Oral drug administration on the test days was supervised by study personnel.

Statistical analyses

Maximal effect values (E_{max}), minimal effect values (E_{min}; only for clonidine), and areas under the effect–time curves were determined with repeated measures. Values from the five studies were separately compared using two-way factorial general linear models repeated-measures analysis of variance (ANOVA), with the factors MDMA (MDMA vs. placebo) and pretreatment (pretreatment vs. placebo), using STATISTICA 6.0 software (StatSoft, Tulsa, OK, USA). Additionally, MDMA and placebo values from all of the studies were pooled and analyzed with MDMA as a single within-subjects factor. Tukey post hoc comparisons were performed based on significant main effects or interactions in the ANOVA. Analyses of the area under the effect–time curve data yielded identical results to those of the maximal values and are, therefore, not shown. Associations between the pharmacodynamic changes and plasma concentration of MDMA were analyzed using Spearman's rank correlations. This first correlation analysis assessed the associations of the parameters between subjects (n=80) for each time point. The mean pharmacodynamic changes after MDMA administration for each time point were then plotted against the respective mean plasma concentrations of MDMA and graphed as hysteresis curves. Correlations between the pharmacodynamic–pharmacokinetic data pairs over time (n=9 time points) were then analyzed using Spearman's rank correlation. Associations between pupillary function parameters and cardiovascular or subjective effects were similarly analyzed (n=10 time points). This second correlation analysis assessed the associations of mean parameter changes from baseline over time within the 16 subjects (n=9 or 10). The criterion for significance was $p<0.05$.

Results

Parameters of pupillary function (placebo condition)

Pupillary function parameters were measured 10 times in 80 subjects after placebo administration. Mean ± SEM values were as follows: pupil size=6.23±0.09 mm, pupil size after light=4.34±0.08 mm, constriction amplitude=1.90±0.01 mm, and recovery time=2.46±0.06 s. Maximal values are shown in Table 1. The diameter of the light-stimulated pupil correlated with the resting pupil size prior to the light stimulus (R_s=0.94, $p<0.001$, n=80).

Effects of MDMA on pupillary function

MDMA increased pupil size both at rest and after the light stimulus and lowered the constriction amplitude compared with placebo (Fig. 3; Table 1). The effect of MDMA on pupil size peaked (mean ± SEM) 2.3±0.2 h after drug administration at the time of the maximal plasma concentration of MDMA and remained high over 6 h in parallel with plasma levels that also remained high over 6 h (Fig. 3a). The effect of MDMA on the constriction amplitude was maximal 1.7±0.1 h after drug administration and decreased to baseline levels over 6 h (Fig. 3b) despite high plasma levels of MDMA. MDMA also prolonged the latency to the pupillary light reflex and shortened the recovery time of the pupillary light reflex response (Table 1).

Subjective effects of MDMA

MDMA produced significant subjective drug effects compared with placebo (Table 1). The peak effect was reached 1.5±0.1 h after MDMA administration (Fig. 3c). The subjective effects of MDMA completely reverted to baseline within 6 h, although the plasma levels of MDMA remained high (Fig. 3c). The effects of the pretreatments on the subjective response to MDMA are reported in detail elsewhere (Hysek et al. 2011, 2012a, b, d). Briefly, reboxetine and duloxetine reduced the subjective effects of MDMA, whereas the other pretreatments overall had no effect on the subjective response to MDMA (Hysek et al. 2012c).

Table 1 Effects of MDMA

		Placebo (mean ± SEM)	MDMA (mean ± SEM)	$F_{1,79}$	p value
Pupil size (mm)	E_{max}	6.60±0.09	7.58±0.07	288.1	<0.001
Pupil size after light (mm)	E_{max}	4.76±0.09	6.86±0.09	646.9	<0.001
Constriction amplitude (mm)	E_{max}	1.76±0.06	0.81±0.12	328.0	<0.001
Latency (s)	E_{max}	0.25±0.00	0.33±0.02	16.2	<0.001
Recovery time (s)	E_{max}	1.74±0.06	1.17±0.07	57.3	<0.001
Subjective drug effect (percent maximum)	E_{max}	3.5±1.7	81.0±2.8	901.2	<0.001
Mean arterial pressure (mmHg)	E_{max}	95.0±1.0	114.5±1.2	339.7	<0.001
Heart rate (bpm)	E_{max}	76.0±1.2	96.2±1.9	138.1	<0.001
Body temperature (°C)	E_{max}	37.3±0.1	37.6±0.1	26.2	<0.001

n=80 (values from all five studies were pooled)

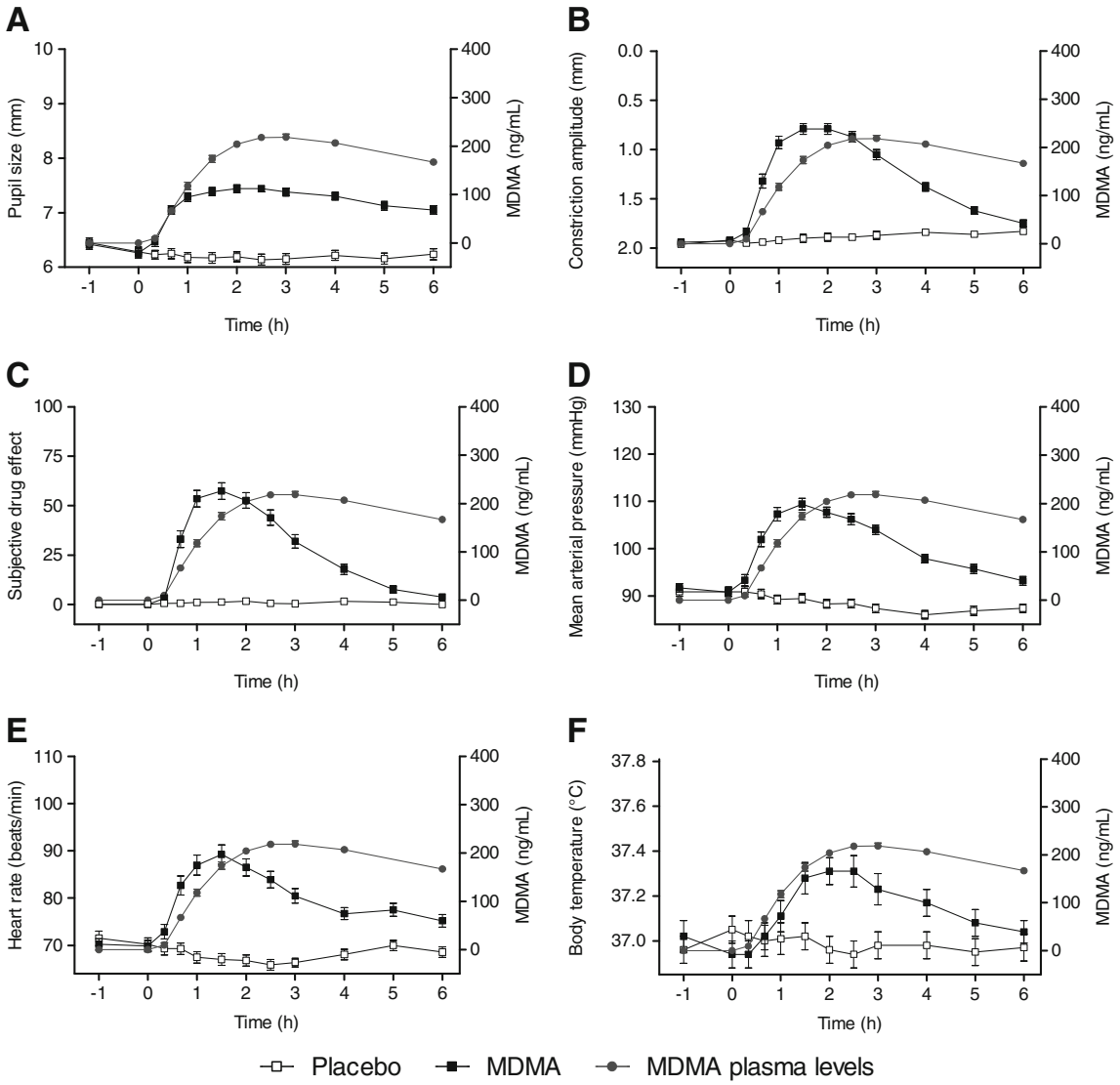

Fig. 3 Acute effects of MDMA on pupil function. Values are expressed as the mean ± SEM of 80 subjects. MDMA increased resting pupil size compared with placebo (**a**). The mydriatic effect of MDMA remained high in parallel with the plasma concentration of MDMA. MDMA reduced the pupil constriction amplitude compared with placebo and this effect decreased more rapidly than the plasma concentration of MDMA (**b**). The subjective (**c**), cardiovascular (**d**, **e**), and thermogenic (**f**) effects of MDMA also disappeared within 6 h when the plasma concentrations of MDMA were still high

Effects of MDMA on blood pressure, heart rate, and body temperature

MDMA significantly increased blood pressure, heart rate, and body temperature compared with placebo (Table 1; Fig. 3d–f). Similar to the subjective effects, MDMA-induced increases in blood pressure and heart rate were short-lasting.

Pharmacokinetics of MDMA

Plasma MDMA concentrations are shown in Fig. 3. The peak plasma MDMA concentration was (mean ± SEM) 243±6 ng/ml. The time to maximum plasma concentration was 2.5±0.1 h.

Pharmacokinetic–pharmacodynamic and pharmacodynamic–pharmacodynamic associations

The relationships between the concentration of MDMA and its pharmacodynamic effects are shown in Fig. 4a–c. The average group pupil size was correlated with the average plasma levels of MDMA over time (R_s=0.77, p<0.01, n=9), with moderate clockwise hysteresis (Fig. 4a). In contrast, the MDMA-induced reduction in constriction amplitude was not significantly associated with the plasma concentrations of MDMA (R_s=0.43, p=0.24, n=9), attributable to pronounced clockwise hysteresis (Fig. 4b). There was a similar marked hysteresis in the relationship between the concentration of MDMA and the subjective drug effects (Fig. 4c) and no correlation between the two (R_s=0.48, p=0.17, n=9). The association between the average subjective effect and pupil size over time was relatively strong (R_s=0.77, p<0.01, n=10), but hysteresis was observed in the relationship between subjective effects and pupil size over time (Fig. 4d), indicating that the subjective effects decreased more rapidly than the mydriasis associated with MDMA. In contrast, little or no hysteresis was observed in the plot of the relationship of subjective effects with constriction amplitude (Fig. 4e), indicating a closer association and more congruent subjective and dynamic pupillary effects of MDMA, also demonstrated by a very strong correlation between the means of these two effects over time (R_s=0.96, p<0.001, n=10; Fig. 4e). There were similar strong associations between MDMA-induced reductions in constriction amplitude and changes in MAP, heart rate, and body temperature (R_s=0.98, 0.92, 0.87; all p<0.001, n=10). Between-subjects correlations further showed that subjective effects were strongly correlated with reductions in the light reflex but not with pupil size (Table 2). MDMA-induced increases in blood pressure and heart rate did not

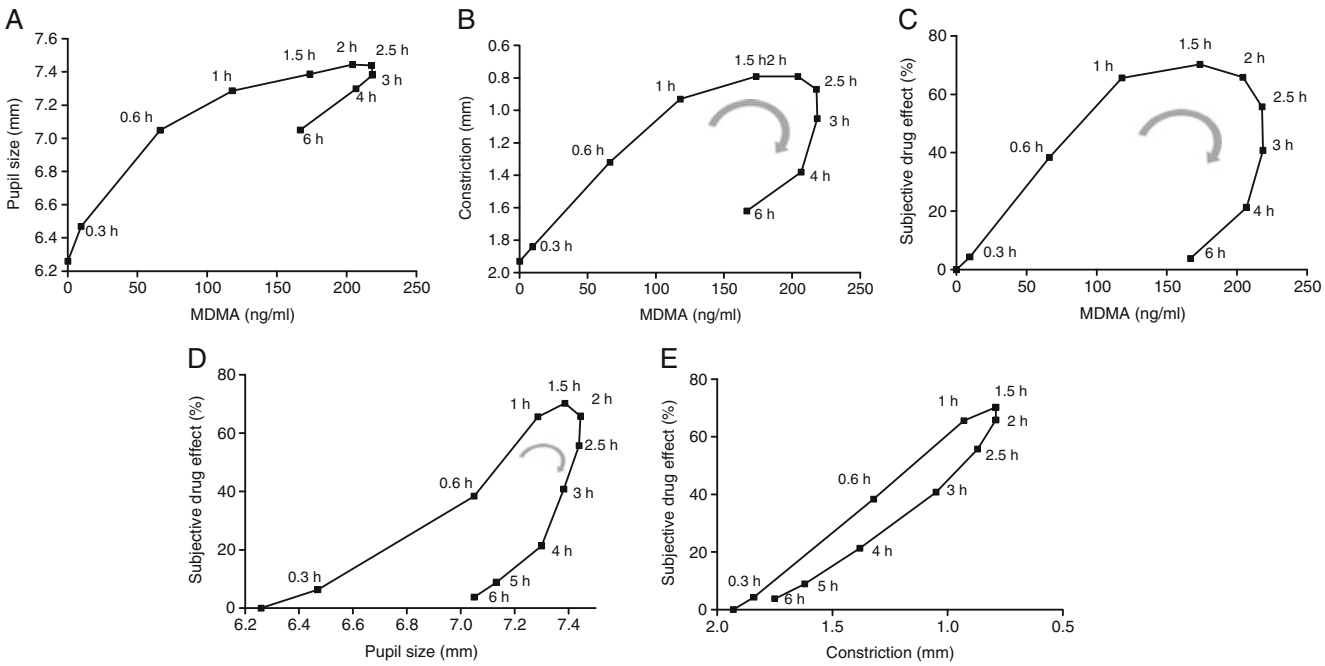

Fig. 4 Pharmacokinetic–pharmacodynamic relationship. MDMA effects plotted against the plasma concentrations of MDMA (**a**–**c**). The values are expressed as the means of 80 subjects, with SEM omitted for clarity. The times of pupillometry and blood sampling are noted next to each point in minutes or hours after MDMA administration. While pupil size (**a**) remained high, constriction amplitude (**b**) and subjective effect (**c**) returned to baseline within 6 h when MDMA concentrations remained high. This clockwise hysteresis was moderate for the mydriatic effect of MDMA, reflecting well the plasma concentration of MDMA (**a**), but pronounced for the impairment in the pupillary reflex response (**b**) and subjective effect of MDMA (**c**). The subjective effect of MDMA returned to baseline faster than the mydriatic response to MDMA (**d**). In contrast, the time course of the subjective effect of MDMA was more congruent with the time course of the MDMA-induced impairment in constriction amplitude (**e**)

Table 2 Correlations between MDMA-induced changes in pupillary function and subjective drug effects

	t=0	*t*=20 min	*t*=40 min	*t*=1 h	*t*=1.5 h	*t*=2 h	*t*=2.5 h	*t*=3 h	*t*=4 h	*t*=6 h
Pupil size (mm)	NS	NS	0.31	0.27	NS	NS	NS	NS	NS	NS
Pupil size after light (mm)	NS	NS	*0.62*	*0.51*	*0.42*	0.26	0.27	NS	NS	NS
Constriction amplitude (mm)	NS	NS	*−0.74*	*−0.61*	*−0.41*	−0.28	−0.28	−0.28	−0.23	NS
Latency (s)	NS	NS	*0.46*	0.29	NS	NS	NS	NS	NS	NS
Recovery time (s)	NS	NS	*−0.42*	−0.31	−0.32	−0.22	*−0.38*	−0.23	−0.28	−0.32

Values are Spearman correlation coefficients for significant correlations ($p<0.05$; $p<0.001$ in italics); $n=80$

NS not significant

correlate with the plasma concentrations of MDMA over time, consistent with the reduced effect over time despite high plasma concentrations of MDMA (Fig. 3d, e).

The findings from the between-subjects analyses of the correlations between the plasma levels of MDMA and pharmacodynamic effects of MDMA for each time point ($n=80$) are shown in Table 3. The MDMA-induced reductions in the constriction amplitude, the pupil size after light, the increase in MAP, and the subjective effects were significantly and strongly associated with the plasma levels of MDMA (Table 3). Weaker correlations were also found between plasma levels of MDMA and the pupil diameter, latency, or heart rate (Table 3). However, these associations were only observed at the beginning of the MDMA effect. Recovery time and body temperature after MDMA administration were not or only weakly and inconsistently associated with plasma MDMA levels (Table 3).

The MDMA-induced reduction in pupil constriction amplitude was significantly greater in subjects with greater MDMA-induced increases in MAP ($R_s=0.56$, $p<0.001$, $n=80$) or more pronounced increases in heart rate ($R_s=0.30$, $p<0.01$, $n=80$) as measured 1 h after MDMA administration. In contrast, MDMA-induced changes in the pupil size were not or only poorly associated with other autonomic changes across subjects.

Pupillary effects of reboxetine, duloxetine, clonidine, carvedilol, and doxazosin alone and on the pupillary response to MDMA

The peak effects of the pretreatments are shown in Table 4. The drug effects on pupil size over time for all five studies are shown in Fig. 5. Both reboxetine and duloxetine increased resting pupil size and pupil size after the light stimulus. Duloxetine also lowered the constriction amplitude (Table 4). The effect of the two monoamine uptake inhibitors on the static pupil diameter was similar in magnitude to the effect of MDMA (Table 4; Fig. 5a, b). In contrast, the effect of MDMA on the constriction amplitude was more pronounced. When duloxetine was administered together with MDMA, the drug effects on all static and dynamic parameters were nonadditive and showed negative synergism, reflected by a significant pretreatment × MDMA interaction in the factorial ANOVA. Thus, duloxetine prevented the effect of MDMA on pupil function, reflected by the absence of a mydriatic effect of MDMA compared with baseline in the duloxetine–MDMA condition and compared with the duloxetine–placebo condition (Fig. 5b). Duloxetine also prevented the MDMA-induced impairment in the pupillary light reflex, although it had a similar effect when

Table 3 Correlations between the effects of MDMA and plasma concentrations of MDMA

	t=0	*t*=20 min	*t*=40 min	*t*=1 h	*t*=1.5 h	*t*=2 h	*t*=2.5 h	*t*=3 h	*t*=4 h	*t*=6 h
Pupil size (mm)	NS	*0.46*	*0.45*	0.35	0.27	NS	NS	NS	NS	NS
Pupil size after light (mm)	NS	*0.50*	*0.64*	*0.57*	*0.54*	*0.53*	*0.49*	*0.47*	0.34	NS
Constriction amplitude (mm)	NS	−0.28	*−0.40*	*−0.55*	*−0.53*	*−0.60*	*−0.55*	*−0.65*	*−0.48*	−0.28
Latency (s)	NS	0.23	*0.46*	0.33	0.35	NS	0.25	0.25	NS	0.25
Recovery time (s)	NS	NS	−0.32	NS	−0.26	NS	*−0.37*	−0.26	−0.26	NS
Subjective drug effect	NS	NS	*0.68*	*0.56*	*0.37*	0.31	*0.44*	0.34	*0.46*	0.31
Mean arterial pressure (mmHg)	NS	0.22	*0.69*	*0.60*	*0.47*	0.35	0.33	0.29	*0.39*	NS
Heart rate (bpm)	NS	NS	*0.61*	*0.47*	*0.46*	0.32	NS	NS	NS	NS
Body temperature (°C)	NS	−0.24	NS	NS	NS	NS	NS	NS	NS	NS

Values are Spearman correlation coefficients for significant correlations ($p<0.05$; $p<0.001$ in italics) between MDMA-induced pharmacodynamic changes and plasma levels of MDMA; $n=80$

NS not significant

Table 4 Effects of pretreatments, of MDMA, and of the combination on pupil function

		Mean ± SEM values				Main effect of MDMA		Main effect of pretreatment		Pretreatment × MDMA	
		Placebo	Pretreatment	MDMA	Pretreatment–MDMA	$F_{1,15}$	p value	$F_{1,15}$	p value	$F_{1,15}$	p value
Pupil size (mm)											
Reboxetine	E_{max}	6.15±0.19	7.22±0.20***	7.32±0.17***	8.08±0.18***, ###	124.8	<0.001	160.4	<0.001	10.7	<0.01
Duloxetine	E_{max}	6.55±0.21	7.30±0.19***	7.52±0.16***	7.65±0.16***	58.9	<0.001	37.0	<0.001	11.2	<0.01
Clonidine	E_{min}	5.86±0.20	4.93±0.22***, ###	7.06±0.17***	6.72±0.15***	87.9	<0.001	59.3	<0.001	7.8	<0.05
	E_{max}	6.75±0.16	6.35±0.22***, ###	7.65±0.15***	7.46±0.16***	68.7	<0.001	12.7	<0.01	2.8	NS
Carvedilol	E_{max}	6.88±0.19	6.66±0.16###	7.63±0.13***	7.66±0.14***	99.5	<0.001	8.0	<0.05	1.2	NS
Doxazosin	E_{max}	6.67±0.20	6.69±0.18###	7.78±0.12***	7.53±0.14***	58.2	<0.001	2.7	NS	2.2	NS
Pupil size after light (mm)											
Reboxetine	E_{max}	4.23±0.17	5.50±0.22***, ###	6.65±0.23***	7.37±0.21***, ###	289.1	<0.001	129.9	<0.001	9.3	<0.01
Duloxetine	E_{max}	4.78±0.22	5.96±0.26***, ###	6.94±0.19***	6.38±0.22***, ##	108.0	<0.001	10.9	<0.01	84.2	<0.001
Clonidine	E_{min}	3.84±0.64	3.15±0.63**, ###	5.31±0.83***	5.08±0.97***	93.1	<0.001	24.0	<0.001	5.2	<0.05
	E_{max}	4.80±0.16	4.47±0.22###	7.01±0.23***	6.83±0.24***	211.2	<0.001	8.9	<0.01	1.2	NS
Carvedilol	E_{max}	5.10±0.25	4.77±0.17###	6.82±0.17***	7.04±0.16***	202.3	<0.001	0.4	NS	2.3	NS
Doxazosin	E_{max}	4.88±0.19	4.88±0.18###	6.86±0.15***	6.79±0.16***	169.4	<0.001	0.1	NS	0.2	NS
Constriction amplitude (mm)											
Reboxetine	E_{min}	1.74±0.06	1.62±0.07###	0.60±0.12***	0.67±0.10***	71.8	<0.001	0.7	NS	3.0	NS
Duloxetine	E_{min}	1.62±0.05	1.27±0.09**, ###	0.52±0.11***	1.18±0.06***, ###	63.9	<0.001	12.4	<0.01	71.2	<0.001
Clonidine	E_{min}	1.81±0.05	1.63±0.08###	0.58±0.11***	0.50±0.09***	72.0	<0.001	7.6	<0.05	1.0	NS
Carvedilol	E_{min}	1.65±0.10	1.75±0.06###	0.68±0.11***	0.49±0.08***	99.5	<0.001	0.7	NS	4.2	NS
Doxazosin	E_{min}	1.76±0.06	1.69±0.05###	0.81±0.12***	0.78±0.09***	79.6	<0.001	1.2	NS	0.3	NS
Latency (s)											
Reboxetine	E_{max}	0.244±0.006	0.254±0.007##	0.303±0.011***	0.306±0.015***	14.8	<0.001	2.8	NS	0.0	NS
Duloxetine	E_{max}	0.245±0.005	0.266±0.007	0.423±0.088*	0.275±0.005	4.8	<0.05	2.2	NS	3.8	0.07
Clonidine	E_{max}	0.252±0.011	0.251±0.023##	0.297±0.033**	0.308±0.061***	30.9	<0.001	0.7	NS	0.4	NS
Carvedilol	E_{max}	0.260±0.007	0.262±0.009##	0.304±0.016**	0.303±0.010##	15.5	<0.001	0.0	NS	0.0	NS
Doxazosin	E_{max}	0.253±0.008	0.249±0.006#	0.325±0.037*	0.291±0.009	10.4	<0.01	1.1	NS	1.1	NS

NS nonsignificant

*p<0.05, **p<0.01, ***p<0.001, compared with placebo; #p<0.05, ##p<0.01, ###p<0.001, compared with MDMA

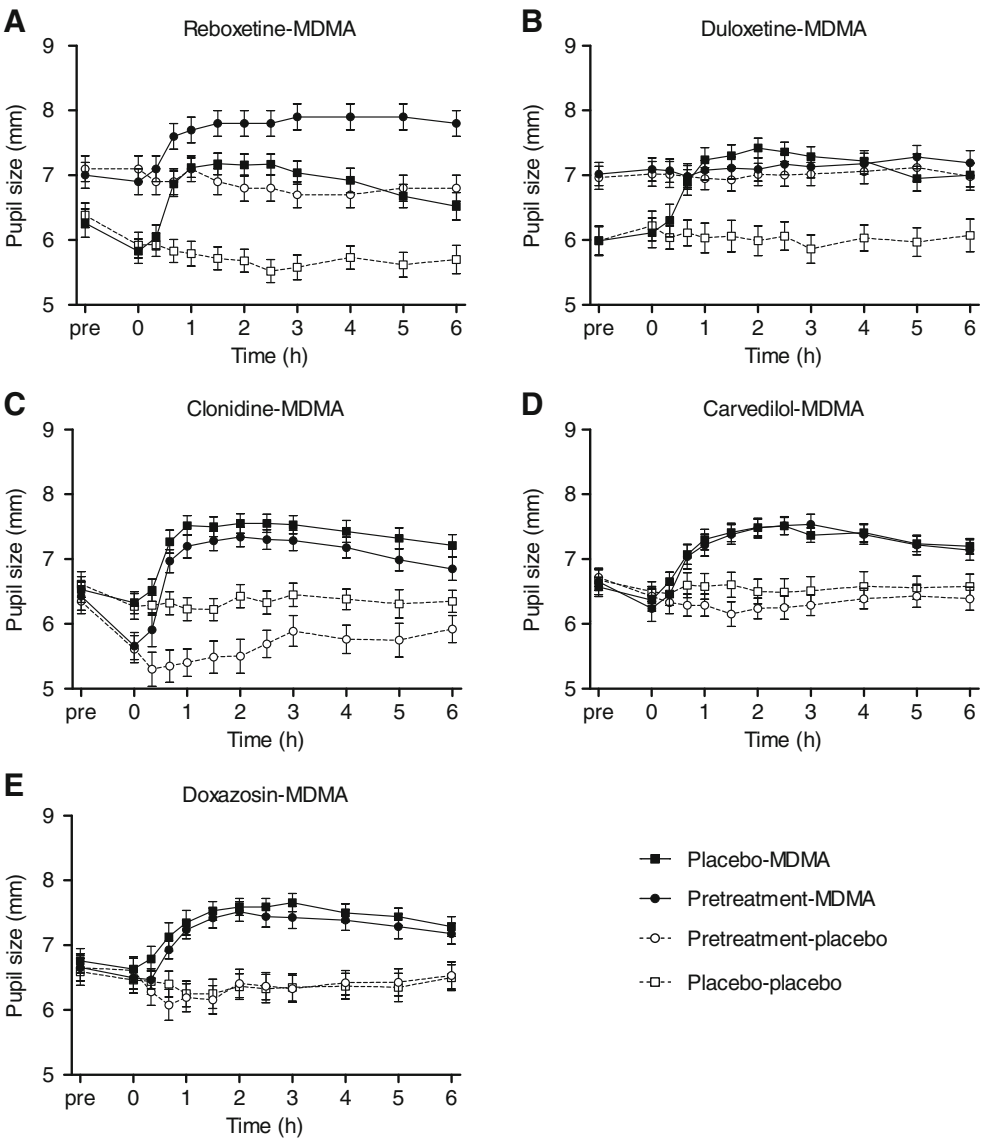

Fig. 5 Drug effects on pupil size over time. MDMA increased pupil size compared with placebo (**a**–**e**). The pretreatment with reboxetine increased pupil size to a similar extent as MDMA alone (**a**). The effect of MDMA on pupil diameter after reboxetine pretreatment compared with reboxetine was significantly smaller than the effect of MDMA compared with placebo (**a**). Duloxetine increased pupil size similar to reboxetine and MDMA (**b**). Duloxetine pretreatment prevented the further increase in pupil size induced by MDMA administration (**b**). Clonidine significantly reduced pupil diameter (**c**). The effects of clonidine and MDMA on pupil size were additive (**c**). Carvedilol nonsignificantly decreased pupil size (**d**). Similar to the effects of clonidine and MDMA, the effects of carvedilol and MDMA on pupil size were additive (**d**). Doxazosin alone had no effect on pupil size compared with placebo, but it tended to nonsignificantly attenuate the mydriatic effect of MDMA (**e**). The data are expressed as the mean ± SEM values in 16 subjects per study

administered alone compared with placebo. The effects of reboxetine and MDMA on pupil size were also nonadditive (Table 4; Fig. 5b). However, resting pupil size and pupil size after the light stimulus were significantly larger after reboxetine plus MDMA compared with MDMA alone. Reboxetine also failed to prevent the effect of MDMA on the pupillary light reflex. In the present study, reboxetine also reduced the cardiostimulant and psychostimulant effects of MDMA (Hysek et al. 2011), and duloxetine nearly completely prevented the cardiovascular, psychotropic, and neuroendocrine effects of MDMA as reported elsewhere (Hysek et al. 2012b, d; Simmler et al. 2011). Clonidine reduced resting pupil size and size after the light stimulus (Table 4; Fig. 5c). This effect of clonidine was antagonistic and overall additive with the effect of MDMA (Fig. 5c). Specifically, clonidine did not significantly reduce the effects of MDMA on any parameter of pupillary function, although it had significant effects alone and reduced the cardiovascular response to MDMA (Hysek et al. 2012a). Clonidine did not significantly reduce the mydriatic effects of MDMA, although it produced significant miosis. Clonidine also had no effects on the psychotropic response to MDMA as previously reported (Hysek et al. 2012a). Carvedilol did not alter the effects of MDMA on pupillary function. In contrast, carvedilol decreased the cardiostimulant and thermogenic effects of MDMA in the same subjects as reported elsewhere (Hysek et al. 2012c). Carvedilol alone decreased pupil size, reflected by a significant main effect of pretreatment in the ANOVA, but the reduction in pupil size after carvedilol–placebo treatment compared with the placebo–placebo condition (Fig. 5d) was not significant in the post hoc test. Doxazosin alone had no effect on

pupil size compared with placebo but slightly and non-significantly reduced the MDMA-induced increase in pupil size (Fig. 5e).

Discussion

In the present study, we showed that MDMA impaired the pupillary reflex response to light, including inducing a longer latency, reducing the constriction amplitude, and reducing the recovery time. MDMA produced mydriasis as previously documented using nonautomated techniques (Farre et al. 2004, 2007; Kolbrich et al. 2008; Mas et al. 1999). MDMA also increased blood pressure, heart rate, and body temperature and produced positive mood effects as described in more detail elsewhere (Hysek et al. 2011, 2012a, b, d).

The analyses of the effects of MDMA over time showed a very strong correlation between the MDMA-induced reduction in constriction amplitude and other autonomic or subjective effects of the drug. The MDMA-induced reduction in the pupillary light reflex normalized over 6 h, similar to the cardiostimulant and subjective drug effects that also largely disappeared over 6 h, although the plasma levels of MDMA remained high. Thus, the reduced reactivity of the pupil to light is relatively short-lasting and subject to acute pharmacological tolerance, similar to the subjective and cardiostimulant effects of MDMA.

Clinical examination of pupil function in cases of drug intoxication typically includes both an estimation of static pupil size and an assessment of the reactivity to a flashlight stimulus. With regard to MDMA intoxication, our findings suggest that the impaired reactivity to light indicates MDMA exposure within the past 1–4 h and is a marker for the acute subjective and autonomic effects of the drug. In contrast, mydriasis lasts at least 6–10 h (Farre et al. 2007; Mas et al. 1999), correlates best with the plasma MDMA concentration changes over time, and shows only moderate pharmacological tolerance. The mydriatic responses to two successive doses of MDMA separated by 24 h were similar, although the peak concentration after the second dose of MDMA increased by 29 %, indicating some degree of tolerance (Farre et al. 2004). Although the mean group changes in pupil size over time reflected the concentration–time curve of MDMA, pupil size did not correlate well with the plasma concentrations of MDMA across subjects at various time points in our study or with MDMA plasma levels 1.25 h after drug administration in a previous study (Kolbrich et al. 2008). This is not surprising because the effects of MDMA on pupil size were maximal at single doses of 75 mg and did not further increase at 125 mg (Mas et al. 1999). Thus, the lack of an association is likely attributable to a ceiling effect of the plasma MDMA concentration–effect curve. In contrast, dynamic impairments of the pupil light reflex response were significantly associated with plasma MDMA levels or the cardiostimulant effects of MDMA across subjects. Evaluating the dynamic pupillary response to light may, therefore, be a better estimation of the time and amount of exposure to MDMA than static pupil size.

Both sympathetic and parasympathetic innervations contribute to the regulation of pupil size and the reflex response (Loewenfeld 1999). At the level of the iris, the latency to and amplitude of the reflex response are mainly determined by parasympathetic activity (Heller et al. 1990), whereas redilation is controlled by sympathetic inputs (Loewenfeld 1999; Morley et al. 1991). Additionally, parasympathetic function is under tonic noradrenergic inhibition centrally at the level of the Edinger–Westphal nucleus where the sympathetic stimulation of α_2-adrenergic receptors may lower parasympathetic output, resulting in "pseudoanticholinergic" mydriasis (Phillips et al. 2000a; Siepmann et al. 2007; Szabadi and Bradshaw 1996). Furthermore, the serotonin system is implicated in pupillary function, possibly via 5-HT_{1A}-mediated stimulation of the release of norepinephrine and consequent activation of α_2-adrenergic receptors (Prow et al. 1996). MDMA mainly releases serotonin and norepinephrine (Liechti and Vollenweider 2001; Rothman et al. 2001; Verrico et al. 2007). Because MDMA affected both the parasympathetic and sympathetic aspects of the pupillary reflex response, all of the aforementioned mechanisms may be involved in the effects of MDMA on pupillary function.

The norepinephrine transporter inhibitor reboxetine significantly increased pupil diameter at rest and after light, consistent with previous studies (Theofilopoulos et al. 1995). Reboxetine did not reduce the mydriatic response to MDMA, but the effects of the two drugs on pupil size were subadditive, indicating that MDMA produces part of its effects on pupil size through the transporter-mediated release of norepinephrine, which is inhibited by reboxetine (Hysek et al. 2011). This finding is consistent with the attenuation of the cardiostimulant and psychostimulant effects of MDMA by reboxetine (Hysek et al. 2011) and supports the view that norepinephrine is involved in the stimulant effects of MDMA.

The α_1-adrenergic receptor inhibitor doxazosin did not affect pupillary function when administered alone but non-significantly reduced the mydriatic response to MDMA. Prazosin did not antagonize mydriasis induced by norepinephrine or phenylephrine in anesthetized cats (Hey et al. 1988; Koss et al. 1988). The data suggest that α_1-adrenergic receptors in the iris may only minimally contribute to mydriasis induced by systemically administered sympathomimetic drugs and that central parasympathetic inhibition may be more relevant.

The $\alpha_1\beta$-adrenergic receptor inhibitor carvedilol had no significant effect on pupil size compared to placebo, consistent with earlier work (Hirohashi et al. 1990) and the absence of effects of the β-adrenergic receptor blocker propranolol on pupillary function (Koudas et al. 2009). Carvedilol did not affect the mydriatic response to MDMA, but it reduced other autonomic effects of MDMA, including increases in blood pressure and body temperature (Hysek et al. 2012c).

Clonidine decreased pupil diameter and enhanced the pupillary reflex, consistent with its known sympatholytic effects (Clifford et al. 1982; Morley et al. 1991; Phillips et al. 2000b, c). Clonidine also lowered the plasma concentrations of norepinephrine and blood pressure in the subjects of the present study (Hysek et al. 2012a). The effect of clonidine on pupil function is thought to involve the stimulation of α_2-adrenergic receptors on central noradrenergic neurons, leading to decreased sympathetic outflow to the iris. The enhancement of the parasympathetic light reflex is consistent with clonidine-induced disinhibition of the noradrenergic central control of parasympathetic outflow (Phillips et al. 2000b). Despite its significant sympathicolytic effects (Hysek et al. 2012a), clonidine failed to significantly reduce the effects of MDMA on pupillary function. Moreover, clonidine did not reduce the MDMA-induced increase in norepinephrine or blood pressure to the same extent as it reduced these parameters when administered alone (Hysek et al. 2012a). Thus, the sympatholytic effects of clonidine and sympathomimetic effects of MDMA were antagonistic in an additive manner, without evidence of interactive effects of the two drugs. The findings indicate that α_2-adrenergic receptors and the vesicular release of norepinephrine are not critically involved in the pharmacological effects of MDMA.

The dual serotonin and norepinephrine transporter inhibitor duloxetine increased resting pupil diameter, prolonged the latency to the light reflex, and reduced the reaction to light. Identical effects on pupillary function have been reported for the serotonin and norepinephrine transporter inhibitor venlafaxine (Bitsios et al. 1999; Siepmann et al. 2007). Serotonin releasers, including fenfluramine (Kramer et al. 1973), meta-chlorophenylpiperazine (Benjamin et al. 1997), and MDMA, and serotonin transporter inhibitors (Nielsen et al. 2010; Noehr-Jensen et al. 2009; Schmitt et al. 2002) also cause mydriasis. Citalopram and paroxetine have also been shown to reduce the constriction amplitude (Nielsen et al. 2010; Noehr-Jensen et al. 2009), similar to previous observations with duloxetine. Duloxetine may, therefore, exert its effects on pupillary function via both noradrenergic and serotonergic mechanisms. Although both duloxetine and MDMA produced mydriasis, pupil size did not further increase after the administration of both drugs, suggesting interactive effects of the two drugs. Moreover, duloxetine almost completely prevented the effects of MDMA on the light reflex. Duloxetine also markedly inhibited the cardiostimulant, psychotropic, and neuroendocrine responses to MDMA in the same subjects (Hysek et al. 2012b, d; Simmler et al. 2011). Selective serotonin transporter inhibitors including citalopram, fluoxetine, and paroxetine have previously been shown to attenuate the physiological and psychological effects of MDMA in humans (Farre et al. 2007; Liechti et al. 2000; Liechti and Vollenweider 2000; Tancer and Johanson 2007). Notably, paroxetine also prevented the mydriatic effects of MDMA (Farre et al. 2007). Together with the interactive effects of duloxetine and MDMA in the present work, the findings provide strong support for a role of serotonin in the mechanism of action of MDMA. The reduction of the effects of MDMA on the pupil light reflex by duloxetine but not reboxetine supports a central modulatory role of serotonin in the effects of MDMA on pupillary function, possibly involving central serotonergic potentiation of noradrenergic outflow (Prow et al. 1996).

In the present study, we assessed pupillary function under dark–light conditions, similar to other studies of the autonomic effects of pharmaceuticals (Bitsios et al. 1999; Nielsen et al. 2010; Noehr-Jensen et al. 2009; Phillips et al. 2000c). The values of the latency to the light reflex and constriction amplitude obtained in the present study were similar to those measured under daylight conditions with the same pupillometer (Taylor et al. 2003), indicating that these parameters may not be critically affected by the light conditions. Overall, our data indicate that the constriction of the pupil represents a measure that is sensitive to pharmacological interventions and may be relatively insensitive to changes in light conditions compared with measures of pupil size.

In summary, MDMA increased pupil size and reduced the response to light. The MDMA-induced prolongation of the latency to the light reflex and reduction in light-induced miosis indicate indirect central parasympathetic inhibition. The faster recovery reflects increased direct sympathomimetic action. Both reboxetine and duloxetine interacted with the effects of MDMA on static and dynamic measures of pupillary function, supporting a role for both norepinephrine and serotonin in the effects of MDMA on pupillary function. MDMA-induced mydriasis was associated with the plasma concentration–time curve of MDMA. The reduced miotic response to light was highly correlated with the cardiostimulant and subjective effects of MDMA and demonstrated acute pharmacological tolerance.

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Discussion

In this project we demonstrated a crucial role for NE in the mechanism of action of MDMA in humans. The study findings are reported and discussed in detail in the published papers presented above. This is a brief discussion of the overall work.

Role of norepinephrine in the psychotropic effects produced by MDMA

Clinical data indicates that 5-HT plays a major role in the mediation of the acute emotional effects of MDMA in humans.[12,68,69] Pretreatment with citalopram attenuated most of the subjective effects induced by MDMA, including positive mood, derealization, and thought disorder.[68] In this project we now describe that also the inhibition of the NE transporter leads to a reduction of a substantial range of MDMA-induced effects in healthy subjects.[99,100] The selective NE transporter blocker reboxetine reduced MDMA-induced subjective effects such as drug high, stimulation, emotional excitation, and anxiety. Similarly, the inhibition of the NE transported also reduced the psychostimulant response to *D*-amphetamine.[92] These findings are consistent with a role of the NE transporter in the mediation of the sympathomimetic stimulant-like aspects of the MDMA effect and are in line with the preclinical data of the strong NE releasing potency of MDMA.[60,78,100] The dual 5-HT and NE transporter blocker duloxetine robustly and almost completely prevented the emotional effects produced by MDMA.[100] The blocking effect of duloxetine on the response to MDMA was more pronounced than that of a selective 5-HT or NE inhibitor supporting to conclusion that both 5-HT and NE contribute to the acute effects of MDMA in humans.

This project also describes that subjective effects induced by MDMA are mainly mediated via a release of the monoamines through the presynaptic transporter site. The α_2 agonist clonidine, which inhibits the vesicular release of NE, did not affect the acute response to MDMA in humans.[101] Taken together, the pronounced effects of duloxetine as well as the effects of citalopram or reboxetine implicate that monoamine transporters are the primary targets of MDMA and physiological impulse-dependent vesicular release of monoamines does not seem to play a critical role for the psychotropic effects of MDMA in humans.

Finally, the α_1 adrenergic receptor might be one of the post-synaptic adrenergic receptors, which is critically involved in psychotropic effects produced by MDMA. We selectively inhibited the α_1 adrenergic receptor with doxazosin and attenuated the heightened mood effects produced by MDMA. Similarly, doxazosin has been shown to reduce subjective liking of the acute effects of cocaine.[102] These findings indicate a possible role for the α_1 adrenergic receptors in the mediation of the mood-enhancing and stimulating effects of psychostimulants and of MDMA in humans.

Role of norepinephrine in the cardiovascular effects produced by MDMA

NE also plays a substantial role in the mediation of the MDMA-induced somatic effects. MDMA highly affects markers for sympathetic system activation, such as increased blood pressure, heart rate, and peripheral NE plasma levels.[99,101,103] We demonstrated that a pretreatment with reboxetine significantly reduced the MDMA-induced increases in NE circulating plasma levels, blood pressure and heart rate[99] and confirmed hereby that the transporter-mediated release of NE plays a crucial role in the cardiovascular response to MDMA. A pretreatment with the post-synaptic β adrenergic receptor antagonist pindolol reduced the heart rate response to MDMA but lead to an enhanced blood pressure reaction.[104] Opposite to the effects of pindolol on the cardiostimulant effects of MDMA, the post-synaptic α_1 adrenergic receptor blocker doxazosin lowered hypertension but enhanced the heart rate response to MDMA. Finally, the combined α_1 and β adrenergic receptor blocker carvedilol prevented both the heart rate and the blood pressure responses to MDMA.[105] Together our findings confirm a central role for β adrenergic receptors in the regulation of stimulant-induced tachycardia and for α_1 adrenergic receptors in the regulation of stimulant-associated hypertension.

The dual inhibition of both the 5-HT and the NE transporter produced an almost complete reduction of the cardiovascular effects produced by MDMA.[103] Thus, in addition to NE, 5-HT release may also contribute to the cardiostimulant effects of MDMA possibly through activation of 5-HT_2 receptors.

Role of norepinephrine in the hyperthermic effects produced by MDMA

We showed that α_1 adrenergic inhibition with doxazosin partly attenuated the MDMA-induced increase in body temperature. Additionally, we found that carvedilol, a combined α_1 and $\beta_{1,2,3}$ adrenergic receptor blocker, significantly reduced the hyperthermic response to MDMA in humans.[105] These findings are consistent with the preclinical which indicate that the MDMA-induced increase in body temperature involves α_1 adrenergic receptor-mediated peripheral cutaneous vasoconstriction with impaired heat dissipation[106] and β_3 adrenergic receptors-mediated heat generation by mitochondrial uncoupling.[106,107] and Interestingly, reboxetine and duloxetine only tended to reduce the MDMA-induced increase in body temperature.[99,100] Thus, a post-synaptic receptor inhibition appears to be more effective than the inhibition of the transporter-mediated monoamine release.

Effects of MDMA on vasopressin

We showed that MDMA increased plasma levels of copeptin, a marker for arginine vasopressin (AVP) secretion, in women but not in men.[108] This sex-difference in MDMA-induced AVP secretion may explain why hyponatremia is typically reported in female ecstasy users.[28 29] The copeptin response to MDMA is likely mediated via MDMA-induced release of 5-HT and/or NE because it was prevented by duloxetine.[108]

Effects of MDMA on emotion recognition (mind reading)

There is evidence that MDMA leads to alterations in the processing of socioemotional information.[109] We illustrated that MDMA enhanced the capacity to read positive emotional cues and impaired to capacity to identify negative emotions from the eye region.[110] MDMA also produced strong subjective pro-social effects, including feelings of being more open, talkative, and closer to others. In addition, MDMA increased in plasma levels of oxytocin, a neuro-peptide, which is thought to mediate interpersonal bonding and to increase empathy.[111,112] Conclusively, the shift in the ability to correctly read socio-emotional information, together with the pro-social feelings produced by MDMA, may enhance social approach behavior and sociability when MDMA is used recreationally and facilitate therapeutic relationships in MDMA-assisted therapy.

Effects of MDMA on pupillary function

MDMA increased the pupil size consistent with its sympathomimetic properties. We found that the MDMA-induced increase in pupil size was lasting and reflected the plasma concentration-time curve of MDMA. In contrast, we observed that the impairment in the reaction to a light reflex was associated with the subjective and other autonomic effects produced by MDMA and exhibited acute tolerance. These findings will aid in the clinical assessment of pupillary changes associated with intoxications with MDMA.

Conclusion & Outlook

Our findings extend the understanding of the mechanism of action of MDMA in humans. We conclude that NE is primarily involved in the mediation of the acute psychostimulant and cardiostimulant aspects of the effects of MDMA in humans. The acute effects evoked by MDMA seem mainly to be mediated via the presynaptic uptake transporters and not by an impulse-dependent vesicular release of monoamines.

Our findings suggest that NE and 5-HT transporter inhibitors and α_1-adrenergic receptor antagonists could be useful in the treatment of stimulant dependence. In cases of sympathomimetic toxicity associated with psychostimulants, combined α and β adrenergic receptor blockers should be used, whereas selective α_1 or β blockers should be withheld due to the risk of enhanced tachycardia or hypertension, respectively.

There are still various research questions to address about the mechanism of action of MDMA. In addition, to the 5-HT and NE transporter, MDMA also binds to the DA transporter where it inhibits the uptake and releases DA from the presynaptic cell.[60,61] DA has been well documented to mediate drug reinforcement and the addictive properties of drugs of abuse but its role in psychostimulant-induced acute emotional effects in humans is still unclear. An interesting target of investigation is the DA transporter which is currently being tested in a similar design as used in the present studies. Further, there is evidence that the genetic background may affect the response to MDMA and other psychostimulants.[113] The role of the genes will also be explored in the present study sample once additional studies have been completed and on the basis of a sufficiently large pooled data sample allowing for pharmacogenetic analyses. Sexual hormones such as testosterone or progesterone have shown to play a role in the mediation of emotions[114] or trust.[115] However, to which extend these hormones play a role in the emotional effects including those induced by MDMA is still unclear. Additionally there are also clinical studies underway investigating the effect of MDMA in the treatment of psychiatric disorders such as PTSD or end-stage cancer (clinicaltrials.gov identifier: NCT01211405, NCT00252174).

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Curriculum Vitae

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Work experience

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Clinical inversitgator course – Clinica Trial Unit, University Hospital Basel	2009

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