J. Membrane Biol. 168, 39–45 (1999)

The Journal of
Membrane
Biology

New Drugs for the Na^+/H^+ Exchanger. Influence of Na^+ Concentration and Determination of Inhibition Constants with a Microphysiometer

H. Fischer[1], A. Seelig[1], N. Beier[2], P. Raddatz[2], J. Seelig[1]

[1]Department of Biophysical Chemistry, Biocentre of the University of Basel, Klingelbergstr. 70, CH-4056 Basel, Switzerland
[2]Preclinical Research Laboratories, Merck KGaA, Frankfurterstr. 250, D-64271 Darmstadt, Germany

Received: 3 September 1998/Revised: 20 November 1998

Abstract. The NHE-1 isoform of the Na^+/H^+ exchanger is excessively activated in cardiac cells during ischemia. Hence NHE-1 specific inhibitors are being developed since they could be of beneficial influence under conditions of cardiac ischemia and reperfusion. In this study, the Cytosensor™ microphysiometer was used to measure the potency of four new drug molecules, i.e., EMD 84021, EMD 94309, EMD 96785 and HOE 642 which are inhibitors of the isoform 1 of the Na^+/H^+ exchanger. The experiments were performed with Chinese hamster ovary cells (CHO K1) which are enriched in the NHE-1 isoform of the Na^+/H^+ antiporter. The Na^+/H^+ exchanger was stimulated with NaCl and the rate of extracellular acidification was quantified with the Cytosensor. The proton exchange rate was measured as a function of the NaCl concentration in the range of 10–138 mM NaCl stimulation. The proton exchange rate followed Michaelis-Menten kinetics with a $K_M = 30 \pm 4$ mM for Na^+. Addition of either one of the four inhibitors decreased the acidification rate. The IC_{50} values of the four compounds could be determined as 23 ± 7 nM for EMD 84021, 5 ± 1 nM for EMD 94309, 9 ± 2 nM for EMD 96785 and 8 ± 2 nM for HOE 642 at 138 mM NaCl, in good agreement with more elaborate biological assays. The IC_{50} values increased with the NaCl concentration indicating competitive binding of the inhibitor. The microphysiometer approach is a fast and simple method to measure the activity of the Na^+/H^+ antiporter and allows a quantitative kinetic analysis of the proton excretion rate.

Key words: Na^+/H^+ exchanger — NHE-1 — Cytosensor — Microphysiometer

Correspondence to: J. Seelig

Introduction

The Na^+/H^+ exchanger (NHE) is a plasma membrane protein consisting of 10 or 12 putative transmembrane domains (Sardet, Franchi & Pouyssegur, 1989). It is present in the cell membrane of most mammalian cells and plays a major role in various cell functions, such as maintenance of the cell volume, control of intracellular pH, initiation of cell growth, and transepithelial Na^+ reabsorption (for a review *see* Grinstein, 1988). The molecular function of NHE is the electroneutral, reversible exchange of Na^+ against H^+. It is driven by the Na^+ concentration gradient across the plasma membrane (Moolenaar et al., 1981*b*; Barber, 1991) and can be inhibited by amiloride and amiloride analogues like DMA (5-N-dimethylamiloride) or MPA (5-N-(methylpropyl)amiloride) (Counillon et al., 1993). The activation of Na^+/H^+ antiporter results in intracellular alkalinization due to the transport of H^+ out of the cell.

At least 4 different NHE isoforms (NHE 1–4) are known to date (Krulwich, 1983; Aronson, 1985). NHE-1 is ubiquitously expressed in mammalian cells (for a review *see* Clark & Limbird, 1991) and has become a focal point of scientific interest. NHE-1 plays an essential role in the development of hypertension (Feig, D'Occhio & Boylan, 1987; Rosskopf, Fromter & Siffert, 1993; Kuroo, 1995) and cardiac ischaemia (Scholz et al., 1995). Selective inhibitors that specifically block the exchange of the NHE-1 subtype have therefore been developed.

The potency of an Na^+/H^+ inhibitor can be expressed by its dose-response curve and its IC_{50} value. In the following it will be shown that the measurement of the extracellular acidification rate by means of a microphysiometer provides a convenient and rapid technique to investigate the potency of drugs to inhibit the Na^+/H^+ antiporter. In addition, the influence of the Na^+ concentration on the rate of Na^+/H^+ exchange is studied in the

concentration range of 10 mM $\leq c_{NaCl} \leq$ 138 mM. The data will be compared with earlier results obtained with the $^{22}Na^+$ isotope uptake assay. The microphysiometer allows a faster measurement and is applicable to a larger concentration range than the isotope exchange assay.

Materials and Methods

Materials

EMD 84021, EMD 94309, EMD 96785 and HOE 642 were from the preclinical research laboratories of Merck, Darmstadt (*cf.* Table for chemical structures). The synthesis of the EMD compounds has been described recently by Baumgarth, Beier and Gericke (1997) whereas HOE 642 has been described by Scholz et al. (1995). All other reagents were commercially available from Fluka (Buchs, Switzerland) and Sigma (Buchs, Switzerland). CHO K1 (wild type) cells were a gift of Molecular Devices, Munich. The mouse fibroblast cell line expressing the human NHE-1 isoform was obtained from Prof. J. Pouysségur, Nice, France.

Cell Culture

CHO K1 cells were grown in monolayer culture in nutrient mixture HAM's F12 with L-glutamine and sodium bicarbonate (Gibco, Basel, Switzerland) supplemented with 10% fetal calf serum, 50 units penicillin, and 50 μg streptomycin per mL medium. The cells were split 1:10 twice a week.

Measurement of Extracellular Acidification Rate

The acidification rate was measured with a recently developed microphysiometer (Cytosensor™, Molecular Devices, Sunnyvale, CA). CHO K1 cells were seeded into 12 mm diameter disposable polycarbonate cell capsules at a density of $3 \cdot 10^5$ cells/capsule in nutrient mixture HAM's F12 as described above. During an incubation time of 12 hr at 37°C in an atmosphere of 95% air and 5% CO_2, the cells attached to the membrane of the capsule cup. The capsule cups were loaded into the measurement chambers of the microphysiometer. The chambers were perfused continuously at a rate of 100 μl/min with flow medium and drugs as indicated below.

For the determination of Na^+ activation and the IC_{50} values of the Na^+/H^+ exchange inhibitors, the flow medium was composed of 138 mM tetramethylammonium (TMA) chloride, supplemented with 10 mM glucose, 5 mM KCl, 1.3 mM $CaCl_2$, 0.81 mM K_2HPO_4, 0.5 mM $MgCl_2$ and 0.11 mM KH_2PO_4 containing no Na^+. After a constant acidification rate (basal acidification rate) was reached, the 138 mM TMA was replaced by a 138 mM NaCl solution in order to stimulate the Na^+/H^+ antiporter. After 6 sec stimulation, the flow medium was stopped and the acidification rate was measured for 20 sec. Next, the cells were again superfused with 138 mM NaCl for further 100 sec and the acidification rate was measured again. Finally, the flow medium was switched back to 138 mM TMA.

The corresponding experiments were also performed at lower Na^+ concentrations (c_{NaCl} = 10 mM − 100 mM) in order to measure the influence of the Na^+ concentration on the acidification rate. In addition, the influence of the stimulation time was also studied, i.e., the perfusion time was varied in the range of 4 to 120 sec at a constant NaCl concentration.

For measurement of IC_{50} values the Na^+/H^+ exchanger inhibitors were added to the TMA solution. After perfusion of the cell chambers for 30 min the cells were again stimulated with 138 mM NaCl. The extracellular acidification rate, AR, was expressed as the percentage of the basal acidification rate according to:

$$AR(\%) = \frac{\text{maximal acidification rate} - \text{basal acidification rate}}{\text{basal acidification rate}} \times 100 \quad (1)$$

The acidification rate was measured as a function of the inhibitor concentration. Again the influence of the NaCl concentration was investigated by performing corresponding experiments at several lower NaCl concentrations.

Sodium Influx into Rabbit Erythrocytes and Mouse Fibroblasts

This NHE exchange inhibition assay with red blood cells and mouse fibroblasts was carried out as described by Baumgarth et al. (1997). Briefly, the $^{22}Na^+$ uptake from the extracellular phase (pH 8.0) into the erythrocyte (pH 6.8) was measured by following the β-decay. An increasing concentration of inhibitor led to a decrease of $^{22}Na^+$ influx into erythrocytes. The NHE-1 isoform was also stably expressed in a mouse fibroblast cell line. The inhibitory potency of the compounds was assessed by determining the EIPA-sensitive $^{22}Na^+$ uptake into the cells after intracellular acidosis (Grinstein & Wieczorek, 1994; Noel & Pouyssegur, 1995; Yun et al, 1995).

Results

Activation of NHE-1 with Na^+

Compared to the isotope exchange method the Cytosensor technique has the advantage of a faster measurement and increased sensitivity. The stimulation with Na^+ can be limited to a few seconds and the change in external pH can be monitored with a time resolution of seconds. A typical experiment for Na^+ stimulation with and without inhibitor is shown in Fig. 1*A*. First, the basal rate of proton excretion in the absence of Na^+ was measured. To this purpose, the CHO K1 cells were superfused with 138 mM tetramethylammonium chloride (TMA) at pH 7.2. The flow was stopped and the acidification of the extracellular space was measured during 20 sec. The basal rate of acidification was defined as 100%. The flow of TMA buffer was then continued for a period of about 60 min. Next, the cells were stimulated with Na^+. The CHO K1 cells were first exposed to a 6-sec pulse of 138 mM NaCl. The flow was then stopped and the acidification rate was measured for 20 sec (first data point (■) in the shaded area of Fig. 1*A*). The superfusion with the NaCl solution was continued for another 2 min and the acidification rate was measured again for 20 sec (2nd data point (■) in shaded area). After NaCl exposure, the cells were superfused again with 138 mM TMA without NaCl. The acidification rate returned to its basal value within 5 min. The stimulation with 138 mM NaCl was repeated three times in intervals of 30 min. The maximum acidification rate was typically 350% (*cf* Fig. 1*A*).

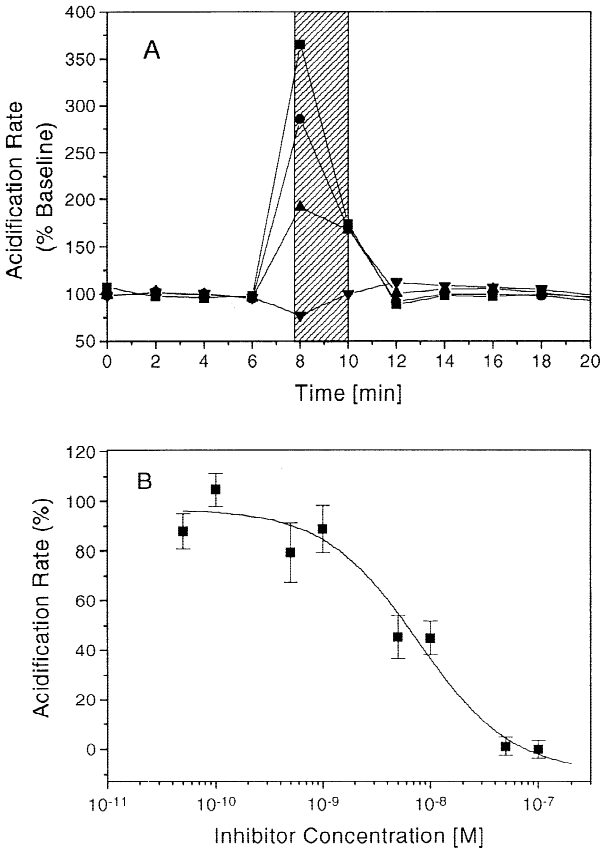


Fig. 1. (*A*) Stimulation of CHO K1 cells with Na^+ and inhibition of the Na^+/H^+ antiporter with EMD 94309. Initially, the cells were superfused with 138 mM TMA at pH 7.2. The basal rate of extracellular acidification of CHO K1 cells was defined as 100%. After a rest period of approximately 60 min, the cells were exposed to 138 mM NaCl for 6 sec and the acidification rate measured (first (■) point in shaded area). NaCl superfusion was then continued for further 2 min (second (■) measurement in shaded area). After NaCl exposure, the cells were superfused again with 138 mM TMA without NaCl. The acidification rate returned to its basal value within 5 min. In further experiments the TMA solution contained, in addition, Na^+/H^+ antiporter inhibitor EMD 94309 at concentrations of 0.5 nM (●), 5 nM (▲), or 50 nM (▼). Otherwise, the experiments were performed exactly as described before. The presence of inhibitor decreased the acidification rate. After complete inhibition of the Na^+/H^+ antiporter, the cells were superfused again with 138 mM TMA for 30 min. Stimulation with 138 mM NaCl lead to an increase of the acidification rate to about 350%, i.e., it returned to the same value as observed before addition of inhibitor. (*B*) Dose-response curve for inhibition of the Na^+/H^+ antiporter by EMD 94309. Data points represent the mean of 3 independent measurements. Solid line calculated according to: Na^+-induced acidification rate % = $100-[(100\ c/IC_{50})/(1+c/IC_{50})]$. The IC_{50} value is 5 ± 1 nM for EMD 94309.

Figure 1*A* demonstrates that an extended exposure to NaCl reduces the H^+ excretion rate of the CHO K1 cells. The dependence on the exposure time was thus investigated in more detail and the results are summarized in Fig. 2. The rate of acidification (basal value 100%) is plotted as a function of the incubation time for stimulation with a 138 mM NaCl solution. Short incubation times of less than 6 sec cause only little stimulation. This can be explained by the time needed for the Na^+ bolus to reach the measuring chamber. Maximum acidification (~350% of basal value) is reached for a NaCl pulse of 6 sec; a further increase in the length of the NaCl pulse reduces the acidification rate. For long exposure times (120 sec) the acidification is only 130% of the basal value and is thus distinctly lower than the maximum value after initial stimulation. In isotope exchange experiments activation times in the order of 2–4 min are usually employed.

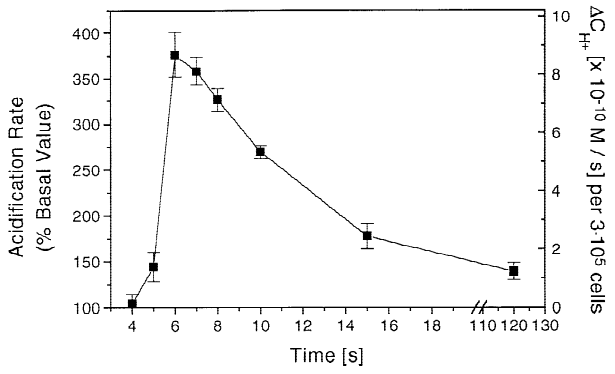


Fig. 2. Acidification rate as a function of Na^+ stimulation time. CHO K1 cells were superfused with 138 mM TMA for about 1 hr. The basal value of the acidification rate was measured and defined as 100%. Next the cells were stimulated with a bolus of 138 mM NaCl for the indicated period of time. Maximum acidification rate was observed for 6 sec stimulation and decreased for increased time of stimulation. Temperature of measurement: 37°C.

Using a Na^+ bolus of fixed pulse length (6 sec), the acidification rate was further investigated as a function of the NaCl concentration (Fig. 3). The acidification rate increased in a nonlinear fashion with increasing c_{NaCl}. A Na^+-induced stimulation of H^+ flux was detectable with a NaCl concentration as low as 10 mM NaCl and increased up to a physiological NaCl solution of 138 mM NaCl. An apparent saturation behavior of the acidification rate at high c_{NaCl} concentrations is suggested by Fig. 3 and a quantitative analysis of the data in terms of Michaelis-Menten kinetics will be discussed below.

Inhibition of the Na^+/H^+ Antiporter with EMD 84021, EMD 94309, EMD 96785 and HOE 642

Exposure of CHO wild-type cells to the putative inhibitors results in a decreased acidification rate upon stimulation with sodium chloride (Fig. 1*A*). The extent of inhibition depends on the inhibitor concentration. This is demonstrated in Fig. 1*B* which shows the dose-response curve of the acidification rate as a function of the inhibitor concentration for EMD 94309. All data points were

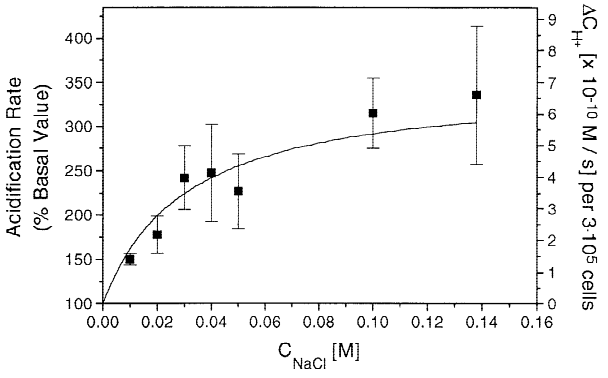


Fig. 3. Acidification rate as a function of Na^+ concentration. Solid line calculated according to Michaelis-Menten kinetics with K_M = 33 mM for Na^+. Temperature of measurement: 37°C. The proton excretion rate (i.e., the change in proton concentration with time) measured with the Cytosensor (right ordinate) after stimulation with 30 mM NaCl is $1.5 \cdot 10^{-10}$ M/sec for $3 \cdot 10^5$ cells (corresponding to $5 \cdot 10^{-16}$ M H^+/sec per cell) and is in good agreement with the results obtained from other assays, e.g., $1.2 \cdot 10^{-10}$ M/sec for $3 \cdot 10^5$ cells (Moolenaar et al., 1981*a*).

measured in triplicate. The IC_{50} value as deduced from the midpoint of the dose-response curve is 5 ± 1 nM for EMD 94309. The IC_{50} values of all compounds measured are summarized in the Table and are compared with the results obtained with the $^{22}Na^+$ isotope assay for erythrocytes and mouse fibroblasts. Dose-response curves were obtained by following the radioactive influx of ^{22}Na into erythrocytes on mouse fibroblasts in the presence of increasing concentrations of inhibitor. The Table demonstrates that the agreement between the Cytosensor measurements and the $^{22}Na^+$ exchange is excellent for three of the drugs and within a factor of 2–3 for one compound.

Reversibility of inhibitor binding was tested by measuring the signal recovery. After complete inhibition of the NaCl stimulated signal the cells were superfused first for 30 min with 138 mM TMA solution and then stimulated once again with 138 mM NaCl as described before. For EMD 84021 and EMD 94309 a nearly 100% signal recovery was observed, i.e., the NaCl-induced signal had the same height as that measured before exposure to the inhibitor. This demonstrates that the measuring protocol did not affect the viability of the cells. EMD 96785 and HOE 642 showed only a reduced signal recovery of 79 and 56%, respectively (Table) (*cf.* below).

Discussion

The Na^+/H^+ exchange protein facilitates the transport of H^+ out of the cell via coupling to an inflow of Na^+. The coupling ratio is 1.0 as determined from flux measurements and electrophysiological studies (reviewed in Aronson, 1985). The simplest scheme which appears to fit most of the kinetic experiments is a single site interaction, i.e., H^+, Na^+, and potential inhibitors compete for a single binding site on the outside as well as on the inside of the cell membrane.

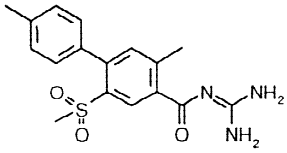
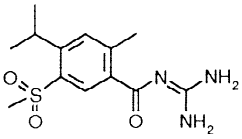
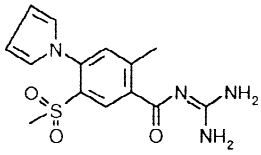
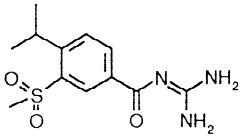
The activity of the Na^+/H^+ exchanger can be measured with several biochemical assays such as the $^{22}Na^+$ influx in reconstituted vesicles (Katz, Pick & Avron, 1989), the determination of the pH inside cells with fluorescent dyes (Ganz, Pachter & Barber, 1990), or the measurement of the Na^+ influx into rabbit erythrocytes by flame spectroscopy (Scholz, 1993). Recently, a microphysiometer (Cytosensor™) was proposed as a new and convenient method to measure the activity of the Na^+/H^+ antiporter (Neve, Kozlowski & Rosser, 1992). In the present study we have first compared the Cytosensor approach with established methods and have then applied it to recently developed NHE-1 inhibitors of clinical interest.

Na^+-Stimulation of the Na^+/H^+ Antiporter

Isotope methods directly detect the $^{22}Na^+$ influx whereas the present methods rely on the excretion of protons. Figure 2 reveals the time response of the Na^+/H^+ antiporter activation. Maximum stimulation of up to 350% of the background acidification rate is achieved immediately after exposure to Na^+. However, as the Na^+ stimulus continues the acidification rate is seen to decrease. After 120 sec stimulation, the acidification rate levels off at about 130% of the basal value. The variation of the proton excretion rate reflects the change of the proton gradient across the membrane. If the running medium is free of Na^+, H^+ appears to accumulate in the cell interior. During the first seconds of stimulation with Na^+ these protons are rapidly released and the H^+ gradient is reduced to a steady-state level where metabolic H^+ production balances H^+ excretion. $^{22}Na^+$ isotope exchange methods have a much lower time resolution; only the overall Na^+ exchange during the first few minutes can be measured. However, since a 1 Na^+: 1 H^+ exchange holds true for the NHE-antiporters, proton efflux is strictly correlated with Na^+ influx. The initial rate of Na^+ influx and H^+ efflux as measured with the Cytosensor can thus be expected to be larger than that derived from isotope measurements.

Figure 3 displays the initial acidification rate of CHO K1 cells after 6 sec stimulation as a function of the applied NaCl concentration. The rate of proton excretion increases with c_{NaCl} up to the highest salt concentration measured. It should be noted that the present measurements extend to c_{NaCl} = 138 mM whereas isotope exchange methods are limited to $c_{NaCl} \approx 40$ mM for technical reasons. The Na^+/H^+ exchange reaction can be described in terms of Michaelis-Menten kinetics according to $V = V_{max}[S]/(K_M + [S])$ where [S] is the sodium

Table. Comparison of the IC_{50} values measured with the Cytosensor™ microphysiometer, rabbit erythrocytes and mouse fibroblast cell lines

Substance	Structure	IC_{50} (CHOK1) [nM]	IC_{50} (rabbit erythrocytes)[a] [nM]	IC_{50} (mouse fibroblast) [nM]	Signal recovery [%]
EMD 84021		23 ± 7	25	33	100
EMD 94309		5 ± 1	2	6	100
EMD 96785		9 ± 2	8	10[a]	79
HOE 642		8 ± 2	26	20[a]	56

[a] Baumgarth et al., 1997.

concentration, K_M the Michaelis-Menten constant for Na^+, and V_{max} the maximum acidification rate. The scatter of the data introduces some ambiguity in the evaluation of V_{max} and K_M. The solid line in Fig. 3 was calculated with K_M = 30 mM and V_{max} = 350%. The % data in Fig. 3 may be used to calculate the H^+ excretion rate (right ordinate in Fig. 3) which can also be represented in a Lineweaver-Burk plot (Fig. 4). The straight line intersects the x-axis at $-1/K_M$ and the ordinate at $1/V_{max}$. The analysis of the data obtained without inhibitor yields K_M = 30 ± 4 mM. The K_M for external Na^+ at physiological external pH has been estimated as 3–50 mM (Aronson, 1985). A more recent measurement for the NHE-1 receptor, using the $^{22}Na^+$ uptake method, led to K_M = 14 mM (Counillon et al. 1997). The K_M value determined with the Cytosensor falls in the middle of the reported K_M values. It relates to the initial acidification rate measured after 6 sec stimulation whereas the ^{22}Na exchange methods represent the time average of the first few minutes. With a K_M of 30 mM it can further be concluded that the NHE-1 antiporter operates at 80% saturation level under physiological conditions.

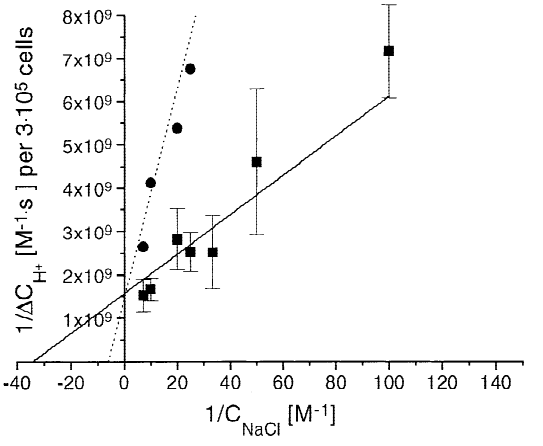


Fig. 4. Lineweaver-Burk plot of the acidification rate as a function of NaCl concentration. (■) no inhibitor present; (●) 10 nM EMD 96785.

In a competitive binding mode, addition of inhibitor should not affect V_{max} but should increase the IC_{50} value. This is indeed borne out experimentally. Figure 4 contains experimental results for Na^+ stimulation in the pres-

ence of EMD 96785 at 10 nM concentration. With competitive inhibition, the Michaelis-Menten model for the acidification rate is given by

$$\frac{1}{V} = \frac{1}{V_{\max}} + \frac{K_M}{V_{\max}} \left(1 + \frac{[I]}{K_I}\right) \cdot \frac{1}{[S]} \quad (2)$$

where [I] is the inhibitor concentration and K_I the inhibitor binding constant. The intersection with the abcissa, *a*, is found as

$$a = -\frac{1}{K_M\left(1 + \frac{[I]}{K_I}\right)} \quad (3)$$

which may be written as

$$\frac{1}{a} = -K_M \left(1 + \frac{[I]}{K_I}\right) \quad (4)$$

A plot of the 1/*a* values for three different inhibitor concentrations (5 nM data not shown) *vs.* [I] yields a straight line with $K_M = 30 \pm 5$ mM and $K_I \approx 2.1$ nM. The inhibition constant of EMD 96785 in terms of the competitive inhibition model is thus $K_I = 2.1$ nM.

The IC_{50} value in the absence of Na^+ is identical to K_I. Upon addition of NaCl, Na^+ competes with the inhibitor and the IC_{50} value increases according to

$$IC_{50} = K_I \left(1 + \frac{[S]}{K_M}\right) \quad (5)$$

At a Na^+ concentration of [S] = 138 mM an IC_{50} value of $IC_{50} = 2.1\ (1 + 138/30) = 11.8$ nM is predicted which is in agreement with the direct measurement of 9 ± 2 nM. Taken together, the quantitative evaluation of the Cytosensor data in terms of Michaelis-Menten kinetics provides a consistent picture of K_M, K_I and IC_{50} values for the compounds investigated.

Inhibition of the Na^+/H^+ Antiporter

The inhibitory potency of EMD 96785 and HOE 642 has been determined before using mouse fibroblast cell lines and measuring the ^{22}Na uptake into the cells after acidosis. The IC_{50} value for the NHE-1 receptor were 10 nM (20 nM) for EMD 96785 (HOE 642) (Baumgarth et al., 1997). No IC_{50} values for mouse fibroblasts were available for EMD 84021 and EMD 94309 which were determined here with the ^{22}Na uptake assay. The Table then compares the IC_{50} values as determined from Na^+ uptake into rabbit erythrocytes and mouse fibroblasts with those of the Cytosensor. Inspection of the Table reveals an excellent agreement between the Cytosensor method and the radioactive assays. Three compounds have IC_{50} values in the range of 5–10 nM whereas EMD 84021 is distinctly less potent with $IC_{50} = 25$ nM. The comparison not only validates earlier measurements but also demonstrates that the microphysiometer provides quantitative dose-response curves in a rather short time period.

An important aspect is the recovery of the CHO K1 cells after the inhibition experiment (last column of Table). For EMD 84021 and EMD 94309 the acidification rate returns to almost its initial value after a 30-min perfusion with TMA indicating that all cells have remained viable during the whole course of the experiment. In contrast, for EMD 96785 and HOE 642 the acidification rate remains distinctly below its initial value after 30 min of perfusion with standard buffer. This suggests either a very slow release of the inhibitor from the receptor or an irreversible change of the CHO K1 cell.

Finally, an important future application of the Cytosensor measurement should be pointed out. It is well established, that G-protein coupled receptors such as the dopamine D_2-, α_2 adrenergic- and also the muscarinic receptor, modulate Na^+/H^+ exchange (Barber, McGuire & Ganz, 1989; Ganz et al., 1990; Neve et al., 1992). Under favorable circumstances the coupling of other receptors with the Na^+/H^+ exchanger should be detectable via the acidification rate. If the drugs of interest develop side effects, i.e., by binding to other receptors than the NHE protein, this could be reflected in the acidification rate and lead to a response of the Cytosensor. An interesting candidate appears to be the coupling between the muscarinic receptor and NHE-1 which is at present under investigation in our laboratory.

This work was supported by the Swiss National Science Foundation Grant 31-42058.94. The excellent technical assistance of Mr. R. Zissel in carrying out the $^{22}Na^+$-uptake studies is gratefully acknowledged.

References

Aronson, P.S. 1985. Kinetic properties of the plasma membrane Na^+-H^+ exchanger. *Annu. Rev. Physiol.* **47:**545–60

Barber, D.L. 1991. Mechanisms of receptor-mediated regulation of Na-H exchange. *Cell Signal* **3:**387–97

Barber, D.L., McGuire, M.E. Ganz, M.B. 1989. Beta-adrenergic and somatostatin receptors regulate Na-H exchange independent of cAMP. *J. Biol. Chem.* **264:**21038-42

Baumgarth, M., Beier, N., Gericke, R. 1997. (2-Methyl-5-(methylsulfonyl)benzoyl)guanidine Na^+/H^+ antiporter inhibitors. *J. Med. Chem.* **40:**2017–34

Clark, J.D., Limbird, L.E. 1991. Na/H exchanger subtypes: a predictive review. *Am. J. Physiol.* **261:**C945–C953

Counillon, L., Noel, J., Reithmeier, R.A., Pouyssegur, J. 1997. Random mutagenesis reveals a novel site involved in inhibitor interaction within the fourth transmembrane segment of the Na^+/H^+ exchanger-1. *Biochemistry* **36:**2951–9

Counillon, L., Scholz, W., Lang, H.J., Pouyssegur, J. 1993. Pharmacological characterization of stably transfected Na^+/H^+ antiporter

isoforms using amiloride analogues and a new inhibitor exhibiting anti-ischemic properties. *Mol. Pharmacol.* **44:**1041–1045

Feig, P.U., D'Occhio, M.A., Boylan, J.W. 1987. Lymphocyte membrane sodium-proton exchange in spontaneously hypertensive rats. *Hypertension* **9:**282–8

Ganz, M.B., Pachter, J.A., Barber, D.L. 1990. Multiple receptors coupled to adenylate cyclase regulate Na-H exchange independent of cAMP. *J. Biol. Chem.* **265:**8989–8992

Grinstein, S. 1988. Na^+/H^+ Exchange. CRC Press, Boca Raton, FL

Grinstein, S., Wieczorek, H. 1994. Cation antiports of animal plasma membranes. *J. Exp. Biol.* **196:**307–18

Katz, A., Pick, U., Avron, M. 1989. Characterization and reconstitution of the Na^+/H^+ antiporter from the plasma membrane of the halotolerant alga Dunaliella. *Biochim. Biophys. Acta* **983:**9–14

Krulwich, T.A. 1983. Na^+/H^+ antiporters. *Biochim. Biophys. Acta* **726:**245–64

Kuro-o, M., Hanaoka, K., Hiroi, Y., Noguchi, T., Fujimori, Y., Takewaki, S., Hayasaka, M., Katoh, H., Miyagishi, A., Nagai, R., Yazaki, Y., Nabeshima, Y. 1995. Salt-sensitive hypertension in transgenic mice overexpressing Na^+-proton exchanger. *Circ. Res.* **76:**148–153

Moolenaar, W.H., Boonstra, J., van der Saag, P.T., de Laat, S.W. 1981a. Sodium/proton exchange in mouse neuroblastoma cells. *J. Biol. Chem.* **256:**12883–7

Moolenaar, W.H., Mummery, C.L., van der Saag, P.T., de Laat, S.W. 1981b. Rapid ionic events and the initiation of growth in serum-stimulated neuroblastoma cells. *Cell* **23:**789–98

Neve, K.A., Kozlowski, M.R., Rosser, M.P. 1992. Dopamine D_2 receptor stimulation of Na^+/H^+ exchange assessed by quantification of extracellular acidification. *J. Biol. Chem.* **267:**25748–25753

Noel, J., Pouyssegur, J. 1995. Hormonal regulation, pharmacology, and membrane sorting of vertebrate Na^+/H^+ exchanger isoforms. *Am. J. Physiol.* **268:**C283–96

Rosskopf, D., Fromter, E., Siffert, W. 1993. Hypertensive sodium-proton exchanger phenotype persists in immortalized lymphoblasts from essential hypertensive patients. A cell culture model for human hypertension. *J. Clin. Invest.* **92:**2553–9

Sardet, D., Franchi, A., Pouyssegur, J. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na^+/H^+ antiporter. *Cell* **56:**271–80

Scholz, W., Albus, U., Linz, W., Martorana, P.A., Englert, H.C., Schölkens, B.A. 1993. Hoe 694, a new Na^+/H^+ exchange inhibitor and its effects in cardiac ischaemia. *Br. J. Pharmacol.* **109:**562–568

Scholz, W., Albus, U., Counillon, L., Gögelein, H., Lang, H.J., Linz, W., Weichert, A., Schölkens, B.A. 1995. Protective Effects of HOE 642, a selective sodium-hydrogen exchange subtype 1 inhibitor, on cardiac ischaemia and reperfusion. *Cardiovasc. Res.* **29:**260–268

Yun, C.H., Tse, C.M., Nath, S., Levine, S.L., Donowitz, M. 1995. Structure/function studies of mammalian Na-H exchangers—an update. *J. Physiol.* **482:**1S–6S