Kinetic Properties of the Na^+/H^+ Antiporter of Lymphocytes from the Spontaneously Hypertensive Rat: Role of Intracellular pH

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Abstract

Enhanced activity of the Na^+/H^+ antiporter is increasingly reported as a feature of cells from hypertensive subjects but the intracellular pH (ipH) dependency of its activity has not been examined. This study was designed to characterize the kinetic properties of the Na^+/H^+ antiporter in lymphocytes from adult spontaneously hypertensive rats (SHR) and in those from age-matched normotensive Wistar-Kyoto (WKY) controls. Steady-state ipH, estimated from the measurement of BCECF fluorescence, was significantly lower in lymphocytes from the SHR than in those from WKY rats (7.09±0.02, n = 17 and 7.17±0.03, n = 19, respectively, $P < 0.025$). The velocity of the antiporter determined from the product of the change in intracellular hydrogen ion concentration (i[H^+]) by the buffering power measured concurrently at each starting ipH exhibited similar kinetic parameters in SHR and WKY cells: V_{max}, 72±18 vs. 79±24 mM H^+/30 s; pK_H, 10.04±0.87 vs. 8.49±0.80; and Hill coefficient, 1.67±0.12 vs. 1.44±0.10, respectively. Likewise, no significant differences were observed between SHR and WKY cells in either the K_m (29±5 and 32±8 mM, respectively) or the V_{max} (6.0±1.0 and 5.53±1.0 mM H^+/30 s, respectively) of the sodium activation curve. We conclude that while the ipH of SHR lymphocytes is reduced, the kinetic properties of the Na^+/H^+ antiporter are virtually identical in SHR and WKY lymphocytes. Consequently, a primary abnormality in the activity of this antiporter is not an inherent feature of lymphocytes from the SHR model of genetic hypertension. We propose that the activity of the Na^+/H^+ antiporter in SHR cells is apt to be increased as a result of reduction in ipH which dictates a higher set point in its steady-state activity. (*J. Clin. Invest.* 1990. 85:1734–1739.)
intracellular pH • hypertension • sodium antiporter exchange

Introduction

A Na^+/H^+ antiporter, which is present in the plasma membrane of virtually all animal cells, is involved in transepithelial ion transport, the control of intracellular pH (ipH),[1] and the regulation of cell volume (1, 2). Activation of the Na^+/H^+ antiporter has been shown to participate in the initiation of cell growth and proliferation and as a mediator of agonist-induced vasoconstriction in vascular smooth muscle cells and glomerular mesangial cells (3–9). A possible role of the Na^+/H^+ antiporter in the pathogenesis of hypertension was postulated by Aronson (10) and further supported by subsequent reports portraying increased activity of this antiporter in cells from the spontaneously hypertensive rats (SHR) (11) and hypertensive humans (12). This notion is particularly attractive in view of evidence showing that the Na^+/H^+ antiporter may correspond to the sodium/lithium countertransport (13), a transporter whose activity is well known to be elevated in red blood cells from a majority of patients with essential hypertension (14, 15). From the evidence just mentioned, it is tempting to postulate that the gene controlling the Na^+/H^+ antiporter may be altered in human primary hypertension.

We have recently shown that steady-state ipH is lower in lymphocytes from the SHR than in those from their normotensive Wistar-Kyoto (WKY) counterparts (16). The basis for this finding and its relationship with the activity of the Na^+/H^+ exchanger, however, remains undefined. A reduction in the activity of the Na^+/H^+ antiporter either qualitative (for instance, a decrease in its affinity for i[H^+]) or quantitative (for example, a decrease in the number of exchangers in the plasma membrane) could result in reduced H^+ extrusion and, thus, reduced ipH. On the other hand, a primary reduction in steady-state ipH could enhance the activity of the Na^+/H^+ antiporter of SHR cells even if the kinetic properties of this exchanger were not different from normal. To investigate these two possibilities the present study was designed to characterize the kinetic properties of the Na^+/H^+ exchanger and its dependence on ipH. The intracellular buffering power was also measured at different levels of ipH to express the velocity of the antiporter as ion (H^+) flux and to determine whether any differences in ipH-dependent cell buffering power exist between SHR and WKY lymphocytes. We reasoned that this approach should be advantageous over previous work portraying a higher Na^+/H^+ exchange activity in cells from hypertensive subjects using more indirect methods (11, 12) which provide only rough estimations for the kinetic parameters of activation of the antiporter.

Methods

Preparation of lymphocytes. SHR and normotensive WKY rats of ~ 16–20 wk of age were obtained from Taconic Farms, Inc. (Germantown, NY) and placed on a standard rat chow diet (Ralston Purina, St. Louis, MO). The thymus gland was removed from anesthetized rats (phenobarbital 5 mg/100 g body weight) and placed in RPMI 1640. The thymus was then cleared of blood vessels, minced and

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1. *Abbreviations used in this paper:* BCECF, 2',7'-bis(2-carboxyethyl)-5,(6)-carboxyfluorescein; ipH, intracellular pH; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto.

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pipetted, and filtered through gauze to remove extraneous tissue. The resulting suspension of thymocytes (essentially T-lymphocytes) was washed twice in RPMI at 150 *g* for 5 min. Cells were then counted and concentrated to 20×10^6 cells/ml and loaded with the acetoxymethyl-ester form of 2',7'-bis(2-carboxyethyl)-5,(6)-carboxyfluorescein (BCECF-AM) (2 μg/ml) at 37°C for 30 min. Cells were washed three times and resuspended in Hepes buffer containing in mM: NaCl, 135.0; KCl, 3.5; $CaCl_2$, 1.25; KH_2PO_4, 1.2; Hepes, 5.0; dextrose, 3; pH = 7.40.

Measurement of ipH. The dye loaded cells were kept under constant magnetic stirring in a thermostatically controlled cuvette (37°C) of a spectrofluorometer (model LS = 5; Perkin-Elmer Corp., Norwalk, CT), connected to a computerized system (PC-XT; IBM Instruments, Inc., Danbury, CT) programmed for data acquisition. The computer is programmed to calculate a fluorescence excitation ratio (500:440) and convert it to ipH measurements based on a calibration curve performed before each experiment (see below). The dye was alternately excited at 500 and 440 nm and its emission was measured at 520 nm. Measurements of BCECF fluorescence after excitation at 500 nm were continuous, whereas at 440 nm were discontinuous (Fig. 1). The emission after excitation at 500 nm is directly proportional to the total amount of unprotonated dye whereas the emission after excitation at 440 nm is proportional to the total amount of dye and remains unchanged as dye protonation changes. The 500:400 ratio generated intermittently is then proportional to the unprotonated dye but is not affected by either the total amount of intracellular dye or the number of cells used in each sample. With the aid of a calibration curve generated for each experiment, each 500:440 ratio of BCECF fluorescence corresponds to an ipH value taken on an average cell.

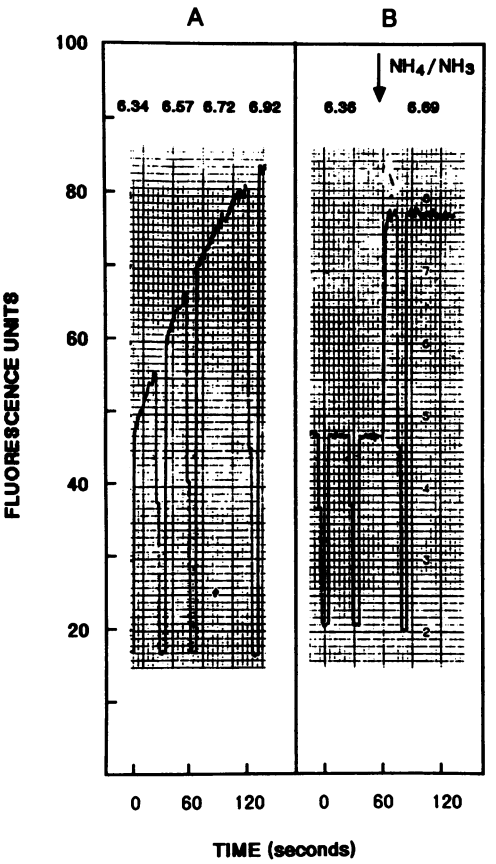

Figure 1. Depicts measurements of ipH from the 500:440 ratio of BCECF fluorescence. (*A*) A representative tracing showing ipH recovery after intracellular acidosis. Cells were acidified using nigericin in Na^+-free Hepes media. After the removal of nigericin, the rate of recovery in the presence of external Na^+ is linear over the first 30 s (data used for kinetic analysis). (*B*) A representative tracing showing no ipH recovery in the absence of external Na^+. NH_4Cl was added at the arrow to assess the buffering power at the same starting ipH.

The calibration procedure was performed using the nigericin technique described by Thomas et al. (17). Our calibration solution contained Hepes (5 mM), 120 mM K^+, and nigericin (2 μg/ml) and was titrated to external pH between 6.2 and 7.6 using dilute acid (HCl) or base (NaOH). Over this pH range the curve generated by plotting the 500:440 ratio against ipH is essentially linear, whereas the curve flattens when ipH is < 6.0 and > 7.8 (18, 21). Accordingly, ipH measurements at very low ipH were not attempted. Leakage of BCECF from cells is slow (18–21). In our preparation of lymphocytes the rate of BCECF leakage is minimal (< 5%/h) (16). Since steady-state ipH measurements were taken shortly after the last wash (10–15 min after dye loading) correction for external dye was not deemed necessary. In experiments assessing ipH recovery from acid loading, the acidified cells were spun down thereby removing all external dye before they were resuspended in the proper media to allow ipH recovery.

Buffering power. The estimation of acid fluxes via Na^+/H^+ exchange from ipH measurements requires a knowledge of the intracellular buffering power. Buffering power was evaluated by exposing the cells to NH_4^+/NH_3 using the NH_4Cl (5 mM) pulse technique (22) (Fig. 1). The buffering power is obtained from $\Delta iNH_4^+/\Delta ipH$ where ΔiNH_4^+ is the amount of iNH_4^+ formed by this alkaline pulse and ΔipH is the change in ipH (ipH final–ipH initial).

Cell volume. Because Na^+/H^+ exchange activity is measured as H^+ flux per volume of intracellular space, it is important to establish the volume of the cells under study. Accordingly, we measured cell volumes in SHR and WKY lymphocytes using a coulter counter (model ZM; Coulter Instruments, Hialeah, FL) as described by Grinstein et al. (19).

Intracellular acidification procedure and ipH recovery. Various degrees of cellular acidification were achieved by exposing the cells to nigericin (0.5 μg/ml) in Na^+-free media containing a K^+ concentration of 2–5 mM.

The acidification process was stopped at the desired ipH level by adding defatted BSA (5 mg/ml) (21). Cells so acidified were spun down and resuspended in a small volume (50 μl) of Na^+-free media. Each aliquot of acidified cells was divided in two. One-half was added to normal media (Na^+, 135 mM) to observe the ipH recovery whereas the other half was added to Na^+-free media to make a concurrent measurement of the buffering power (or capacity) at exactly the same starting ipH (Fig. 1). The kinetic analysis of the Na^+/H^+ antiporter was based on data obtained during the initial 30 s of ipH recovery. The rate of ipH recovery in the presence of external Na^+ appeared linear over the first 30 s as observed on a chart recorder hooked to the spectrofluorometer (Fig. 1 *A*). In the nominal absence of HCO_3, this initial (30 s) rate of ipH recovery in lymphocytes is virtually dependent on Na^+/H^+ exchange as it can be completely obliterated by amiloride and more specific inhibitors of the antiporter such as EIPA (16, 20, 21). In the absence of external Na^+, the antiporter is inhibited and ipH recovery is therefore not observed (Fig. 1 *B*).

The Na dependency of ipH recovery was examined in separate experiments in cells acidified to an ipH of ~ 6.6 and exposed to media containing different Na^+ concentrations (0, 9, 27, 54, 81, 108, and 135 mM) with choline substituting for Na^+ to maintain isoosmolarity.

Parameters of activation of the Na^+/H^+ exchanger. The speed of recovery from an acid load (mmol H^+/liter cells/30 s) was determined from the product of the change in $i[H^+]$ observed over the first 30 s by the cell buffering capacity measured in the same sample at the same starting ipH. The parameters of proton activation of the Na^+/H^+ exchanger were obtained according to the Hill kinetic model:

$$V = \frac{V_{max} \times i[H^+]^{n_H}}{K_H + i[H^+]^{n_H}} \qquad \text{(model 1)}$$

or

$$V = \frac{V_{max} \times 10^{(-n_H \times ipH)}}{10^{(-pK_H)} + 10^{(-n_H \times ipH)}},$$

where V is the initial velocity measured over the first 30 s; V_{max} is maximal initial velocity; n_H is the Hill coefficient of cooperativity; and K_H is the Hill constant; $pK_H = -\log(K_H)$.
The ipH at which $V = \frac{1}{2}V_{max}$ is referred to as pKm, calculated as pK_H/n_H. The standard error of pKm is calculated as:

$$\frac{SE\ pKm}{pKm} = \frac{SE\ pK_H}{pK_H} - \frac{SE\ n_H}{n_H}.$$

The activation of net transport by external Na^+ ($[Na^+]_0$) was fit by Michaelis-Menten kinetics ($n_H = 1$) and the parameters were obtained according to the model:

$$V = \frac{V_{max} \times [Na^+]_0}{K_m + [Na^+]_0}, \quad \text{(model 2)}$$

where V is initial velocity measured over the first 30 s; V_{max} is the maximal initial velocity; and K_m is the Michaelis-Menten constant, (i.e., the concentration of $[Na^+]_0$ at which $V = \frac{1}{2}V_{max}$).

Measurements were done in duplicate samples for each level of ipH within each experiment. Experimental data from individual rats were represented by the same number of recovery samples (usually four to five). All experimental data obtained within each strain were pooled for nonlinear analysis. Nonlinear regressions were performed by a NONLIN procedure of SYSTAT. Data were analyzed using the unpaired t test. Results are presented as mean±SE.

Results

Steady-state ipH and cell volume. In lymphocytes from the adult SHR, ipH was lower than in those from WKY rats (7.09±0.022, $n = 17$ and 7.17±0.03, $n = 19$, respectively, $P < 0.025$). Cell volume was not significantly different in lymphocytes from the two groups (SHR, 114±5.4 fl, $n = 6$; and WKY, 119±3.9 fl, $n = 6$). The systolic blood pressure, measured before each experiment by the awake tail cuff method, was significantly higher in SHR than in WKY rats (176±6 mmHg, $n = 17$ and 99±3 mmHg, $n = 19$, respectively, $P < 0.001$).

Buffering power. As anticipated, buffering power expressed as millimolar H^+/pH unit, displayed an ipH-dependent pattern (Fig. 2). No significant differences in intrinsic buffering power (i.e., that contributed by buffers other than HCO_3/CO_2) were observed between SHR and WKY cells at any of the levels of ipH studied (range 6.2 to 7.2).

Table I. Parameters of Na^+ Activation of the Na^+/H^+ Exchanger Obtained from Simple Michaelis-Menten Kinetics

	Initial ipH	K_m	V_{max}	V_{max}
		mM	*pH/30 s*	*mM H^+/30 s*
SHR (5)	6.64±0.01	29±5	0.23±0.045	6±1
	NS	NS	NS	NS
WKY (7)	6.66±0.02	32±8	0.26±0.015	5.5±1.0

Activation of Na^+/H^+ exchange by external Na^+. For these experiments, lymphocytes from SHR and WKY rats were acidified to about the same level of ipH (Table I). ipH recovery in the presence of external sodium (135 mM) was similar in SHR and WKY lymphocytes (ΔipH = 0.19±0.02 and 0.22±0.01 pH units/30 s, respectively). Activation of H^+ transport (i.e., H^+ extrusion rate) after intracellular acid loading exhibited saturability characteristics and first order dependence on external sodium (Fig. 3). The kinetic parameters of Na^+ activation of the Na^+/H^+ exchanger were derived according to simple Michaelis-Menten kinetics. No significant differences in either the K_m or V_{max} of the $[Na^+]_0$ activation curve were observed between SHR and WKY lymphocytes (Table I).

Activation of Na^+/H^+ exchange by ipH. Activation of the antiporter by internal H^+ (i.e., ipH), measured as H^+ efflux, exhibited a non-Michaelis-Menten behavior as suggested by the concavity of the activation curve (Fig. 4) and evidenced by a degree of cooperativity > 1 by Hill kinetic analysis. (Table II). No significant difference in the ipH dependent velocity of the antiporter was observed between SHR and WKY lymphocytes as the curves generated using lymphocytes from each strain appeared indistinguishable (Fig. 4). The parameters of activation of the Na^+/H^+ exchanger by ipH obtained from the

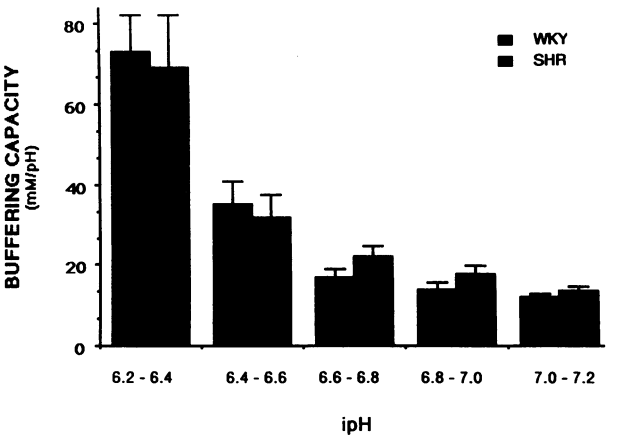

Figure 2. Intracellular buffering power of SHR and WKY lymphocytes at different ipH levels. The buffering power was evaluated by exposing the cells to 5 mM NH_4Cl. No differences were found between SHR and WKY cells.

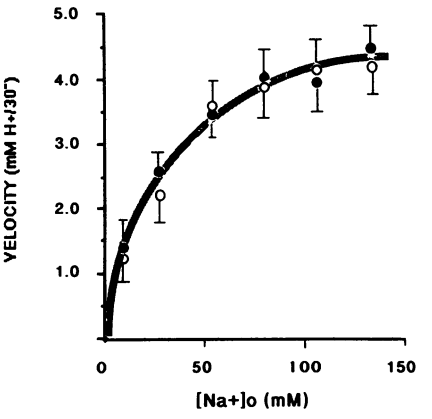

Figure 3. Na^+ activation curve of the Na^+/H^+ exchanger. The 30-s rate of ipH recovery from acid loading (initial ipH ~ 6.6) is plotted against different Na^+ concentrations. The curve exhibits saturability characteristics and first order dependence on external Na^+. No significant differences were observed between SHR and WKY cells. ○ SHR; ● WKY.

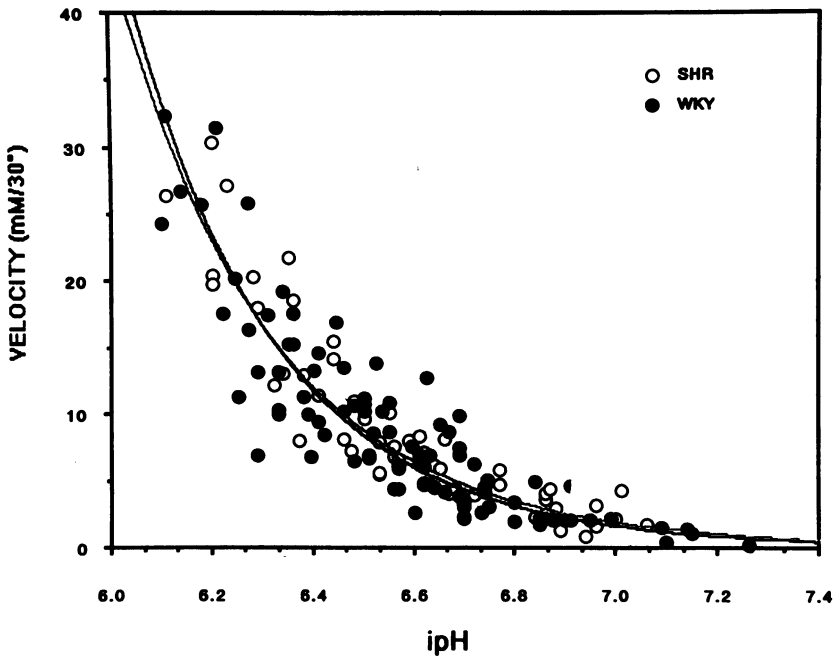

Figure 4. ipH dependent activation curve of the Na^+/H^+ exchanger. The velocity of recovery at each starting ipH was obtained from the product of the change in $i[H^+]$ noted over the first 30 s by the buffering power ($\Delta[NH4^+]i/\Delta i[H^+]$) measured simultaneously at the same starting ipH. The cooperative behavior is demonstrated by the concavity of the curve. No differences were found between SHR and WKY cells as shown by the respective activation curves which are virtually indistinguishable.

Hill kinetic model were virtually identical between SHR and WKY lymphocytes (Table II).

It should be noted that our calculations of V_{max} and n_H are extrapolations of the data. Ideally, data points closer to the V_{max} should be obtained but ipH values below 6.0 will certainly strain the BCECF method of ipH measurement because the linearity of the 500:440 ratio as a function of ipH falls when pH is < 6.2. Additionally, such severe levels of intracellular acidosis would probably involve the disturbance of several cell structures and functions such that the relevance of these conditions to normal physiology would be questionable. The essential point of similarity of the kinetic properties of the Na^+/H^+ exchanger in SHR and WKY cells can be inferred from the mere inspection of the data points (Fig. 4). Our data were fitted in a widely accepted kinetic model of the Na^+/H^+ exchanger (23–25), and the parameters thus generated were virtually identical in SHR and WKY cells.

Discussion

The present study demonstrates that the parameters of activation of the Na^+/H^+ exchanger by either intracellular hydrogen ion concentration (i.e., ipH) or external sodium are virtually identical in SHR and WKY lymphocytes. Thus, when lymphocytes from SHR and WKY rats are acidified to the same extent, their respective rates of H^+ extrusion and their dependence on either external Na^+ (Fig. 3) or ipH (Fig. 4) are remarkably similar. In addition, no significant differences in cell buffering power were observed between SHR and WKY lymphocytes at various levels of ipH (Fig. 2). This suggests that cells from these two strains have basically the same intracellular make-up of buffering systems. Cell volume was found to be similar in SHR and WKY cells in agreement with a previous study using lymphocytes (11).

Our data also reflect on the mode of operation of the Na^+/H^+ exchanger in lymphocytes. A greater than first order dependence on the internal H^+ concentration is evidenced by a degree of cooperativity greater than one obtained by Hill analysis. Other investigators have reported similar findings in various cell types (20, 23–28). This may suggest more than one transport site for internal H^+. Since Na^+/H^+ exchange is electroneutral with a Na^+/H^+ coupling ratio of 1 (20, 23–29), the cooperative behavior of the antiporter would imply that its stoichiometry is 2:2, 3:3, etc. The Na^+ activation curve of the antiporter, however, exhibited simple Michaelis-Menten kinetics and first order dependence on external Na^+ which argues strongly against the possibility of more than one transport site. These findings are best explained on the basis of the modifier site theory proposed by Aronson et al. (23). That is, the exchanger possesses only a single transport site for internal H^+ but one or more modifier sites at which internal H^+ binds and thereby activates the exchanger without being transported.

To our knowledge, the present study is the first in examining the kinetic parameters of intracellular hydrogen ion activation of the Na^+/H^+ antiporter in a hypertensive model. This analysis is particularly critical since we found that steady-state ipH of SHR lymphocytes is reduced as compared to that of WKY controls. Importantly, this finding is not peculiar to the SHR as a reduction in ipH has been recently reported in eryth-

Table II. Parameters of Proton Activation of the Na^+/H^+ Exchanger Obtained from Hill Kinetic Model

	V_{max}	pK_H	n_H	pK_m
	mM H^+/30 s			
SHR (15)	72±18	10.04±0.87	1.67±0.12	6.0±0.09
	NS	NS	NS	NS
WKY (17)	79±24	8.49±0.80	1.44±0.10	5.9±0.15

n_H, Hill coefficient of cooperativity; K_H, Hill constant; and $pK_H = -\log(K_H)$.
The ipH at which $V = \frac{1}{2}V_{max}$ is referred to as pK_m, calculated as pK_H/n_H.

rocytes from humans with essential hypertension (30). This finding in cells from hypertensive subjects assumes importance given the exquisite sensitivity of the activity of the antiporter to internal H^+. That is, a reduction in ipH dictates a concurrent enhancement in the activity of the Na^+/H^+ antiporter (Fig. 4). Thus, even though the parameters of activation of the Na^+/H^+ antiporter are virtually identical in SHR and WKY cells, its steady-state velocity has to be higher in the cells with the lower ipH. For the sake of illustration, the velocity of the exchanger at the steady-state ipH prevailing in SHR (7.09±0.02) and WKY cells (7.17±0.03) can be estimated at 1.29 mmol H^+/liter cells/30 s and 0.89 mmol H^+/liter cells/30 s, respectively (data derived from the parameters given in Table II). Accordingly, the existing difference in steady-state ipH between SHR and WKY cells in and of itself may result in a distinct difference in the activity of the Na^+/H^+ antiporter. In the presence of a chronic mechanism of intracellular acid accumulation in SHR cells, possibly increased metabolic acid production or other mechanism(s) of sustained acid accumulation, a new set-point is reached whereby ipH is reduced and Na^+/H^+ activity is secondarily elevated.

While the precise mechanism whereby ipH is reduced in lymphocytes from the SHR remains to be determined, it is clear from the ipH dependency of the Na^+/H^+ antiporter that such alteration is apt to result in an obligatory increase in its activity. In the present study we deliberately used a bicarbonate-free medium to examine the kinetic properties of the Na^+/H^+ antiporter in the absence of other pH regulatory transport systems. We wish to emphasize that a critical role of ipH as a driving force for the activity of the exchanger is likely to prevail under more physiologic conditions as we have recently demonstrated that in the presence of bicarbonate in the medium, ipH is also reduced in lymphocytes from the SHR (16).

Our findings of similar kinetic properties of the Na^+/H^+ antiporter in SHR and WKY lymphocytes stand in apparent contrast with previous investigations portraying an elevated activity of this exchanger in lymphocytes from the SHR (11) and in platelets from hypertensive subjects (12). There are significant methodological differences, however, in the approach used in those previous investigations and in the present study. Feig et al. (11) and Livne et al. (12) used an indirect method that is based on cellular volume changes after activation of the antiporter by cell acidification with sodium propionate. Using this methodology, the V_{max}, but not the K_m, of the Na^+ activation curve of the Na^+/H^+ exchanger was found higher in lymphocytes from the SHR as compared to WKY cells (11). The estimation of Na^+/H^+ exchange activity from cellular osmotic swelling, however, has major limitations. The increase in cell volume that follows the incubation of cells in a weak acid, such as sodium propionate, is contributed to, in large part, by the increase in the intracellular content of nondiffusible solute which necessitates an increase in cell water content to maintain isoosmolality (31, 32). This methodology is also confounded by the lack of direct ipH measurements to control the precise extent of cellular acidification. Further, the accurate estimation of Na^+/H^+ exchange activity from changes in cell volume after the activation of the exchanger, may be obscured by the contribution of other mechanisms of Na^+ transport that contribute to cellular osmotic swelling. Alterations in the Na^+/K^+ pump and other sodium transporters have been well described in hypertension (for review see reference 33). It is, thus, conceivable that such alteration(s), rather than an intrinsic overactivity of the Na^+/H^+ antiporter, may have accounted for the abnormal fractional osmotic swelling reported in these previous studies.

By measuring the rate of H^+ efflux, which is essentially linear over the first 30 s after the activation of the Na^+/H^+ exchanger, the method we used provides a more direct evaluation of the activity of the antiporter and permits the assessment of its ipH dependency. It should be noted that the dependency of the Na^+/H^+ antiporter on ipH is of greater physiologic relevance than its dependence on external Na^+. As shown in Fig. 3, the K_m of the Na^+ activation curve is low (~ 30 mM) so that the effect of changes in the concentration of extracellular Na^+ within the physiologic range on the activity of the Na^+/H^+ exchanger is of limited physiologic relevance.

Recent studies in cultured vascular smooth muscle cells from the SHR have revealed a higher V_{max} of the Na^+ activation curve of the antiporter (9, 34). The K_m, however, was similar to that of cells from the WKY rat. There is abundant evidence for accelerated growth in culture of VSMC from the SHR (9, 35, 36), and several studies have shown participation of the Na^+/H^+ antiporter in the initiation of cell growth (3, 4, 37). It is conceivable that increased growth rate in SHR cells may require recruitment of a greater number of antiporters in the plasma membrane or enhancement of their activity. Either circumstance would translate into increased V_{max}. This characteristic, if acquired in vitro, does not necessarily reflect that the kinetic properties of the antiporter of SHR cells are altered in vivo.

In summary, our finding of identical Na^+/H^+ kinetic parameters in SHR and WKY lymphocytes indicates that a primary alteration of this antiporter is not an inherent feature of cells from this model of genetic hypertension. We therefore conclude that the elevated activity of the Na^+/H^+ antiporter previously noted to be associated with hypertension (10–13), is not a primary abnormality. From the finding of similar kinetic properties of the Na^+/H^+ antiporter in SHR and WKY cells, it could be inferred that the gene controlling this transporter is not likely to be abnormal in the SHR and, by analogy, in individuals with primary hypertension. Thus, rather than a genetic alteration in the Na^+/H^+ antiporter itself, an enhancement in its activity as a physiologic response to a reduction in steady-state ipH is more likely. A role for ipH in hypertension is further suggested by our recent finding of reduced ipH in lymphocytes from the Dahl salt sensitive rat as compared to the salt-resistant strain (38). To the extent that data from lymphocytes can be extrapolated to cells directly involved in blood pressure regulation, our data suggest that a secondary enhancement in the activity of the Na^+/H^+ exchanger imposed by the reduced steady-state ipH (i.e., a higher set point) could play a role in the development of hypertension. This reduction in ipH, if extrapolated to renal cells, could very well account for enhanced Na^+ reabsorption and contribute to the development of salt-sensitive hypertension (10, 39). If manifested in smooth muscle cells enhanced Na^+/H^+ exchange activity could be involved in the development of hypertension either by causing augmented cell proliferation or by increasing vascular tone directly or via changes in intracellular calcium (16). Studies targeted to examine Na^+/H^+ exchange activity and ipH in vascular and renal tissue are clearly needed to substantiate or refute this contention.

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References

1. Mahensmith, R. L., and P. S. Aronson. 1985. The plasma membrane Na^+/H^+ exchanger and its role in physiological and pathophysiological processes. *Circ. Res.* 56:773–788.

2. Aronson, P. S. 1985. Kinetic properties of the plasma membrane Na^+/H^+ exchanger. *Annu. Rev. Physiol.* 47:545–560.

3. Moolenaar, W. H., R. Y. Tsien, P. T. Van Der Saag, and S. W. Delaat. 1983. Na^+/H^+ exchange and cytoplasmic pH in the action of growth factors in human fibroblasts. *Nature (Lond.).* 304:645–648.

4. Ganz, M. B., G. Boyarsky, R. B. Sterzel, and W. F. Boron. 1989. Arginine vasopressin enhances pHi regulation in the presence of HCO3 by stimulating three acid-base transport systems. *Nature (Lond.).* 337:648–651.

5. Smith, J. B., and T. A. Brock. 1983. Analysis of angiotensin-stimulated sodium transport in cultured smooth muscle cells from rat aorta. *J. Cell. Physiol.* 114:284–290.

6. Huang, C. L., M. G. Cogan, E. J. Cragoe, Jr., and H. E. Ives. 1987. Thrombin activation of the Na^+/H^+ exchange in vascular smooth muscle cells: evidence for a kinase C-independent pathway which is Ca-dependent and pertussis toxin sensitive. *J. Biol. Chem.* 262:14134–14140.

7. Mitsuhashi, T., and H. E. Ives. 1988. Intracellular calcium requirements for activation of the Na^+/H^+ exchanger in vascular smooth muscle cells. *J. Biol. Chem.* 263:8790–8796.

8. Hatori, N., B. P. Fine, A. Nakamura, E. Cragoe, Jr., and A. Aviv. 1987. Angiotensin II effect on cytosolic pH in cultured rat vascular smooth muscle cells. *J. Biol. Chem.* 262:5073–5078.

9. Berk, B. C., G. Vallega, A. J. Muslin, H. M. Gordon, M. Canessa, and R. W. Alexander. 1989. Spontaneously hypertensive rat vascular smooth muscle cells in culture exhibit increased growth and Na^+/H^+ exchange. *J. Clin. Invest.* 83:822–829.

10. Aronson, P. S. 1982. Red cell sodium-lithium countertransport and essential hypertension. *N. Engl. J. Med.* 307:317. (Letter.)

11. Feig, P. U., M. A. D'Occhio, and J. W. Boylan. 1987. Lymphocyte membrane Na^+/H^+ exchange in spontaneously hypertensive rats. *Hypertension (Dallas).* 9:282–288.

12. Livne, A., J. W. Balfe, R. Veitch, A. Marquez-Julio, S. Grinstein, and A. Rothstein. 1987. Increased platelet Na^+/H^+ exchange rates in essential hypertension: application of a novel test. *Lancet.* i:533–536.

13. Canessa, M. L., K. Morgan, and A. Semplicini. 1988. Genetic differences in lithium-sodium exchange and regulation of the sodium-hidrogen exchanger in essential hypertension. *J. Cardiovasc. Pharmacol.* 12(Suppl.):S92–S98.

14. Canessa, M., N. Adragna, H. S. Solomon, T. M. Connolly, and D. C. Tosteson. 1980. Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N. Engl. J. Med.* 302:772–776.

15. Cooper, R., D. LeGrady, S. Nanas, M. Trevisan, M. Mansour, P. Histand, D. Ostrow, and J. Stamler. 1983. Increased sodium-lithium countertransport in college students with elevated blood pressure. *JAMA (J. Am. Med. Assoc.).* 249:1030–1034.

16. Batlle, D. C., A. Saleh, and G. Rombola. 1990. Reduced intracellular pH in lymphocytes from the spontaneously hypertensive rat. *Hypertension.* 15:97–103.

17. Thomas, J. A., R. N. Buchhbaum, A. Zimniak, and E. Racker. 1979. Intracellular pH measurements in Ehrich ascites tumor cells utilizing spectrospopic probes generated in situ. *Biochemistry.* 18:2210–2218.

18. Grinstein, S., S. Cohen, and A. Rothstein. 1984. Cytoplasmic pH regulation in thymic lymphocytes by an amiloride sensitive Na^+/H^+ antiport. *J. Gen. Physiol.* 83:341–369.

19. Paradiso, A. M., R. Y. Tsien, and T. E. Machen. 1984. Na^+/H^+ exchange in gastric glands as measured with a cytoplasmic trapped, fluorescent pH indicator. *Proc. Natl. Acad. Sci. USA.* 81:7436–7440.

20. Grinstein, S., J. D. Goetz, and Rothstein. 1984. Na fluxes in thymic lymphocytes. II. Amiloride-sensitive Na^+/H^+ exchange pathway: reversibility of transport and asymmetry of the modifier site. *J. Gen. Physiol.* 84:585–600.

21. Grinstein, S., and W. Furuya. 1986. Characterization of the amiloride-sensitive Na^+/H^+ antiport of human neutrophils. *Am. J. Physiol.* 250:283–291.

22. Roos, A., and W. F. Boron. 1981. Intracellular pH. *Physiol. Rev.* 61:296–434.

23. Aronson, P. S., J. Nee, and M. A. Suhm. 1982. Modifier role of internal H^+ in activating the Na^+/H^+ exchanger in renal microvillus membrane vesicles. *Nature (Lond.).* 229:161–163.

24. Miller, R. T., and A. S. Pollock. 1987. Modification of the internal pH sensitivity of the Na^+/H^+ antiporter by parathyroid hormone in a cultured renal cell line. *J. Biol. Chem.* 262:9115–9120.

25. Warnock, D. G., and A. S. Pollock. 1988. Na^+/H^+ exchange in epithelial cells. *In* Na^+/H^+ Exchange. S. Grinstein, editor. CRC Press, Inc., Boca Raton, FL. 77–87.

26. Boron, W., and W. L. Boulpaep. 1983. Intracellular pH regulation in the renal proximal tubule of the salamander Na^+/H^+ exchange. *J. Gen. Physiol.* 81:29–52.

27. Vigne, P., C. Frelin, and M. Lazdunski. 1984. The Na^+ dependent regulation of the internal pH in chick skeletal muscle cells. The role of the Na^+/H^+ exchange system and its dependence on internal pH. *EMBO (Eur. Mol. Biol. Organ.) J.* 3:1865–1870.

28. Murer, H., U. Hopfer, and R. Kinne. 1976. Sodium/proton antiport in brush bordeer membrane vesicles isolated from rat small intestine and kidney. *Biochem. J.* 154:597–604.

29. Kinsella, J. L., and P. S. Aronson. 1982. Determination of the coupling ratio for Na^+/H^+ exchange in renal microvillus membrane vesicles. *Biochim. Biophys. Acta.* 689:161–164.

30. Resnick, L. M., R. K. Gupta, E. Sosa, M. L. Corbett, and J. H. Laragh. 1987. Intracellular pH in human and experimental hypertension. *Proc. Natl. Acad. Sci. USA.* 84:7663–7667.

31. Cooke, K. R., and A. D. C. Macknight. 1984. Effects of medium acetate on cellular volume in rabbit renal cortical slices. *J. Physiol. (Lond.).* 349:135–156.

32. Macknight, A. D. C., C. W. McLaughlin, and R. J. Scott. 1988. Sodium-hydrogen exchange in rabbit renal cortical slices incubated in acetate media. *J. Physiol. (Lond.).* 398:523–541.

33. Haddy, F. J., M. B. Pamnani, and D. L. Clougue. 1987. Pathophysiological role of cation transport and natriuretic factors in hypertension. *Hypertension.* 10(Suppl.):101–107.

34. Kuriyama, S., T. N. Denny, and A. Aviv. 1988. Na^+ and Rb^+ Transport in vascular smooth muscle cells of SHR, wistar kyoto and wistar rats. *J. Cardiovasc. Pharmacol.* 11:722–729.

35. Yamori, Y., T. Igawa, M. Tagami, T. Kanbe, Y. Nara, M. Kihara, and R. Horie. 1984. Humoral trophic influence on cardiovascular structural changes in hypertension. *Hypertension.* 6(Suppl. III):27–32.

36. Haundenschild, C. C., J. Grunwald, and A. V. Chobanian. 1985. Effects of hypertension on migration and proliferation of smooth muscle in culture. *Hypertension.* 7(Suppl.):101–104.

37. L'Allemain, G., A. Franchi, E. Cragoe, Jr., and J. Pouyssegur. 1984. Blockade of the Na^+/H^+ antiport abolishes growth factor induced DNA synthesis in fibroblasts. *J. Biol. Chem.* 259:4313–4319.

38. Sobrero, M. I., A. Saleh, and D. C. Batlle. 1989. Decreased intracellular pH (ipH) in lymphocytes from the salt-sensitive Dahl rat. *Clin. Res.* 37:905A. (Abstr.)

39. Aviv, A., and A. Livne. 1988. The Na^+/H^+ antiport, cytosolic Ca^{++} and essential hypertension: a reassessment and a hypothesis. *Am. J. Hypertens.* 1:410–413.