Acid-Base Status and Intracellular pH Regulation in Lymphocytes From Rats With Genetic Hypertension

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ABSTRACT
This article reviews work from this laboratory dealing with acid-base status and intracellular pH (pH$_i$) regulation in rat genetic models of hypertension. With freshly isolated thymic lymphocytes, pH$_i$ and its regulation were examined in the spontaneously hypertensive rat (SHR). In this rat model, pH$_i$ was found to be reduced as compared with that of lymphocytes from normotensive Wistar-Kyoto (WKY) rats. The activity of the Na$^+$/H$^+$ antiporter assessed after stimulation by acute cell acidification was similar in lymphocytes from SHR and WKY rats both in the nominal absence of HCO$_3^-$ and in media containing HCO$_3^-$ (22 mM). The kinetic properties of the Na$^+$/H$^+$ antiporter, examined as a function of pH$_i$ with the Hill kinetic model, revealed no significant differences between lymphocytes from SHR and WKY rats. The kinetic properties of the Na$^+$/H$^+$ antiporter and Na-independent Cl$^-$-HCO$_3^-$ exchangers, examined as a function of extracellular Cl$^-$, were also virtually identical in lymphocytes from SHR and WKY rats. Unlike the Na$^+$/H$^+$ exchanger and the Na-independent Cl$^-$-HCO$_3^-$ exchanger, which had their highest activities at extremes of pH$_i$ (low pH$_i$, Na$^+$/H$^+$ exchanger; high pH$_i$, Na-independent Cl$^-$-HCO$_3^-$ exchanger), the Na-independent Cl$^-$-HCO$_3^-$ exchanger had its maximal activity near steady-state pH$_i$. In Dahl/Rapp salt-sensitive rats with hypertension, the pH$_i$ of thymic lymphocytes was also reduced as compared with that of normotensive salt-resistant animals. In this model, renal net acid excretion in salt-sensitive rats was augmented as compared with that of salt-resistant rats. The increase in renal acid excretion was due to an increase in both ammonium and titratable acid excretion and was observed while animals were placed on high, normal and low salt diets. The findings of intracellular acidosis and enhanced renal acid excretion suggest that cellular acid overproduction is augmented in salt-sensitive hypertension.

Key Words: Acid base, intracellular pH$_i$, acid excretion, ammonium, salt-sensitive hypertension, Na$^+$/H$^+$ antiporter, Cl$^-$/HCO$_3^-$ exchanger, spontaneously hypertensive rat

The contributions of Dr. Jacob Lemann and his associates to the acid-base literature have been important and will be long lasting. This article, which is written to honor Dr. Jacob Lemann, will hopefully show that alterations in acid-base metabolism may be present in animal models of genetic hypertension and perhaps are relevant to the pathogenesis of human essential hypertension. Our recent finding that, in the salt-sensitive Dahl/Rapp rat, renal net acid excretion is augmented, possibly as a result of endogenous acid overproduction, will be discussed (1). Studies performed in our laboratory dealing with intracellular pH (pH$_i$) and its regulation by three plasma membrane pH, regulatory proteins (Na$^+$/H$^+$ antiporter, Na-dependent Cl$^-$/HCO$_3^-$ exchanger, and Na-independent Cl$^-$/HCO$_3^-$ exchanger) using thymic lymphocytes from the spontaneously hypertensive rat (SHR) will also be reviewed (2-4). In freshly isolated lymphocytes, pH$_i$ can be measured with minimal disruption of cellular structure and function. We thus think that unstimulated lymphocytes best portray the cellular acid-based environment prevailing in vivo in the whole animal. Work from other laboratories and clinical studies involving the Na$^+$/H$^+$ antiporter in cells from patients with essential hypertension will not be discussed in this article, which has been put together as a review of work from a single laboratory to honor Dr. Lemann's contributions to the acid-base literature.

Intracellular pH and Na$^+$/H$^+$ Antiporter Activity in Lymphocytes from the SHR

Various reports have claimed that the activity of the Na$^+$/H$^+$ antiporter is increased in circulating cells from hypertensive rats and subjects with essential hypertension (5-10). Because a low pH$_i$ in itself stimulates the activity of the Na$^+$/H$^+$ antiporter, we reasoned that pH$_i$ could be reduced in SHR cells and thereby act as a driving force for increased Na$^+$/H$^+$ exchange. Accordingly, we measured pH$_i$ in thymic lymphocytes obtained from SHR and WKY rats, as well as in freshly isolated Wistar-Kyoto (WKY) control rats using 2',7'-bis carboxyethyl-5,6-carboxyfluorescein, a pH-sensitive fluorescence probe. When the rats were age 16 to 20 wk, the pH$_i$ of lymphocytes suspended in a
HCO₃⁻-free N-hydroxyethylpiperazine-N′-2-ethanesulfonic acid-buffered solution was lower in SHR than in WKY rats (7.07 ± 0.02, N = 16 and 7.22 ± 0.01, N = 15, respectively; P < 0.001) (2). In rats less than 5 wk of age, the pH, was also lower in SHR than in WKY rat lymphocytes (7.12 ± 0.04, N = 11 and 7.23 ± 0.04, N = 11, respectively; P < 0.05), although at this age, systolic blood pressure was not different between the two groups (87 ± 4.0 and 85 ± 3.0 mm Hg, respectively).

In lymphocytes suspended in a more physiologic HCO₃⁻/CO₂-buffered solution, the pH, was again lower in adult SHR than in adult WKY rats (7.18 ± 0.02, N = 16 and 7.31 ± 0.02, N = 16, respectively; P < 0.001) (2). The blockade of Na⁺/H⁺ exchange by a specific inhibitor, ethyl-isopropyl-amiloride (EIPA), or by exposure to Na⁺-free media resulted in intracellular acidification in both SHR and WKY rat lymphocytes. The lower pH, in lymphocytes from SHR rats as compared with those from WKY rats persisted after the blockade of the Na⁺/H⁺ exchanger by EIPA (7.09 ± 0.04 versus 7.20 ± 0.03; P < 0.05) and by the removal of external Na⁺ (7.04 ± 0.05 versus 7.19 ± 0.02; P < 0.005) respectively (2). The persistence of the reduced pH, in SHR cells, as compared with WKY rat cells, after Na⁺/H⁺ blockade suggested that a primary abnormality in the activity of the Na⁺/H⁺ exchanger was unlikely to account for the observed reduction in the steady-state pH, of SHR cells (2). The possible basis for this alteration will be discussed later in this article.

In a subsequent study (3), we examined the kinetic parameters of intracellular hydrogen ion activation of the Na⁺/H⁺ antipporter in lymphocytes from the SHR. A reduction in pH, leads to an increase in the activity of the Na⁺/H⁺ exchanger both by providing a greater availability of substrate (H⁺ ions) and by titrating an internal modifier site that further enhances the Na⁺/H⁺ exchanger. The activation of the antipporter 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 (Figure 1) and as evidenced by a degree of cooperativity of more than 1 by Hill kinetic analysis (3). No significant difference in the pH₁-dependent activity of the antipporter was observed between SHR and WKY lymphocytes because the curves generated with lymphocytes from each strain appeared indistinguishable (Figure 1). The parameters of activation of the Na⁺/H⁺ exchanger by pH₁ obtained from the Hill kinetic model were virtually identical between SHR and WKY lymphocytes (3).

In this study, we again found that the steady-state pH₁ in SHR lymphocytes was reduced as compared with that of WKY controls (3). This finding is not peculiar to the SHR because a reduction in pH₁ has been reported by Resnick et al. (11) in erythrocytes from humans with essential hypertension. A low pH₁ in cells from hypertensive subjects could assume physiologic significance, given the exquisite sensitivity of the activity of the antipporter to internal H⁺. That is, a reduction in pH₁ could dictate a concurrent enhancement in the activity of the Na⁺/H⁺ antipporter under steady-state conditions (2,3). Thus, even though the parameters of activation of the Na⁺/H⁺ antipporter were found to be virtually identical in SHR and WKY cells, its steady-state activity ought to be higher in the cells with the lower pH₁. We therefore proposed that chronic intracellular acidosis in SHR cells results in a secondary increase in Na⁺/H⁺ activity (2,3).

Na⁺/H⁺ Exchange Activity in Cells Assayed in the Presence of HCO₃⁻/CO₂

Previous studies that had evaluated the Na⁺/H⁺ antipporter in cells from hypertensive subjects had been performed under conditions where bicarbonate/carbon dioxide (HCO₃⁻/CO₂), the physiologic buffer system, was absent from the assay media (5–10). This, by design, results in an overestimation of the role of the Na⁺/H⁺ antipporter in the regulation of pH₁ and ignores the existence of other HCO₃⁻-dependent transporters that contribute not only to pH₁ regulation but also to the regulation of cell volume, cell growth, and intracellular sodium (4,12–15). The overactivity of either one or both of these transporters, particularly if accompanied by reduced sodium extrusion via the plasma membrane Na⁺/K⁺ ATPase pump, would explain the elevated levels of intracellular sodium recently reported by us in lymphocytes from the SHR (16) and by others in the red blood cells of subjects with primary hypertension (17).
We thus undertook further studies to examine the activity of the Na+/H⁺ antipporter in the presence of HCO₃⁻/CO₂ and the activity and kinetic properties of the Na⁺-dependent and the Na⁺-independent Cl⁻/HCO₃⁻ exchangers (4). Freshly isolated thymic lymphocytes from SHR and WKY rats were also used for these studies. Young animals with early hypertension (6 to 8 wk of age) were used, because in older animals, the involution of the thymus gland starts occurring and this limits the number of cells that can be harvested. As previously reported by us in lymphocytes assayed in a HCO₃⁻-free solution (2,3), the steady-state pHᵢ in cells from the SHR (N = 11) was lower than that of cells from age-matched control WKY rats (N = 11) (7.21 ± 0.01 and 7.29 ± 0.02, respectively; P < 0.05) (4). In lymphocytes assayed in an HCO₃⁻/CO₂-containing solution, the pHᵢ also was lower in SHR (N = 11) than in WKY rats (N = 11) (7.09 ± 0.03 and 7.14 ± 0.01, respectively), but in this study, the difference did not reach statistical significance (4). It is pertinent to note here that, in platelets from humans with essential hypertension and in cultured vascular smooth muscle cells from the SHR, the overactivity of the Na⁺/H⁺ antipporter has been described in association with a normal (18) or a high pHᵢ (7). These seemingly contradictory findings can be explained, in our opinion, on the basis of in vitro platelet hyperactivity, which has been described in platelets from hypertensive subjects (19), or the stimulation of the antipporter during enhanced growth in culture, a feature of vascular smooth muscle cells from the SHR (7,20). The overactivity of the antipporter in these cell models may result from protein kinase C activation (21).

The possibility of cell-specific differences in pHᵢ between hypertensive and normotensive subjects, however, needs to be considered as well. The differential regulation of the antipporter by acid feeding has been reported by Moe et al. (22). Those authors have shown that acid feeding increases Na⁺/H⁺ antipporter mRNA levels in renal cells but decreases it in fibroblasts (22). We have further shown that, in cultured vascular smooth muscle cells, lowering the pH of the media results in decreased Na⁺/H⁺ exchange activity (23).

To study the activity of pHᵢ regulatory transporters involved in the recovery from cell acidification, pHᵢ was lowered to about 6.4 by the NH₄Cl technique (4). pHᵢ recovery in the presence of HCO₃⁻/CO₂ was fast in the first minute and usually complete by 10 min (Figure 2). The removal of Na⁺ from the recovery solution (choline replacement) completely obliterated the initial rate of pHᵢ recovery, and in fact, pHᵢ continued to fall during the first minute. In the presence of EIPA, pHᵢ recovery was also obliterated during the initial first minute, but thereafter, a progressive increase in pHᵢ was observed. Thus, pHᵢ recovery at 10 min was not completely blocked by EIPA (Figure 2). The absence of external Na⁺ produced a greater degree of inhibition than EIPA. The addition of DIDS had no effect on the initial pHᵢ recovery (first minute), although it had a slight but consistent inhibitory effect as pHᵢ was returning to steady state (Data not shown in Figure 2).

The activity of the Na⁺/H⁺ antipporter was derived from data obtained in the first 40 s of pHᵢ recovery. The velocity of recovery was expressed as H⁺ flux derived from the product of the change in pHᵢ observed in the first 40 s times the buffering power (millimoles of H⁺ per liter per ΔpHᵢ) measured at the departing pHᵢ (approximately 6.4). Buffering power was measured as previously described (24).

As expected, the activity of the Na⁺/H⁺ antipporter during pHᵢ recovery, expressed as H⁺ flux, was dependent on pHᵢ, such that its activity markedly decreased as pHᵢ increased toward steady-state pHᵢ (Figure 3). As a function of pHᵢ, SHR and WKY rat lymphocytes displayed a virtually identical behavior in terms of Na⁺/H⁺ exchange activity, evaluated as EIPA-sensitive pHᵢ recovery (Figure 3).
Na⁺-Dependent Cl⁻/HCO₃⁻ Exchange Activity in Lymphocytes from the SHR

The activity of this transporter was studied in experiments where Cl⁻ was removed from the external media (4). In response to Cl⁻ removal from the media (replaced by aspartate, pH 7.40), there was a rapid increase in pH (from about 7.1 to 7.6) that reached a plateau after about 4 min (Figure 4A). This acute alkalization was unaltered by the presence of EIPA (20 µmol/L) but was completely prevented by the addition of DIDS (400 µmol/L) (Figure 4A).

An increase in pH was seen with Cl⁻ concentrations below 132 mM such that concentrations below this level resulted in rapid and larger increases in pH (HCO₃⁻ in, Cl⁻ out of the cell) (Figure 4B). In further experiments, we examined the dependency of the Na-dependent Cl⁻/HCO₃⁻ exchanger on the prevailing pH (Figure 5). In these studies, pH was preset at various levels by the nigericin technique (3,4). The pH increase was maximal near steady-state pH (-7.0 to

Figure 3. pH dependency of the activity of the Na⁺/H⁺ antiporter (EIPA-sensitive H⁺ flux) and the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger (EIPA-resistant H⁺ flux). Data are expressed as H⁺ flux and are plotted as a function of the pH, prevailing during recovery from cell acidification. Reproduced from Reference 4 with permission.

Figure 4. (A) Representative examples of pH changes elicited by Cl⁻ removal from the media (replaced by aspartate, pH 7.4). Note that the rapid rise in pH induced by Cl⁻ removal was not modified by the presence of EIPA (20 µmol/L) but was obliterated by DIDS (400 µmol/L) and by the removal of Na⁺ from the medium. (B) Representative examples showing that changes in the external chloride concentration clearly affect the rate of rise in pH, in a concentration-dependent manner. The changes in pH observed are consistent with HCO₃⁻ entry via a Na⁺-dependent Cl⁻/HCO₃⁻ exchanger (big circle) rather than the reversal of the Na⁺-independent Cl⁻/HCO₃⁻ exchanger (small circle). Reproduced from Reference 4 with permission.
Figure 5. Effect of starting pH$_i$ on the alkalinization produced by Cl$^-$/removal. (A) pH$_i$ was first set at various levels by the K$^+$ nigericline technique in the continuous presence of HCO$_3^-$/CO$_2$. The pH$_i$ change produced by Cl$^-$/removal was then monitored for 5 min in the presence of EIPA (20 $\mu$/mol/L) to ensure that the Na$^+$/H$^+$ exchanger was blocked. (B) $\Delta$ pH$_i$ observed during the first 40 s of Cl$^-$/removal plotted against the prevailing pH$_i$ in a group of pooled experiments performed as described in Panel A. The maximal rise in pH$_i$ attributable to HCO$_3$ entry via the Na$^+$-dependent Cl$^-$/HCO$_3$ exchanger (big circle) occurs near steady-state pH$_i$. Reproduced from Reference 4 with permission.

The aggregate of these findings indicates that the initial change in pH$_i$ observed when the external Cl$^-$ concentration is reduced reflects the activation of a Cl$^-$/HCO$_3$ exchanger that is Na$^+$ dependent, DIDS sensitive, EIPA resistant, and maximally active near steady-state pH$_i$ (~7.10). We thus use only the initial phase of the pH$_i$ increase observed 40 s after Cl$^-$/removal as an index of the stimulated activity of the Na$^+$-dependent Cl$/HCO_3$ exchanger. This approach also allowed us to calculate $\Delta$H$^+$/40 s $\times$ millimoles of H$^+$/L per pH$\Delta$H) on the basis of the buffering power measured at the departing steady-state pH$_i$.

After Cl$^-$/removal, pH$_i$ increased in a virtually indistinguishable fashion in SHR and WKY rat cells (4). The activity of the Na$^+$-dependent Cl$^-$/HCO$_3$ exchanger was not significantly different between SHR and WKY rat cells ($\Delta$H$_{\text{Na-pi}}$ 0.11 ± 0.01 and 0.13 ± 0.02 pH U/min, respectively). Likewise, H$^+$ flux was similar between SHR and WKY rat cells (2.23 ± 0.26 and 2.50 ± 0.43 mmol of H$^+$/L per minute, respectively). The kinetic behavior of the Na$^+$-dependent Cl$^-$/HCO$_3$ exchanger as a function of external Cl$^-$ was also examined. The $V_{\text{max}}$ was not significantly different between SHR and WKY rat cells (1.89 ± 0.32 and 2.19 ± 0.43 mmol of H$^+$/L per minute, respectively). The $K_{\text{m}}$ was also similar—74 ± 3 and 75 ± 2 mM in SHR and WKY rat cells, respectively (4).

Although the activity of this exchanger could be inhibited by increasing external Cl$^-$ concentrations at a Cl$^-$ concentration of around 100 mmol/L, the physiologic level prevailing in the extracellular space, this
transporter remained quite active. Importantly, the optimal pH for the activity of this transporter was found to be near to steady-state pH (7.20) (see Figure 5B) (4).

**Na⁺-Independent Cl/HCO₃ Exchange in Lymphocytes from the SHR**

For these experiments, cells were first alkalinized by the removal of external Cl⁻, as shown in Figure 4. This method resulted in a pH increase with rapid stabilization in about 5 min (4). After the resuspension of lymphocytes in a Cl⁻-containing solution, pH recovery was extremely fast, EIPA resistant, Na⁺ independent, and totally DIDS sensitive (Figure 6A). The initial rate of pH recovery was clearly dependent on external Cl⁻ (Figure 6B). These findings are all consistent with the operation of an Na⁺-independent Cl⁻/HCO₃⁻ exchanger. To calculate maximal Cl⁻/HCO₃⁻ exchange activity, we used the fall observed during the first 40 s after the addition of Cl⁻. H⁺-equivalent flux was then calculated as ΔpH per minute × buffering power measured at the departing alkaline pH and in the presence of HCO₃⁻/CO₂ (4).

The pH recovery from an alkaline pH in lymphocytes from SHR \(N = 11\) and WKY rats \(N = 11\) is shown in Figure 7 (4). No differences were observed in the rate of pH recovery between SHR and WKY rat cells (ΔpH at 40 s, 0.21 ± 0.01 and 0.19 ± 0.02, respectively). The dependency of this exchanger on pH was also virtually the same in SHR and WKY rat cells, such that H⁺ flux rapidly approached 0 when pH returned to steady-state (4).

The activation of the Na⁺-independent Cl⁻/HCO₃⁻ exchanger by extracellular Cl⁻ exhibited characteristics of first-order dependency with no differences observed between lymphocytes from SHR \(N = 9\) and
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Intracellular pH and Acid Excretion in the Dahl Rat Model of Salt-Sensitive Genetic Hypertension

We studied the parameters of intracellular and extracellular acid-base status, as well as renal acid excretion in the Dahl/Rapp salt-sensitive (SS) rat as compared with its genetically salt-resistant (SR) control (1). These studies were performed in SS rats made hypertensive by exposure to a high-salt diet and in SS rats in which the development of hypertension had been prevented by severe salt restriction. To rule out differences in food intake as a confounding factor, additional studies were conducted in SS and SR rats under strict pair-feeding conditions and while on a more physiologic level of salt intake (1%)(1).

In rats on a high-salt diet, the pH, of thymic lymphocytes was significantly lower in SS than in SR rats. This difference in pH, was observed both in the presence and in the absence of HCO3/CO2, in the media (1). In rats on a low-salt diet, the mean pH, was also lower in the SS than in SR rats, but the difference did not reach statistical significance. No significant differences in blood pH and blood bicarbonate were found between anesthetized SS and SR rats on either the high or low-salt diets.

Under the very low-salt diet, SS rats also excreted more ammonium and titratable acidity than did SR rats. Although the difference in either of these two parameters did not achieve statistical significance, net acid excretion was significantly higher in SS than in SR rats. Under the high-salt diet, SS rats developed marked hypertension and had markedly higher rates of acid excretion than did SR rats, and this difference was due to higher rates of both ammonium and titratable acid excretion (1) (Figure 9).

In rats on a 1% salt diet, the urinary excretion of titratable acid, ammonium, and net acid were also higher in hypertensive SS rats than in normotensive SR rats (Figure 9). Arterial acid-base parameters, evaluated after the completion of the metabolic study in anesthetized rats, revealed no significant difference in either blood pH or blood bicarbonate between SS and SR rats (1). Figure 9 summarizes the data on net acid excretion, corrected per 100 g body wt, of SS and SR rats placed on the three different levels of salt intake (0.08, 1, and 8% salt diets). When compared with their SR counterparts, hypertensive SS rats placed on either a normal rat chow (1% salt) or an extremely high-salt diet (8%) had a markedly elevated rate of renal acid excretion. A similar alteration (i.e.,
higher acid excretion in SS than SR rats) was observed when the development of hypertension in SS rats was prevented by extreme dietary salt deprivation (1).

DISCUSSION

The studies just described using lymphocytes from SHR and WKY rats provide insight into the relative activity and kinetic properties of the three main transporters involved in the control of pH: the Na⁺/H⁺ exchanger, the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger, and the Na⁺-independent Cl⁻/HCO₃⁻ exchanger. The activity of each one of these transporters was found to be distinctively dependent on the prevailing pH (3,4). The activity of the Na⁺/H⁺ antiporter was the highest at an acidic pH (3,4), whereas the activity of the Na⁺-independent Cl⁻/HCO₃⁻ exchanger was maximal at an alkaline pH (4). The activity of both the Na⁺/H⁺ antiporter and the Na⁺-independent Cl⁻/HCO₃⁻ exchanger decreased markedly as pH approached steady-state pHᵢ. By contrast, we found that an Na⁺-dependent Cl⁻/HCO₃⁻ exchanger was maximally stimulated near the physiologic range of steady-state pHᵢ (7.0 to 7.3) (4).

If intracellular pH or the Na⁺/H⁺ exchanger is to be implicated in the pathogenesis of hypertension, then HCO₃⁻-dependent transport processes cannot be ignored. Whenever the external media lack HCO₃⁻/CO₂, one essentially eliminates, by design, the operation of all HCO₃⁻-dependent transporters and overestimates the role of the Na⁺/H⁺ exchanger in pHᵢ regulation. The lack of studies other than our own assessing the activity of HCO₃⁻-dependent transporters likely reflects methodologic difficulties with HCO₃⁻/CO₂ media. The solutions lacking HCO₃⁻/CO₂ that are usually used to examine Na⁺/H⁺ exchanger activity are easy to handle, and one does not have to worry about the operation of HCO₃⁻-dependent transport processes. This, although convenient and valid to evaluate maximal activity, may not convey what is happening in physiologic conditions. We found that the rate of proton extrusion observed at various degrees of cell acidification is virtually identical between lymphocytes from SHR and WKY rats (3,4). In the presence of HCO₃⁻, like in its absence, the Na⁺/H⁺ exchanger has a steep pHᵢ dependency and there are no significant differences in the activity of this exchanger between lymphocytes from SHR and WKY rats (4).

The tendency toward intracellular acidosis in lymphocytes from the SHR should enhance not only Na⁺/H⁺ exchange but Na⁺-dependent Cl⁻/HCO₃⁻ exchange as well (the two transport systems that defend the cell against acid loads). The secondary activation of these two transporters acting in concert would attenuate intracellular acidosis and in the process would tend to increase intracellular sodium. We have thus proposed that chronic intracellular acidosis in lymphocytes from the SHR dictates a secondary elevation in the activity of the Na⁺/H⁺ antiporter, which results in enhanced Na⁺ accumulation and the extrusion of acid equivalents (2,3).

The Na⁺-dependent Cl⁻/HCO₃⁻ exchanger, owing to its activity near steady-state pHᵢ, is a prime candidate to adjust its activity during physiologic perturbations in cell acid-base production (4). Accordingly, the activity of the Na⁺-dependent Cl⁻/HCO₃⁻ exchanger, a transporter that brings HCO₃⁻ into the cell, is likely to be higher in SHR than in WKY cells near steady-state 

Figure 9. Summary of net acid excretion (NAE) data in SS and SR rats obtained at the end of 16 to 21 days on a low-salt diet (0.08%), a normal salt diet (1%), and a high-salt diet (8%). Significant differences were observed at all levels of salt intake by t test (unpaired data analysis). Reproduced from Reference 1 with permission.
vided conclusive data that essentially excluded the NH1-1 form of the antiporter as a candidate gene in human hypertension (25). We interpret our findings as suggestive that the plasma membrane proteins that subserve CI-/HCO3- exchange are not likely to be different in cells from SHR and WKY rats. By extrapolation, we have proposed that primary alterations in the structure of these pHi regulatory proteins are also not likely in human essential hypertension (3,4). Their regulation in vivo, however, may differ, depending on the prevailing pHi and other factors that influence their activity (3,4).

We interpret our finding of enhanced renal acid excretion in Dahl/Rapp SS rats as being secondary to increased metabolic acid production (1). This is suggested by our finding of a decreased pHi in SS as compared with SR rats. In the face of intracellular acidosis, the addition of acid equivalents into the systemic circulation would result in the titration of plasma bicarbonate, leading to systemic acidosis. A significant difference in extracellular acid-base status between the SS and SR rats, however, could not be demonstrated in our studies (1). It should be noted that, in the face of a renal adaptive increase in acid excretion, changes in extracellular acid-base parameters could be minimal or even completely lacking. Overt metabolic acidosis does not need to be a feature accompanying chronic acid overproduction for the same reason that exogenous acid administration may not result in sustained metabolic acidosis when renal compensation has taken place (1).

It should be noted that increased renal acid excretion in the SS rat was not due to increased food intake, as demonstrated by our pair-feeding studies (1). If dietary acid excess from food intake is excluded, then a perturbation of cellular metabolism, resulting in an increased production of proton equivalents, seems likely in SS rats. According to our hypothesis, intracellular acidosis and the attendant addition of acid equivalents into the circulation, even when unaccompanied by a detectable extracellular acidosis, would be the signal for the stimulation of renal acid excretion (1). In this context, enhanced acid excretion by the kidneys may provide a total body marker for the enhanced cell metabolism associated with hypertension.

How could our findings of increased renal acid excretion and reduced pHi relate to hypertension in the SS rat? One possibility is that intracellular acidosis could stimulate both renal bicarbonate and sodium reabsorption. In response to acid loading or metabolic acidosis, the activity of the Na+/H+ exchanger in renal proximal cells increases (26,27). One can thus envision a situation where increased activity of the Na+/H+ antipporter, resulting from increased cellular acid production, could play a role in the initiation of hypertension by contributing significantly to renal sodium retention in SS rats. We think that our findings may explain previous observations that the provision of sodium as sodium bicarbonate or sodium citrate, unlike sodium chloride, does not increase blood pressure in SS individuals (28). These alkalinizing agents could suppress the overactivity of the Na+/H+ antipporter by neutralizing acid overproduced by these hypertensive individuals. Thus, we have proposed that intracellular acidosis stimulates the stimulus for exaggerated renal sodium retention via Na+/H+ exchange in SS individuals and that such stimulus is removed by the provision of alkali (1).

Another link between acid overproduction and hypertension can be envisioned at the level of cells other than kidney cells where reduced pHi would also stimulate Na+/H+ exchange, thereby promoting cell Na+ influx and intracellular Na+ accumulation (2). This increase in cell Na+ could also contribute to an increase in intracellular Ca2+ via a plasma membrane Na+/Ca2+ exchanger (Figure 10) (2). Intracellular acidosis may be part of a generalized alteration in cellular ionic composition prevailing in some forms of genetic hypertension that may be expressed at the whole-animal level by enhanced renal acid excretion. Whether enhanced acid excretion is a feature of human salt sensitivity and whether it occurs in types of hypertension other than the SS Dahl rat will need to be investigated.

Before assuming that our observations are relevant to the pathogenesis of hypertension, a word of caution is necessary. Rat strains that predictably develop hypertension as an inherited characteristic provide a convenient experimental cohort for the study of potential putative factors but present multiple problems as well. One problem that has been recognized in recent years, particularly with respect to the SHR, is one of "contamination" during the breeding process (29–33). This has resulted in genetic heterogeneity among different rat colonies of supposedly identical genetic rat models of hypertension. Differences among SHR and even much more dramatic differences in its genetic control, the WKY rat, have been well described by Kurtz and others (29–33). This genetic divergence

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**Figure 10.** Schematic drawing of hypothetical scheme of interactions between pHi, intracellular Ca2+ (Ca2+), and intracellular Na+ (Na+) in SHR cells. Reduced pHi is proposed as a driving force for both increased Na+ via stimulation of Na+/H+ exchange, and increased Ca2+ via Ca2+/H+ exchange. Reproduced from Reference 2 with permission.
between the two strains is obviously problematic for genetic studies. This is not to say, however, that studies using the WKY rat as a control are useless for physiologic studies. Differences that emerge in a population not perfectly inbred are more likely to assume significance in terms of blood pressure and better reflect the heterogeneity of human essential hypertension.

The Dahl/Rapp rat, a model of SS genetic hypertension is particularly useful to study genetic hypertension because the inbred SS and SR rats are genetically identical at 80% of the defined loci, whereas SHR and WKY rats have a more heterogenous background, as stated above. However, a contamination involving the Dahl rat model of SS hypertension has been recently detected as well (34). This involved the SS strain of Dahl rats that had been bred to homozygosity by Dr. John Rapp. This contamination occurred several years later while these animals had become commercially available. Our work in the Dahl/Rapp rat described above was performed before this contamination took place (animals were studied during the period from 1990 to 1991), and all SS animals without exception developed accelerated hypertension on either a normal (1%) or a high-salt diet (8%). It is obviously critical to know the source and date of birth of the rat strains being studied as a way of checking for problems derived from the contamination of inbred strains and thus a meaningful interpretation of the data. Even when this critical information is available and even if one can be reasonably certain of genetic homogeneity, there is a fundamental limitation inherent to purely comparative studies between a genetic hypertensive strain and its normotensive control (i.e., SHR versus WKY or SS versus SR). This is because these strains not only differ with regard to the hypertension-causing gene(s), but they also differ in a vast array of additional loci that were altered by chance in the process of generating the two strains (hypertensive versus normotensive) (33). This results in a multitude of differences that are in all likelihood unrelated to hypertension. This is an inherent limitation of the inbreeding process for genetic studies. However, comparative studies between hypertensive and nonhypertensive strains remain, in our opinion, critically important in unraveling physiologic/metabolic differences that may be relevant to hypertension, particularly if they are found in subsequent cosegregation studies.

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