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Kidney International, Vol. 42 (1992), pp. 1355–1362

Red cell sodium-proton exchange is increased in Dahl salt-sensitive hypertensive rats

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Red cell sodium-proton exchange is increased in Dahl salt-sensitive hypertensive rats. To investigate the relationship between red blood cell Na^+/H^+ exchange (EXC) and genetic factors in hypertension, we studied the maximal rate of the antiporter (mmol/liter cell × hr; flux units = FU) in three strains of genetically hypertensive rats. Salt-resistant Dahl rats (DR) were normotensive under low (0.02%) and high (8%) NaCl diets, while salt-sensitive Dahl rats (DS) became markedly hypertensive after four weeks on the high-NaCl diet. Na^+/H^+ exchange did not differ between DR and DS rats when both were fed with the low-NaCl diet (mean ± SE, 31 ± 3, $N = 15$, vs. 29 ± 3 FU, $N = 14$). On the high-NaCl diet, the DR strain did not exhibit significant changes in blood pressure and antiporter activity, but the DS rats significantly increased their blood pressure and Na^+/H^+ exchange (57 ± 4 FU, $N = 13$) versus DR rats (38 ± 3 FU, $N = 15$, $P < 0.02$). DS rats also significantly increased blood pressure and antiporter activity when fed with high-NaCl diet for one week. These data indicate that high NaCl intake *per se* does not increase Na^+/H^+ EXC because the control DR strain did not exhibit transport and blood pressure alterations as observed in the DS strain. Milan hypertensive and spontaneously hypertensive rats (Charles River substrain) had higher blood pressures than Milan and Wystar-Kyoto normotensive rats when they were maintained for four weeks on a 1.5% NaCl diet; however, no differences were seen among normotensive and hypertensive strains in Na^+/H^+ exchange activity. When the four strains were fed for four weeks with a low-NaCl diet, blood pressure and Na^+/H^+ exchange activity did not change in any of these strains. Na^+/H^+ exchange activity in the three hypertensive strains did not correlate with previously reported measurements in kidney brush border membrane vesicles, a finding suggesting that measurements in intact cells reveal antiporter regulatory mechanisms. Our data indicate that elevated Na^+/H^+ exchange is not due solely to hypertension or high-NaCl diet *per se*, since these alterations were not shared by all genetic rat models of hypertension. The development of hypertension with a high-NaCl intake in the DS strain followed by a stimulation of RBC Na^+/H^+ exchange indicate that the antiporter is up-regulated by salt-sensitive elevation of blood pressure.

Genetic factors, salt intake, and sodium transport abnormalities interact in the development of essential hypertension. Furthermore, in human hypertension the blood pressure response to high salt intake is heterogeneous, and the mechanism(s) for such differences in salt sensitivity remain to be fully elucidated [1]. Genetically hypertensive rats represent an useful model to investigate the relationship of Na^+ transport alterations to the pathophysiological mechanisms involved in the development of hypertension. Three models of genetically hypertensive rats [salt-sensitive Dahl (DS) rats, spontaneously hypertensive rats (SHR), and Milan hypertensive (MHS) strains] also differ with respect to the salt-sensitivity of their blood pressure [2, 3]. As has been shown in the DS strain, elevation of the NaCl intake from 0.04 to 8% for four weeks dramatically raises the blood pressure in comparison to that of the salt-resistant strain (DR) [3]. In the SHR, genetically determined hypertension is associated with increased sympathetic nervous activity, and no response of blood pressure to dietary NaCl loading has been observed in the sub-strain provided by the Charles River Breeding Co. [4]. In the MHS strain, the development of low-renin hypertension is accompanied by faster excretion of a sodium load and low kidney to body weight ratio [5].

Recently, an increased activity of plasma membrane Na^+/H^+ exchange (Na^+/H^+ EXC) has been reported in several blood cell types—platelets, lymphocytes, and erythrocytes—in humans [6–9] as well as in SHR [10–13]. Similar alterations have been described in brush border vesicles from kidney tubular cells [14] of the SHR strain, and therefore they might play a role in the development or maintenance of hypertension. However, little information is available on the relationship between salt intake and Na^+/H^+ EXC in the pathogenesis of hypertension.

The present study was designed to investigate red blood cell (RBC) Na^+/H^+ EXC in genetically different strains of hypertensive rats that are known to have different pathophysiological mechanisms of hypertension. Our results indicate that increased RBC Na^+/H^+ EXC activity is not shared by all strains of genetically hypertensive rats and therefore is not the result of high blood pressure *per se*. Elevated Na^+/H^+ EXC activity occurred only in the DS and not in the DR strain when a high-NaCl diet elevated blood pressure, and thus appears

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Received for publication December 9, 1991
and in revised form July 23, 1992
Accepted for publication July 25, 1992

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Table 1. Red blood cell Na/H exchange in Dahl rats fed with different NaCl intakes

Strain	% NaCl diet	Blood pressure *mm Hg*	Na^+/H^+ exchange *mmol/liter cell × hr*
DR ($N = 15$)	0.02	109 ± 9	31.5 ± 3.0
DR ($N = 15$)	8.00	125 ± 4	37.7 ± 3.1
DS ($N = 14$)	0.02	157 ± 12	29.1 ± 3.1
DS ($N = 13$)	8.00	204 ± 9	57.2 ± 3.8

The V_{max} of Na^+/H^+ EXC activity was measured at pH_i 6.0, pH_0 8.0 as indicated in **Methods** section. Values are mean ± SE. Low- and high-NaCl diet were fed for four weeks to the rats. Age of the animals was 11 to 12 weeks. N = number of animals.

The blood pressure and Na^+/H^+ EXC activity for DR in low- and high-NaCl diets were not significantly different. Blood pressure between DR and DS rats were significantly different ($P < 0.01$) in low- and high-NaCl diets. Na^+/H^+ EXC activities were significantly different ($P < 0.01$) between DS and DR in high-NaCl diet and in DS in low- and high-NaCl diet.

Table 2. Red blood cell Na^+/H^+ exchange in Milan normotensive and hypertensive rats fed with different NaCl intakes

Strain	% NaCl diet	Blood pressure *mm Hg*	Na^+/H^+exchange *mmol/liter cell × hr*
MNS ($N = 12$)	0.02	153 ± 5	62.3 ± 7.5
MNS ($N = 10$)	1.5	162 ± 3	44.4 ± 4.5
MHS ($N = 12$)	0.02	211 ± 19	51.2 ± 8.3
MHS ($N = 10$)	1.5	208 ± 14	44.1 ± 4.5

Data are means ± SE; rats were aged 14 to 16 weeks. Transport activity in low NaCl diet, $N = 6$. Rats were maintained in low and high sodium diets for four weeks. N = number of animals. Blood pressure between MNS and MHS were significantly different ($P < 0.01$) in both NaCl diets. P values for Na^+/H^+ EXC activities between MNS and MHS in both diets were not significantly different.

strongly associated to the development of hypertension in this genetic rat model.

Methods

Animals

The experiments were performed on Dahl salt sensitive (DS/JR) and Dahl salt resistant (DR/JR) rats (Harlan Sprague-Dawley Inc., Indianapolis, Indiana, USA), spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto (WKY) rats (Charles River Breeding Laboratories, Boston, Massachusetts, USA), and Milan hypertensive strain (MHS) and Milan normotensive strains (MNS). The Milan rats were supplied by Dr. Giuseppe Bianchi, Milan, Italy. The age and the blood pressure are given in Tables 1, 2 and 3. During the studies the rats were maintained in accordance with the Guidelines of the Committee on Animals at the Harvard Medical School, which were adapted from those prepared by the Committee on the Use of Laboratory Animals at the Institute of Laboratory Animal Resources, National Research Council, USA.

All animals were housed at constant temperature (21 ± 2°C) and relative humidity (55 to 56%). At the beginning of the study each strain of rats was randomly divided into two groups and allocated either to a high-NaCl diet (1.5% NaCl, 0.62% KCl) or a low-NaCl diet (0.02% NaCl, 0.62% KCl; Bioserv Inc., Frenchtown, New Jersey, USA) for a period of four weeks.

To study Dahl rats, protocols previously described by Rapp [3] were followed. Rats were placed on the above low NaCl diet (0.02% NaCl) or a high NaCl diet containing 8% NaCl for a period of one and four weeks. The high-sodium diet consisted of the above high-sodium diet supplemented by 0.8% NaCl as drinking water. A 8% NaCl diet is recommended to be maintained for at least four weeks to achieve adequate salt loading and elevated blood pressure in the DS strain [15–17].

Systolic blood pressure was measured weekly by the tail-cuff microphonic manometer method with the rats under light ether anesthesia. Values for blood pressure were defined from the average of four measurements, with variability not exceeding ±5% for each rat. All rats were sacrificed by guillotine, and blood was collected into heparinized tubes and used the same day for the transport experiments.

Measurement of Na^+/H^+ exchange activity

Preparation of RBCs. Blood was centrifuged at 2000 g for four minutes at 4°C, the plasma and buffy coat were removed by aspiration and the RBC were then washed three times with ice-cold (4°C) Mg wash solution (MgWS) containing (mM): 75 $MgCl_2$, 85 sucrose, 10 TRIS-MOPS, pH 7.4 at 4°C before being resuspended to approximately 50% hematocrit (Hct) with MgWS. Aliquots of this suspension were then diluted with 0.02% Acationox detergent (American Scientific Products, McGaw Park, Illinois, USA) in double-distilled water for determinations of hemoglobin (Hb) by optical density (OD) at 540 nm and Na^+ concentration by atomic absorption spectrophotometry (Perkin-Elmer 3030B).

Modification of RBC pH. For studying the cell pH activation of the antiporter, RBCs with pH_i varying from 6.0 to 7.0 were prepared as previously described [18, 19]. For V_{max} measurements, cells were loaded to pH_i 6.0 with the acid-loading solution at pH 5.8. In brief, RBC (5% Hct) were incubated at 37°C for 10 minutes in an acid-loading solution containing (mM): 170 KCl, 0.15 $MgCl_2$, 0.1 ouabain, 0.1 bumetanide (prepared in DMSO and added just prior to the addition of RBCs), 10 glucose, 40 mM sucrose, and 20 TRIS-MES (adjusted to pH 5.8 at 37°C). The osmolarity of the acid-loading solution was adjusted to 360 mOsm to avoid acid swelling. Following the 10 minute preincubation with different acid-loading solutions, 100 μM DIDS (to inhibit the anion exchanger) and 200 μM neptazane (to inhibit carbonic anhydrase) was added in order to "clamp" pH_i. The cells were incubated at 37°C for a further 20 minutes and then washed three times with ice-cold pH wash solution containing (mM): 170 KCl, 0.15 $MgCl_2$, and 40 sucrose. After the final wash, the cells were resuspended to approximately 50% Hct with pH wash solution and stored on ice until use. Aliquots of the cell suspensions were used for determination of Hb, Hct, and intracellular Na^+ content. The pH_i was determined by measuring with a pH electrode the pH of a cell lysate made with four volumes of 0.02% Acationox detergent. The cellular Na^+ content was determined by atomic absorption

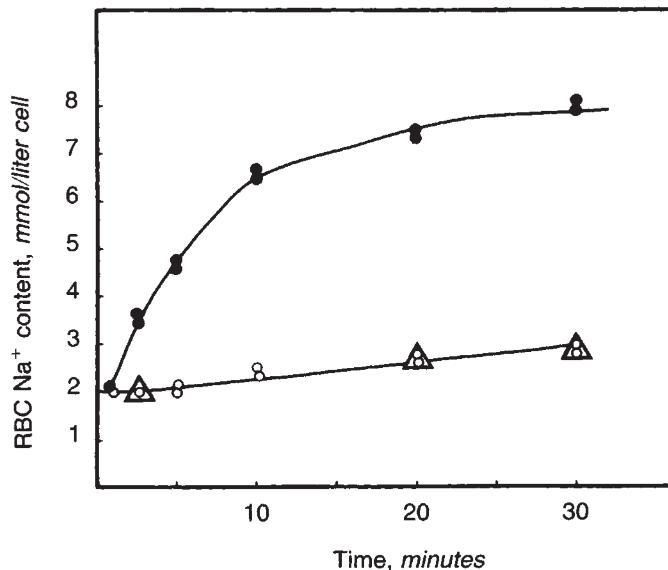

Fig. 1. *Time course of net Na^+ influx into acid loaded RBC from a MNS rat.* The cellular pH was 6.1 and the flux media contained (mM): 150 NaCl, 20 KCl, 0.15 $MgCl_2$, 0.1 ouabain, 0.1 bumetanide, 0.4 neptazane, 10 glucose, 40 sucrose and either 10 TRIS-MOPS pH 8.0 (○–○) or 10 Tris-Mes pH 6.0 (-●-●-) at 37°C, hematocrit 2%. Notice that at pH_o 8.0, Na^+ influx (40 mmol/liter cell × hr) increases rapidly between 0 and 10 minutes and proceeds at slower rates between 10 and 30 minutes. At pH_o 6.0, Na^+ influx has significantly lower (7.5 mmol/liter cell × hr) rate than at pH_o 8.0 at it is linear up to more than 20 minutes. The Na^+/H^+ EXC activity (ΔpH_o 8.0 to 6.0) was 32.5 mmol/liter cell × hr. Na^+ influx at pH_o 8.0 was almost completely inhibited by 1.0 mM amiloride (△–△).

spectroscopy with suitable standards prepared in double-distilled and deionized water. The cation content of acid-loaded cells was expressed per liter of original volume, as determined by relating the OD 540 nm of the RBC lysate to that of a known volume of RBCs. The cell volume was estimated by relating the Hb per liter of the loaded cells with that of the fresh cells.

Sodium influx measurements. Na^+/H^+ EXC was estimated as net Na^+ influx into acid-loaded cells driven by an outward H^+ gradient (that is, ΔpH_o Na^+ influx) as previously reported [18, 19]. To this end, initial rates of net Na^+ influx were measured incubating RBC in two different media, one with pH 8.0 and another with pH 6.0 (Fig. 1). The difference between both media determines the fraction of Na^+ influx driven by an outward H^+ gradient (ΔpH_o Na^+ influx). This assay avoids the use of amiloride which causes lysis at 1 mM concentrations. The Na^+ influx medium (2 ml) contained (mM): 150 NaCl, 20 KCl, 0.15 $MgCl_2$, 0.1 ouabain (to block the Na-K pump), 0.1 bumetanide (to block the Na-K-Cl cotransport), 0.4 neptazane (to block carbonic anhydrase), 10 glucose, 40 sucrose, and either 10 TRIS-MOPS, pH 8 at 37°C, or 10 TRIS-MES, pH 6.0 at 37°C, 360 mOsm. To start the transport reaction, 100 μl of acid-loaded RBCs was added to both influx media. At timed intervals (1, 6, and 11 min for pH_o 8.0 and 1 and 21 min for pH_o 6.0), duplicate 250 μl aliquots were pipetted into precooled Eppendorf tubes containing 0.7 ml of a solution composed of (mM) 80 choline chloride, 80 KCl, 0.25 $MgCl_2$, 10 TRIS-MOPS pH 7.4 at 4°C, and 40 sucrose layered over 0.4 ml of dibutylphthalate oil. The transport reaction was terminated by immediate centrifugation at 14,000 g for 10 seconds at room temperature. The supernatants were thoroughly removed by aspiration, the internal walls cleaned with cotton swabs and the external surface of the tubes was wiped dry to avoid sodium contamination; this step is important to achieve good reproducibility of the assay. The bottom of the tube containing the RBC pellet was then cut off and placed into 1 ml of 0.02% Acationox to lyse the cells. The cell lysates were vortexed vigorously and centrifuged at 2,000 g for five minutes at 4°C; the hemoglobin concentration was determined after dilution of the lysate with 0.02% Acationox. The lysate Na^+ concentration was determined by atomic absorption spectroscopy with use of appropriate standards prepared in double-distilled water. The RBC Na^+ content was calculated [18, 19] from the Na^+ concentration (μM) of the lysate, the optical density of Hb from the lysate of the flux media sample, the optical density of Hb from the lysate of the fresh RBC suspension and Hct of the fresh RBC suspension. The slope of the regression line of cell Na^+ content versus time was calculated with the least square method. Net Na^+ influx was expressed in mmol/liter cell × hr defined as a flux unit (FU).

Statistical analyses

The data was analyzed in a computer facility of our Hospital which runs a Clinfo Software statistical program (Bolt, Beranek and Newman, Cambridge, Massachusetts, USA) in Digital Equipment Corporation Computer (Maynard, Massachusetts, USA). The data are reported as mean ± standard error (SE). The Student's *t*-test was used to compare intra-strain differences in blood pressure and Na^+/H^+ exchange with salt intake because both groups exhibited similar variance. The null hypothesis was rejected when $P < 0.05$.

Chemicals

NaCl, $MgCl_2$, dibutylphthalate and glucose were obtained from Fisher Scientific Company (Fairlawn, New Jersey, USA). Ouabain, TRIS, MES, MOPS, DIDS, and albumin (bovine fraction V) were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA); KCl was obtained from Mallinckrodt, Inc. (St. Louis, Missouri, USA); neptazane from Lederle Laboratories, Division of the American Cyanamid Co. (Pearl River, New Jersey, USA); bumetanide from Leo Laboratories (Vernouillet, France) and Acationox from Scientific Products (McGraw Park, Illinois, USA).

Results

To measure Na^+/H^+ EXC activity in rat RBCs, cell pH was varied between 6.0 and 7.0 and clamped by blocking the anion exchanger with DIDS. Since cellular acification induced swelling, the osmolarity of the acid loading and flux media was adjusted at 360 mOsm. When acid loaded RBC (pH_i 6.0) were incubated in Na^+ media with pH 6.0, net Na^+ gain proceeded at low rate (Fig. 1) because Na^+/H^+ EXC was fully inhibited by acid external pH and there was no outward H^+ gradient. Under this condition, Na^+ influx was linear up to 20 minutes. When acid-loaded RBC were incubated in Na^+ media with pH 8.0, net Na^+ influx was much faster than at pHo 6.0, and initial rates were held between 0 and 6 minutes. The difference in Na^+ influx between both media (ΔpH_o) provided a measurement of Na^+/H^+ EXC as H-gradient driven Na^+ influx. Although the

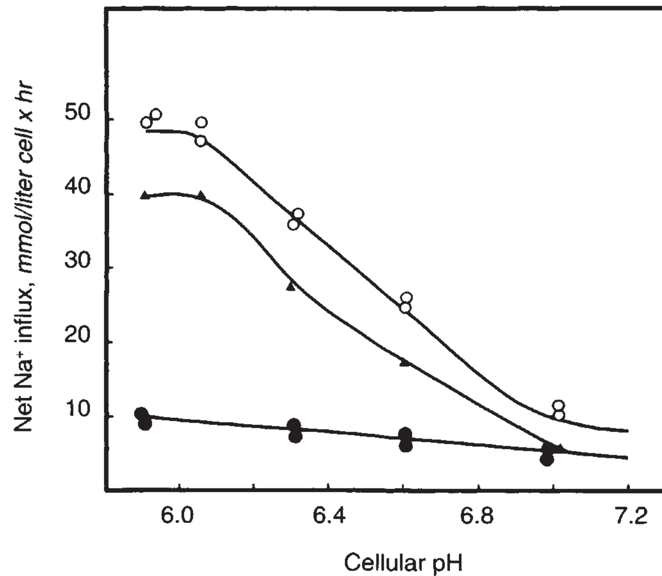

Fig. 2. *Activation of net Na^+ influx by intracellular pH in RBC of a DS rat.* At pH_o 8.0 (○), Na^+ influx increased sigmoidally with a fall in cell pH and reached maximal values between pH_i 5.9 and 6.1. At pH_o (●), Na^+ influx was very low and it increased in a linear fashion with a fall in cell pH. The Na^+/H^+ EXC activity (ΔpH_o, ▲) reached maximal activity at pH_i 6.0.

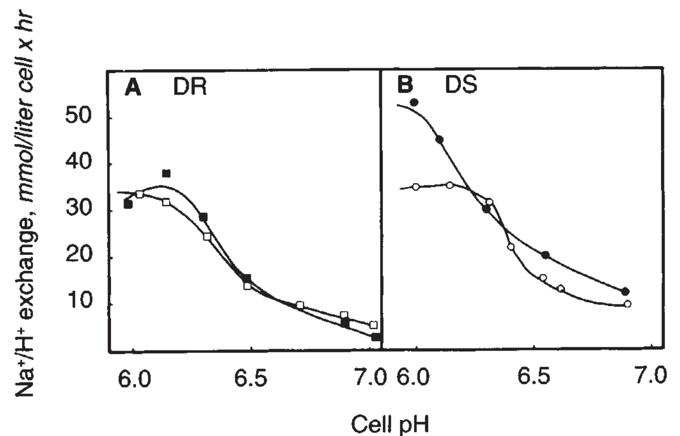

Fig. 3. *Activation of red blood cell Na^+/H^+ exchange by cellular acidification.* **A.** Results obtained in one DR rat on high- (8%) [-■-■-] and one DR rat on a low- (0.02%) [□-□] NaCl diet. Na^+/H^+ EXC was measured as the Na^+ influx driven by an outward H^+ gradient (difference between Na^+ influx at pH_o 8.0 and pH_o 6.0 [14]). A Hill plot analyses of the data (log $v/(V_{max-v})$ vs. log H_i) gave the following kinetic parameters for high-NaCl diet: K_m 302 nM and n_{app} 2.0, and for low-NaCl diet, K_m 265 nM and n_{app} 2.0. **B.** Results obtained in one DS rat on a high (●-●) and one DS rat on a low (○-○) NaCl diet. A Hill plot analyses of the data (log v/V_{max} − v vs. log H_i) gave the following kinetic parameters for high-NaCl diet: K_m 292 nM and n_{app} 1.2, and with low-NaCl diet: K_m 243 nM and n_{app} 2.7. The differences between these n_{app} values was significantly different ($P < 0.01$). The standard errors of the influx at every pH_i were less than 10%.

the antiporter activity of RBCs was 50 to 100 times lower than of kidney cells and vesicles, as in the case of the Na-K pump, Na^+ transport kinetics could be studied with great precision because a large number of cells with low passive permeability could be used in the assays while the intracellular composition for H^+ and Na^+ could be modified as required.

Figure 1 also shows that 1 mM amiloride almost completely inhibited the antiporter activity. However, this high concentrations of amiloride very often induced cell lysis, and for this reason was not used in all experiments. Furthermore, measurements of ΔpH_o Na^+ influx could be performed simultaneously in many RBC samples, while measurements of Na-dependent H^+ efflux under pH-stat conditions needed to be determined in sequence.

Figure 2 shows the cell pH dependence of net Na^+ influx at pH_o 8.0 and 6.0 in RBCs of a Dahl salt-sensitive rat. Notice that at pH_o 6.0, Na^+ influx was linearly dependent from intracellular pH_i, while at pH_o 8.0 was sigmoidally activated by cellular acidification and reached very high flux rates. The ΔpH_o for Na^+ influx reached maximal values at pH_i between 5.9 and 6.1, as previously reported for human RBC [18]. Therefore, to assay the maximal antiporter activity, RBCs were acid-loaded to pH_i 6.0 and assayed at pH_o 8.0 and 6.0 for net Na^+ influx.

The kinetics of Na^+/H^+ EXC activation by acid cellular pH was studied in RBCs of the DS and DR rats on both high- (8%) and low- (0.02%) NaCl diets. Figure 3A shows a plot of the H^+ gradient-driven Na^+ influx as a function of cellular pH in the DR strains maintained in low and high-NaCl diet. It can be seen that, as the cell H^+ concentration was increased and the cell pH (pH_i) decreased, net Na^+ influx markedly increased and reached maximal rates around pH_i 6.0 and half maximal (pK) at pH_i 6.5. A Hill plot analysis of these data was used to calculate the Hill coefficient (n_{app}) for cell pH activation from the slope of log $v/(V_{max} - v)$ versus log H_i (plot not shown) using a rearranged form of the Hill equation.

$$-\log \frac{v}{(V_{max}) - v)} = n_{app} \log (H_i) - \log K'$$

A plot of log $(v)/V_{max})$ of ΔpH_o Na^+ influx (v) versus log H_i yielded a straight line with slope equal to the Hill coefficient (n_{app}); the intercept at x-axis at y = 0 gives log (H_i), that is, the logarithm of the substrate concentration that yield 50% of the V_{max}, which for the sake of simplicity, we call K_m. The constant $K' = [(H_i)_{0.5}]^{napp}$.

The steep increase in the Na^+ influx with increasing H_i was reflected by a high n_{app} for pH_i activation in RBC of DR rats on low- and high-NaCl diets (Fig. 3A). The V_{max} of Na^+/H^+ EXC, the Hill coefficient (n_{app}) and the pK were similar in RBCs of DR rats on low- and high-NaCl diet (Fig. 3A). In RBCs of DS rats (Fig. 3B), not only did the V_{max} increase with the high-NaCl diet, but the n_{app} was reduced from 2.7 to 1.2 ($P < 0.01$ for the differences between the slopes); there was no change in the K_m. We measured the V_{max} of Na^+/H^+ EXC at pH_i 6.0 in 15 DR and DR rats maintained on low- and high-NaCl diet.

DR rats exhibited similar blood pressure values on a low- and high-NaCl intake (Table 1). In contrast, DS rats fed a low-NaCl diet (157 ± 12 mm Hg) became markedly hypertensive when fed for four weeks with a high-NaCl diet (204 ± 9 mm Hg). The V_{max} of Na^+/H^+ EXC for DR rats on high- and low-NaCl intake did not differ significantly (Table 1, Fig. 4). In contrast, after four weeks on a high-NaCl diet, the DS strain showed significantly higher Na^+/H^+ EXC activity than in the low NaCl diet (Table 1, Fig. 3); furthermore, DS rats on the high-NaCl diet

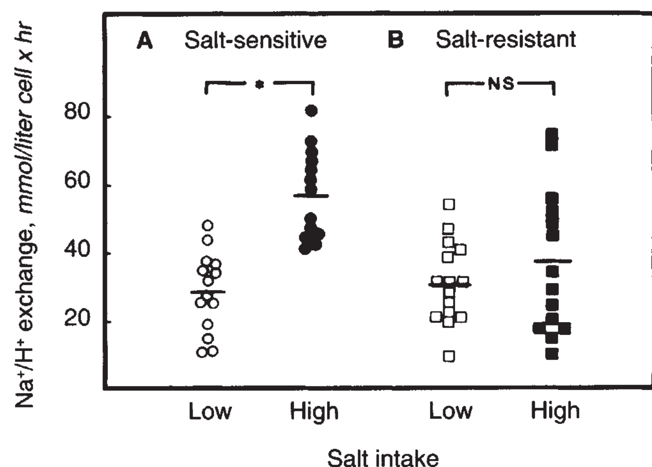

Fig. 4. *Scatter diagram of the maximal transport rate (V_{max}) of RBC Na^+/H^+ exchange in Dahl salt-sensitive (DS) rats on low- and high-NaCl diets and in Dahl salt-resistant (DR) rats on low- and high-NaCl diets for four weeks.* Low-NaCl diet was 0.02% NaCl, high-NaCl diet was 8% NaCl. Mean values in each group are indicated by horizontal lines. High-NaCl diet significantly increased the antiporter activity in DS ($P < 0.001$) but not in DR rats.

exhibited significantly higher V_{max} of Na^+/H^+ EXC than the DR strain. As shown in Figure 4, only 14% of DS rats with low-NaCl intake exhibited V_{max} values >40 FU, while all DS rats with high-NaCl intake exhibited values >40 FU. It should be also noted that after four weeks of the low NaCl diet, there was a significantly higher mean blood pressure in the DS than in DR rats. As previously reported by Rapp [3], the rise in blood pressure with age is accelerated in the DS strain with a high NaCl intake.

We also examined the impact on blood pressure and antiporter activity of a high-NaCl diet maintained for only one week. Na^+/H^+ EXC, as well as blood pressure in the DS strain were significantly higher compared with values monitored on a low-NaCl diet (43.6 ± 6 vs. 29.1 ± 3 FU, $P < 0.05$, $N = 5$).

Similar studies were performed in the MHS and MNS rats and the blood pressure, age, and diet are reported in Table 2. Blood pressure was significantly higher in MHS than in MNS rats ($P < 0.01$) on high- (1.5%) or low- (0.02%) NaCl diet. The cell pH activation of Na^+/H^+ EXC exhibited similar kinetic parameters in these strains (data not shown). However, there was no difference in the V_{max} of RBC Na^+/H^+ EXC activity between the normotensive and hypertensive strains (Table 2). It should be noted that MHS and MNS rat RBCs have significantly higher antiporter activity than DS and DR strains maintained on the low-NaCl diet.

WKY rats were normotensive on both low- and high-NaCl diets, while SHRs were hypertensive on both diets (Table 3). These results are in agreement with previous reports, indicating that the development of hypertensive in the SHR sub-strain bred by Charles River Breeding Co. (SHR/CR) is independent of variation in salt intake between 0.04 and 1.4% [4]. The V_{max} of Na^+/H^+ EXC was not significantly different in RBCs of SHRs and those of WKY rats on low- or high-NaCl intake.

Table 3. Red blood cell Na^+/H^+ exchange in WKY, and SHR strains fed with differnet NaCl intakes

Strain	% NaCl diet	Blood pressure *mm Hg*	Na^+/H^+exchange *mmol/liter cell × hr*
WKY (N = 15)	0.02	148 ± 4	30.6 ± 4.8
WKY (N = 7)	1.50	149 ± 10	40.6 ± 5.1
SHR (N = 9)	0.02	185 ± 4	27.8 ± 3.8
SHR (N = 12)	1.50	199 ± 12	33.1 ± 5.5

Data are mean ± SE of V_{max} of Na^+/H^+ exchange. Rats were aged 12 weeks; N = number of animals. Low- and high-NaCl diets were maintained for four weeks. For WKY vs. SHR, $P < 0.01$. Blood pressure between WKY and SHR were significantly different ($P < 0.01$) in both NaCl diets. P values for Na^+/H^+ EXC activities between strains in both diets were not significantly different.

Discussion

Our studies indicate that the V_{max} of RBC Na^+/H^+ EXC activity was markedly elevated in the Dahl salt-sensitive genetic model of hypertension when those rats consumed a high-NaCl diet. The increase in antiporter activity paralleled the development of salt-sensitive hypertension in DS rats; in the control DR rats, neither blood pressure nor Na^+/H^+ EXC changed when fed with an 8% NaCl diet.

The abnormality of RBC Na^+/H^+ EXC activity is not a common feature of all the different genetic rat models of hypertension. Both Milan strains exhibited higher antiporter activity than the Dahl strains maintained on the low-NaCl diet. Therefore, it is possible that the exchanger is already maximally stimulated so that a high NaCl diet cannot further activate it. Our data shows that there are marked differences in antiporter activity between rat strains. Of the three hypertensive strains studied only the DS strain on high NaCl intake exhibited elevated Na^+/H^+ EXC activity. The finding that MHS and SHR/CR have antiporter activity similar to the normotensive controls (MNS and WKY) indicate that this Na^+ transport alteration is not due to the elevated blood pressure *per se*. It appears, therefore, that the antiporter activity may vary according to different pathogenetic mechanisms in the genetic models of hypertension. Our findings indicate that the RBC antiporter abnormality is tightly linked to gene-salt intake interactions that contribute to the development of salt-sensitive hypertension in the Dahl strain.

The lack of effect on Na^+/H^+ EXC of an increase in sodium diet from 0.02 to 1.5% in the WKY/SHR and MNS/MHS strains indicates that a high-salt diet *per se* does not necessarily influence the activity of the exchanger and blood pressure in the hypertensive rats. We did not study the SHR, WKY, and Milan strains fed with an 8% NaCl diet, and therefore it might be argued that only such high salt intake and not high blood pressure can influence RBC Na^+/H^+ EXC activity. We do not think this is the case because when the DR normotensive control rats were fed with 8% NaCl diet, they did not increase blood pressure or Na^+/H^+ EXC. Furthermore, feeding the Dahl strains for only one week with an 8% NaCl diet induced a significant elevation of blood pressure and Na^+/H^+ EXC activity.

Previous studies have shown that the SHR/CR sub-strain does not increase blood pressure even with an 8% NaCl intake as the Taconic SHR sub-strain does [4]. It would be important

to test the response of the Taconic SHR sub-strain, which has been shown to increase blood pressure when NaCl intake was raised up to 8% [4]. However, very often, as in the case of the Milan strains, testing of the effect on blood pressure of sodium intakes higher than 1.5% cannot be performed because they drink less saline than tap water compared to the normotensive rats [5].

Orlov et al [20] also reported no difference in antiporter activity in a small number of WKY, SHR, MNS, and MHS rats whose NaCl intake and original supplier were not specified. In that report, Na^+/H^+ EXC activity was determined by measuring Na^+ influx after cell shrinkage was induced by the addition of valinomycin in the presence and absence of 0.5 mM amiloride. Thus, these flux measurements largely reflect the operation of the "volume-stimulated" Na^+/H^+ EXC involved in the volume regulatory increase response to cell shrinkage. In a subsequent study, the same authors reported higher values of RBC Na^+/H^+ EXC in SHR than in WKY rats (95 ± 7.0 FU, $N = 6$ vs. 61 ± 5.7 FU, $N = 5$) when amiloride-sensitive H^+ efflux was measured at pH_i 6.5 to 6.7 [21]. Those values are surprisingly high in comparison to their and our data (Tables 2 and 3), particularly since the antiporter is only half-maximally activated at pH_i 6.5.

The mechanism by which DS rats become hypertensive remains to be fully elucidated. Renal, humoral and sympathetic neural mechanisms appear to be implicated in the genetic predisposition to hypertension in this rat strain [17, 22]. When fed a low-NaCl diet, DS rats slowly develop hypertension and survive well [3]. DS rats fed a high-NaCl diet develop fulminant hypertension and are usually dead by eight weeks of treatment, while DR remain normotensive. Several studies indicate that an abnormality in kidney function is responsible for salt-induced hypertension in the DS strain [23]. Indeed, an increased Na^+ reabsorption and reduced natriuretic capacity of the kidney may lead to blood volume expansion and hypertension [23]. Several studies have also shown that high sodium intake induces increased urinary calcium losses in the three models of hypertension [24–26]. Abnormalities in Ca^{2+} metabolism such as elevated cytosolic Ca^{2+} in platelets, are also present in these three models, but the DS strains exhibit the highest values [26, 27, 28]. Because cytosolic Ca^{2+} also modulates Na^+/H^+ EXC activity in platelets [9] and human RBCs [28–30], it may be possible that Ca^{2+}-dependent mechanisms are involved in the RBC transport abnormality exhibited by the DS rat. We have shown that basal cytosolic Ca^{2+} levels are an important determinant of the Hill coefficient for cell pH activation of the antiporter in human RBC [30]. Alternatively, it is also possible that the number of antiporter sites is increased after four weeks of high-NaCl diet, because rat RBCs have a half-life of 40 days.

Because Na^+/H^+ EXC activity in kidney cells is involved in Na^+ reabsorption at the proximal tubule, the question arises: Does elevated RBC Na^+/H^+ EXC in DS rats reflect a similar abnormality in the kidney? As shown in Table 4, the antiporter activity has been studied in the brush border vesicles from kidney proximal tubules of the three strains of genetically hypertensive rats. In the Milan strains, Na^+/H^+ EXC activity was not significantly different in RBCs and kidney membranes [31]. SHR kidney vesicles exhibited increased V_{max} of H^+ gradient-driven Na^+ influx in comparison with the normotensive control [14], while in our study RBCs did not show a similar alteration. Finally, Na^+/H^+ EXC in kidney vesicles of DS rats maintained for one week on the 8% NaCl diet [32] was not different than what was observed in the RBCs of DR rats (Table 3). Notably, the DR but not the DS strain was able to decrease its antiporter activity when the NaCl intake was increased [32]. It appears, therefore, that RBC Na^+/H^+ EXC does not necessarily reflect the activity of kidney brush border membranes. The different response of the antiporter might be related to the fact that we have assayed the antiporter activity in intact cells that contain all the cytoplasmic biochemical machinery for regulation of transport turnover rate; alternatively, the kidney tubule may posses a different antiporter isoform that the one present in erythrocytes.

Table 4. Na^+/H^+ exchange in kidney brush border membrane vesicles and vascular smooth muscle cells in different strains of genetically hypertensive rats

Cell type	Reference	Na^+/H^+ exchange		*P* values
A. Kidney BBMV				
1. Morduchowicz et al	[14]	SHR 2.1 ± 0.27	WKY 0.70 ± 0.30	<0.01
2. Hanozet et al	[31]	MHS 10.6 ± 0.3	MNS 7.7 ± 0.8	NS
3. Lewis et al	[32]	DS 30 ± 3	DR 31.6 ± 6	NS
B. Cultured VSM cells				
1. Berk et al	[33]	SHR 12.8 ± 4.3	WKY 6.5 ± 2.3	<0.05
2. Socorro et al	[34]	MHS 23.8 ± 2.2	MNS 16.2 ± 2.1	NS

Abbreviations are: BBMV, brush border membrane vesicles; VSM, vascular smooth muscle cells.

Flux units for A1 and A2 = nmol Na/mg protein × 5 sec; A3 not reported. Sodium diet: A1 = 0.4% NaCl, A2 not reported and A3 = 8% NaCl.

A1:V_{max} of amiloride-sensitive Na^+ influx driven by an outward H^+ gradient in nmol Na/mg protein × 5 sec. A2: V_{max} of H^+-gradient driven Na^+ influx nmol/mg protein × 3 sec. A3: V_{max} of Na-dependent fluorescent changes/mg protein × sec.

B1 and B2: Na^+/H^+ EXC measured as dimethyl-amiloride sensitive Na-influx at pH_i 6.8 in nmol/mg protein × min. NS = not significant.

Might elevated RBC Na^+/H^+ EXC in DS rats reflect a similar abnormality in vascular smooth muscle cells? The antiporter activity has been studied in cultured aortic cells grown with serum medium in SHR/WKY models [33], and in the Milan strains [34] but not in the Dahl strains. As shown in Table 3, the antiporter activity in RBCs correlates with the vascular smooth muscle cell activity in the Milan strains but not in the SHR, which shows elevated activity in serum-grown cells only at early passages [33]. Thus, Na^+/H^+ EXC activity exhibit tissue-specific changes in the different genetic models of hypertension that might be related to different genes controlling its expression or to post-translational modifications regulating its turnover rate. Considering that a variety of tissue-specific regulatory mechanisms of Na^+/H^+ EXC are observed in the rat models, the elevated RBC activity does not necessarily reflect similar kidney and vascular smooth muscle abnormalities.

Recent studies have also shown that the SHR and MHS strains have increased insulin and triglycerides as observed in many hypertensive subjects [35, 36]. These abnormalities appear to be compensatory to the resistance to insulin-stimulated glucose uptake exhibited by adypocytes and skeletal muscle.

Similar studies in the Dahl strains showed that DS and DR rats had significantly higher plasma insulin and triglyceride concentrations than did Sprague-Dawley rats when fed with 0.6% NaCl intake [37]. However, only triglycerides were significantly elevated in DS in comparison to DR rats [37]. These findings suggest that the interaction between salt and insulin sensitivity deserves further studies of RBC Na^+/H^+ EXC in Dahl rats.

Elevated RBC Na^+/H^+ EXC activity has been reported in some patients with essential hypertension [6–9, 38, 39] and insulin-resistant glucose disposal [39], but it is not yet clear whether this abnormality is a characteristic of salt-sensitive patients. Recent studies in young Blacks have demonstrated an increased antiporter activity in RBC of hyperinsulinemic, insulin-resistant hypertensives [39]. Because human RBCs possess insulin receptors and insulin activates the antiporter in vitro [30], the increased Na^+/H^+ EXC activity in these hypertensive patients appear to be sensing the associated hyperinsulinemia of their insulin-resistant state.

In conclusion, RBC Na^+/H^+ EXC is only elevated in the Dahl salt-sensitive hypertensive model when the hypertensive state is established by a high-Na Cl diet. These findings indicate that this abnormality is due to gene-environmental interactions specific for this strain. In addition, excess salt intake and hypertension *per se* do not induce elevation of RBC Na^+/H^+ EXC in DR, MHS, MNS, SHR/CR, and WKY strains. This increased RBC Na^+/H^+ EXC activity appears to be a marker of salt-sensitive hypertension in the rat models of hypertension.

Acknowledgments

This work was supported in part by the following NIH-NHLBI grants: HL 35664, P50-HL 366568, HL 42120. The results were analyzed in a CLINFO facility supported by NIH Grant 02635. We are very grateful to Drs. G. Bianchi and Patricia Ferrari for kindly providing the Milan rat strains. A preliminary report of this study was presented at the 24th Annual Meeting of the American Society of Nephrology held in Baltimore, Maryland, November 1991.

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