Reduced Sodium-Proton Exchange Activity in Lymphocytes From Transgenic Rats

Martin Tepel, Torsten Klaus, Stephanie Laukemper, Walter Zidek

Abstract We investigated sodium-proton (Na\(^+\)-H\(^+\)) exchange activity in transgenic TGR(mRen-2)27 rats, a strain showing fulminant hypertension after the mouse Ren-2 renin gene has been integrated into its genome, in age-matched normotensive Sprague-Dawley (SD) rats, in spontaneously hypertensive rats (SHR) from the Münster strain, and in normotensive Wistar-Kyoto (WKY) rats. From each strain Na\(^+\)-H\(^+\) exchange activity was determined in lymphocytes using the pH-sensitive fluorescent dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) by measuring the recovery rate of cytosolic pH (pH\(_c\)) after intracellular acidification. Resting pH\(_c\) was not significantly different in transgenic rats (n=10) compared with SD rats (n=10) (7.305±0.038 versus 7.337±0.031; mean±SEM), but resting pH\(_c\) was significantly lower in lymphocytes than in their normotensive counterparts (n=12) (7.232±0.030 versus 7.377±0.022; P<.01). Na\(^+\)-H\(^+\) exchange activity was significantly lower in lymphocytes from transgenic rats compared with SD rats (5.102±0.561 versus 7.385±0.491×10\(^{-3}\) dpH/s; P<.01), whereas Na\(^+\)-H\(^+\) exchange was significantly enhanced in lymphocytes from SHR compared with WKY rats (5.564±0.432 versus 7.387±0.433×10\(^{-3}\) dpH/s; P<.05). The apparent half-maximal activation of Na\(^+\)-H\(^+\) exchange was not significantly different in the strains tested. The present study indicates that hypertension in transgenic rats is not related to Na\(^+\)-H\(^+\) exchange overactivity. (Hypertension. 1994; 24:357-361.)

Key Words • calcium channels • ion exchange • lymphocytes • animals, transgenic

In spontaneously hypertensive rats (SHR), an increased sodium-proton exchange (Na\(^+\)-H\(^+\) exchange) was observed in both blood cells and vascular smooth muscle cells (VSMCs). These findings have led to the speculation that an increased Na\(^+\)-H\(^+\) exchange activity might play an important role in the development or maintenance of primary hypertension. The increased Na\(^+\)-H\(^+\) exchange was explained by either a decreased intracellular pH\(_c\) or an increased sensitivity of the Na\(^+\)-H\(^+\) exchange to intracellular pH\(_c\). It is still unclear how Na\(^+\)-H\(^+\) exchange is related to hypertension. In cultured VSMCs of SHR, Na\(^+\)-H\(^+\) exchange activity was observed that was increased and, after several passages, similar to that in normotensive rats. Antihypertensive treatment normalized Na\(^+\)-H\(^+\) exchange in VSMCs of SHR. The Na\(^+\)-H\(^+\) exchange activity may play a permissive role for cell proliferation, eg, in VSMCs. Therefore, Na\(^+\)-H\(^+\) exchange may be causally related to hypertension. On the other hand, an increased Na\(^+\)-H\(^+\) exchange may be just coincided with hypertension, without any causal relation.

We studied Na\(^+\)-H\(^+\) exchange in lymphocytes from transgenic TGR(mRen-2)27 (TGR) rats and from SHR of the Münster strain to answer the question of whether Na\(^+\)-H\(^+\) exchange activity is increased in models of hypertension other than the SHR. In the TGR rats, an additional mouse Ren-2 gene has been integrated into the genome, and TGR rats show a marked increase in blood pressure. Compared with the respective control strain, Na\(^+\)-H\(^+\) exchange was found to be decreased in TGR rats and increased in SHR.

Methods

TGR and Sprague-Dawley (SD) rats were purchased from Moellegaard Breeding Center Deutschland GmbH (Schönwalde, Germany) and from Institut für Versuchstierkunde (Hannover, Germany), respectively. SHR from the Münster strain as previously described and the normotensive Wistar-Kyoto (WKY) control strain were obtained from Medizinische Poliklinik, Münster, Germany. The rats were fed a standard pellet diet and allowed tap water ad libitum. Blood was obtained from 10 TGR rats (8 weeks old; weight, 250 to 300 g; systolic blood pressure, 224±13 mm Hg), 10 SD rats (8 weeks old; weight, 250 to 300 g; systolic blood pressure, 114±5 mm Hg), and 12 WKY rats (8 weeks old; weight, 250 to 300 g; systolic blood pressure, 114±5 mm Hg). All animal procedures were approved by the local authority for animal research.

Preparation of Lymphocytes

Lymphocytes were obtained from heparinized blood according to previously described methods. Briefly, blood was centrifuged at 240g for 15 minutes, and the upper two thirds of the supernatant was aspirated. The remaining blood was mixed 1:1 with Hank's balanced salt solution (HBSS) containing (mmol/L) NaCl 136, KCl 5.4, KH\(_2\)PO\(_4\) 0.44, Na\(_2\)HPO\(_4\) 0.34, CaCl\(_2\) 1.0, d-glucose 5.6, and HEPES 10, pH 7.4. Lymphocytes were prepared after centrifugation of blood on a Ficoll gradient (Lymphoprep, Boehringer Mannheim; 5.6% [wt/vol] Ficoll; density, 1.077 g/mL). The lymphocyte interphase was carefully aspirated, washed three times (400g for 5 minutes), and suspended in HBSS. The lymphocyte viability was greater than 95% as determined by the trypan blue exclusion test.

Measurement of Cytosolic pH (pH\(_c\)) in Lymphocytes

Measurements of pH\(_c\) were done according to established methodology using a pH-sensitive fluorescent dye. A stock
solution of the pH-sensitive fluorescent dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM) at a final concentration of 1 mmol/L was prepared in dimethyl sulfoxide. The lymphocyte suspension (1×10^6 lymphocytes/mL) was incubated with 10 μmol/L of cell-permeant BCECF-AM for 15 minutes at 37°C. After centrifugation at 240g for 15 minutes to remove extraneous dye, the lymphocyte pellet was again resuspended in HBSS. The lymphocyte counts were adjusted to 1×10^6 lymphocytes/mL. The fluorescence intensity of a 100 μL suspension of BCECF-loaded lymphocytes in a thermostated quartz cuvette with constant stirring was measured at 37°C according to established methods. Briefly, lymphocytes were resuspended in a buffer solution in which NaCl had been replaced by KCl (KCl final concentration, 141.4 mmol/L) containing 5 μmol/L nigericin. The calibration was performed by sequential titrations with 0.1 mol/L potassium hydroxide or HCl while fluorescence intensity and extracellular pH were recorded using an electrode connected to a pH meter. The determination was linear in the pH range of 6.3 to 7.6, with correlation coefficient values greater than 0.95. The pH calibration curves were not significantly different among the rat strains studied.

Na^+-H^+ exchange was activated by the addition of various amounts of sodium propionate solution from a 1 mol/L stock solution, pH 7.4 (final concentrations, 5 to 100 mmol/L). Osmotic activation of the Na^+-H^+ exchange by 100 mmol/L propionic acid appeared unlikely, because the addition of 100 mmol/L NaCl did not change the pH in any strain. Furthermore, addition of 100 mmol/L LiCl, which shows an intracellular/extracellular distribution in the range of 2:1, and of 200 mmol/L urea did not change pH. The recovery of pH was initiated in the absence of extracellular sodium, where Na^+ had been replaced by choline, indicating that the recovery of pH after intracellular acidification is mediated by the Na^+-H^+ exchange.

The rate of pH recovery after intracellular acidification was calculated using the software GRAPHPAD-INPLOT 4.02 (GraphPad Software Inc) and expressed as dpH/s (change in pH per second). Rate constants of the pH recovery after intracellular acidification were obtained through curve fitting of the experimental data to an exponential curve.

Concentration-response curves were obtained by addition of various amounts of propionic acid and plotting the achieved pH versus the initial rate of the pH recovery. To obtain the kinetic parameters of the pH recovery rate in lymphocytes from each strain, the data pairs of pH and initial pH recovery rate were pooled for each of the four strains tested. The initial rate of pH recovery increased with decreasing pH, reaching a maximum at a pH of approximately 6.6 to 6.8. The pH of 6.8 or less was always obtained after addition of the maximal concentration of 100 mmol/L propionic acid. According to previous reports, the relation between pH and initial rate of pH recovery can be fitted to a sigmoidal curve using nonlinear regression analysis. The initial rate of pH recovery after maximal stimulation of lymphocytes by 100 or 25 mmol/L propionic acid was used for the comparison of the Na^+-H^+ exchange activity of the lymphocytes from the strains tested.

Statistics
Data are presented as mean±SEM. Groups were compared with ANOVA with Student-Newman-Keuls post hoc test using INSTAT 2.02 (GraphPad). Two-tailed probability values less than 0.05 were considered significant.

Results
Resting pH in Rat Lymphocytes
Resting pH was not significantly different in lymphocytes from hypertensive TGR rats compared with their normotensive SD counterparts (7.305±0.038 versus 7.337±0.031). On the other hand, resting pH was significantly lower in SHR compared with their normotensive WKY counterparts (7.232±0.030 versus 7.377±0.022; P<0.01, Fig 1).

Na^+-H^+ Exchange Activity in Rat Lymphocytes
Fig 2 shows representative fluorescence tracings of BCECF-loaded lymphocytes after intracellular acidification with propionic acid. After the addition of propionic acid, pH dropped immediately. The fall in pH,
activates the Na⁺-H⁺ exchange, which in turn extrudes intracellular H⁺ for extracellular Na⁺ in order to reestablish the original pH. The pHᵢ recovery after intracellular acidification was prevented in the absence of extracellular sodium, where Na⁺ had been isosmotically replaced by choline, supporting the notion that the pHᵢ recovery is due to activation of the Na⁺-H⁺ exchange.

The pHᵢ recovery rates immediately after intracellular acidification were determined from the original experimental data using a computerized calculation procedure. Fig 3 shows pHᵢ recovery rates immediately after intracellular acidification. The pHᵢ recovery rates were distinctly different in lymphocytes from TGR compared with SD rats and in lymphocytes from SHR compared with WKY rats. The Table gives the mean values of the initial rates of pHᵢ recovery after intracellular acidification with 25 or 100 mmol/L propionic acid, respectively. After intracellular acidification with 25 mmol/L propionic acid, the pHᵢ recovery rate was significantly reduced in lymphocytes from TGR compared with SD rats (P<.01, Fig 4). On the other hand, the pHᵢ recovery rate was significantly enhanced in lymphocytes from SHR compared with WKY rats (P<.05), although the difference was less pronounced than that between TGR and SD rats. Note that no significant differences of the pHᵢ recovery rate could be observed between the two hypertensive strains (TGR rats versus SHR), whereas the pHᵢ recovery rate was significantly different in the two normotensive strains (SD versus WKY rats, P<.01). These data suggest the existence of a reduced Na⁺-H⁺ exchange activity in TGR rats and an increased Na⁺-H⁺ exchange activity in SHR compared with their respective normotensive counterparts.

Fig 5 shows the changes of lymphocytic pHᵢ immediately after addition of propionic acid. After intracellular acidification of lymphocytes by addition of 25 mmol/L propionic acid, the changes of lymphocytic pHᵢ were similar in all strains tested. The mean changes of lymphocytic pHᵢ were 0.491±0.037, 0.433±0.032, 0.426±0.016, and 0.445±0.014 for TGR rats, SD rats, SHR, and WKY rats, respectively (P=.281). When maximal concentrations of 100 mmol/L propionic acid were used for intracellular acidification, no significant differences of the changes of lymphocytic pHᵢ among the rat strains tested could be observed either (0.656±0.041, 0.669±0.026, 0.576±0.026, and 0.641±0.027 for TGR rats, SD rats, SHR, and WKY rats, respectively; P=.135).

As indicated in the literature, the changes of lymphocytic pHᵢ immediately after addition of similar amounts of propionic acid are a reflection of the cytosolic buffer...
capacity for H⁺. The apparent half-maximal activation of Na⁺-H⁺ exchange was not significantly different in the strains tested (TGR rats, 6.9±0.1; SD rats, 6.7±0.1; SHR, 6.8±0.2; and WKY rats, 6.9±0.2). Since resting pH values were similar in TGR and SD rats, the significantly reduced activity of the Na⁺-H⁺ exchange in lymphocytes from TGR compared with SD rats cannot be attributed to an acidic shift of the resting pH in TGR rats nor to changes in the buffer capacity for H⁺ in TGR rats.

**Discussion**

We performed the measurements of pH, and Na⁺-H⁺ exchange in lymphocytes to selectively assess the genetically determined deviations of Na⁺-H⁺ exchange in both SHR and TGR rats. To stimulate Na⁺-H⁺ exchange, we used propionic acid, as this method can easily be performed in cell suspensions. A potential weakness of the propionic acid method compared with the NH₄Cl prepulse or nigericin method is that the latter methods allow a more accurate determination of maximal Na⁺-H⁺ exchange activity because of a more pronounced intracellular acidification.

SHR showed a decreased pHᵢ, but in TGR rats pHᵢ was similar to that in SD rats. This finding is in accordance with those of Saleh and Battle and Battle et al, who found a decreased pHᵢ in SHR of another strain. In TGR rats, Na⁺-H⁺ exchange was significantly reduced compared with their control strain. Since pHᵢ values and intracellular buffer capacities were similar in both strains, an intrinsic abnormality of Na⁺-H⁺ exchange is likely. On the other hand, in SHR Na⁺-H⁺ exchange was increased compared with WKY rats. Assumimg that at resting pHᵢ Na⁺-H⁺ exchange is active, the lower pHᵢ can explain the increased Na⁺-H⁺ exchange activity in SHR, although this issue is controversial.

The most trivial cause of different pHᵢ recoveries after intracellular acidification might be differences in the intracellular buffer capacity for H⁺ in lymphocytes from the strains tested. However, as identical amounts of propionic acid induced identical acidification in lymphocytes, this explanation can largely be ruled out. It may be noted that under the conditions used in the present study the buffer capacity is predominantly determined by the total cytosolic protein concentration. It appears unlikely that an enhancement of pHᵢ recovery in lymphocytes from SD rats by a factor of about 1.4 is caused by a corresponding 30% reduction in cytosolic protein concentration.

A comparison of the findings in SHR and TGR rats underscores the different pathomechanisms in both models, because hypertention in TGR rats is associated with a decreased and in SHR with an increased Na⁺-H⁺ exchange activity. In the literature, it was speculated that by a hypothetical effect on vascular growth, Na⁺-H⁺ exchange could have an impact on blood pressure. On the other hand, the findings by Alexander et al, who found that Na⁺-H⁺ exchange activity was not increased in SHR compared with Wistar rats, also suggested that there is no relation between Na⁺-H⁺ exchange activity and hypertension.

In TGR rats the cause of decreased Na⁺-H⁺ exchange is not apparent. TGR rats show a low plasma renin activity, an increased adrenal renin activity, and a decreased renal renin activity, but the pathophysiological basis for hypertension in these animals is unclear. As to the alterations in Na⁺-H⁺ exchange, no direct link to the insertion of the mouse renin gene in these animals is apparent. The findings therefore suggest that in TGR rats other alterations than those caused by the mouse renin gene may exist, whether they are independent of the presumed genetic alteration or somehow indirectly related.

In summary, the data showed that in TGR rats lymphocytic Na⁺-H⁺ exchange is markedly reduced, indicating that Na⁺-H⁺ exchange can vary independent of blood pressure in various rat strains.

**References**


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