Impaired Na\(^{+}\)-H\(^{+}\) Exchanger Activity of Hepatocytes in Chronic Renal Failure

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Abstract. Available data indicate that cation transport is impaired in many cells in chronic renal failure (CRF). The information on the activity of the Na\(^{+}\)-H\(^{+}\) exchanger in CRF is variable, and both increased and reduced activity have been reported. The mechanisms through which CRF may exert an effect on the Na\(^{+}\)-H\(^{+}\) transport are not known. Data exist indicating that PTH inhibits the Na\(^{+}\)-H\(^{+}\) exchange in kidney and liver, and this action of hormone is most likely due to its ability to raise cytosolic calcium ([Ca\(^{2+}\)]\(_{i}\)). Therefore, it is possible that excess PTH in CRF may adversely affect the activity of the Na\(^{+}\)-H\(^{+}\) antiport. This study examines the activity of Na\(^{+}\)-H\(^{+}\) antiport, intracellular pH (pH\(_{i}\)), and buffering capacity of hepatocytes obtained from rats after 6 wk of CRF, from CRF parathyroidectomized animals, and from CRF rats and normal rats treated with verapamil. The pH\(_{i}\) and the buffering capacity of hepatocytes were not different in all groups of animals. The activity of the Na\(^{+}\)-H\(^{+}\) antiport of hepatocytes from CRF animals was significantly (P < 0.01) lower than in hepatocytes from normal rats, CRF parathyroidectomized rats, CRF rats treated with verapamil, and normal rats treated with verapamil, and the values in the latter four groups of animals were not different. This impaired activity of Na\(^{+}\)-H\(^{+}\) antiport in CRF was observed in all external concentrations of sodium (25, 50, 75, 100, 125, and 150 mM). Thus, CRF altered the kinetics of the transporter in that its V\(_{\text{max}}\) decreased and its K\(_{m}\) increased.

The data show that: (1) CRF is associated with reduction in the activity of Na\(^{+}\)-H\(^{+}\) antiport in hepatocytes; (2) this defect is due to the state of secondary hyperparathyroidism of CRF; and (3) excess PTH mediates its effect by elevating [Ca\(^{2+}\)]\(_{i}\) of hepatocytes because treatment of CRF animals with verapamil, which blocks the PTH-induced rise in [Ca\(^{2+}\)]\(_{i}\), of these cells, prevented the impairment in the activity of the Na\(^{+}\)-H\(^{+}\) antiport. (J Am Soc Nephrol 8: 929–934, 1997)

A large body of evidence indicates that cation transport is impaired in a variety of cells in chronic renal failure (CRF). The V\(_{\text{max}}\) of Ca\(^{2+}\) ATPase is reduced in brain synaptosomes, pancreatic islets, cardiac myocytes (1), adipocytes (2), and hepatocytes (3) of CRF rats and in red blood cells of patients with CRF (4). Similar observations were reported regarding the V\(_{\text{max}}\) of Na\(^{+}\)-K\(^{+}\) ATPase (1,3–5) and Na\(^{+}\)-Ca\(^{2+}\) exchanger (1–3). The available data on the effect of CRF on the activity of the Na\(^{+}\)-H\(^{+}\) antiport are not uniform. Corry et al. (6) found an increase in activity of Na\(^{+}\)-H\(^{+}\) antiport of red blood cells from patients treated with hemodialysis, and Cohn et al. (7) reported a similar observation in renal brush border vesicles of dogs with renal insufficiency. In contrast, Rombola et al. (8) reported reduced activity of Na\(^{+}\)-H\(^{+}\) exchanger in lymphocytes from hemodialysis patients, and Greiber et al. (9) found that the activity of this exchanger in thymocytes from CRF rats is decreased. It is possible that the effect of CRF or other factors such as metabolic acidosis on the Na\(^{+}\)-H\(^{+}\) antiport varies among tissues, as suggested by Moe et al. (10). Therefore, examination of the effect of CRF on the Na\(^{+}\)-H\(^{+}\) exchanger of other cells is warranted.

Michnowska et al. (11) reported that the exposure of hepatocytes to PTH initially activated the Na\(^{+}\)-H\(^{+}\) antiport, which was followed by marked inhibition; these effects of PTH were due to the PTH-induced rise in cytosolic calcium ([Ca\(^{2+}\)]\(_{i}\)) of hepatocytes because both verapamil and nifedipine blocked the effects of PTH on the Na\(^{+}\)-H\(^{+}\) exchanger. PTH also inhibits the activity of the Na\(^{+}\)-H\(^{+}\) antiport of renal brush border membrane (12) and of cultured American opossum kidney cells (13). Therefore, it is plausible to propose that the state of secondary hyperparathyroidism of CRF, which is associated with elevation of basal levels of [Ca\(^{2+}\)]\(_{i}\) (1), would inhibit the activity of the Na\(^{+}\)-H\(^{+}\) antiport in CRF.

The present study examined the effect of CRF on the Na\(^{+}\)-H\(^{+}\) antiport of hepatocytes and evaluated the role of chronic excess of PTH in CRF in the genesis of the potential changes of the Na\(^{+}\)-H\(^{+}\) exchanger activity of these cells in CRF.

Materials and Methods

A total of 80 Sprague-Dawley rats weighing 320 to 400 (354 ± 3.8) g were used. The animals were housed in individual cages and fed normal rat chow (Wayne Research Animal Diets, Chicago, IL) throughout the study. The diet contained 1.4% calcium, 0.97% phosphorus, and 4.4 IU of vitamin D/g. Studies were performed in five groups of animals: (1) normal rats; (2) rats with CRF of 6 wk duration; (3) normocalcemic parathyroidectomized CRF (CRF-PTX) rats of 6
wk duration; (4) CRF rats of 6 wk duration treated with verapamil (CRF-V; 0.1 μg/g body wt), which was given subcutaneously twice a day from day 1 of CRF; and (5) normal rats treated with verapamil (normal-V), as described above for 6 wk.

CRF was produced by 5/6 nephrectomy; the animals underwent right 2/3 nephrectomy through a flank incision and 1 wk later, a left nephrectomy was done. PTX was performed by electrocautery, and the success of the procedure was ascertained by a decrease in plasma levels of calcium of at least 2 mg/dl. The PTX rats were allowed to freely drink water containing 5% calcium gluconate. This procedure is adequate to normalize serum calcium in the PTX rats. Seven days after PTX, the rats were subjected to 5/6 nephrectomy as described above. Two days before being euthanized, the animals were housed in metabolic cages, and two consecutive 24-h urine collections were obtained for the measurement of creatinine clearance. The animals were killed by decapitation on day 42 after the completion of the 5/6 nephrectomy in CRF rats (CRF, CRF-PTX, CRF-V) or after the beginning of the treatment with verapamil in normal rats.

Hepatocytes were isolated using a modification of the method of Seglen (14). The animals were injected with 5000 U of sodium heparin (ICN Biochemical, Irvine, CA) and 15 min later, the rats were killed by decapitation. The chest was opened and the aorta was cut and catheterized. Subsequently, the liver was perfused with 200 ml of cold (4°C) oxygenated calcium-free Joklik medium (Sigma Chemical Co., St. Louis, MO) supplemented with 10 mM Na HEPES, 10 mM glucose, 0.5% bovine serum albumin, and 1 mM EGTA (pH, 7.4) for 2 min. At the end of this procedure, the liver was uniformly pale. Both superior and inferior vena cava were ligated, and a PE 20 tube was placed in the superior vena cava below the ligation. The liver was then removed and perfused through the vena cava catheter with warm (37°C) oxygenated calcium-free Joklik medium with no EGTA, but containing 150 U of collagenase type II/ml (Worthington Biochemical, Freehold, NJ) and 0.6 mg of hyaluronidase/ml (Sigma) (pH 7.4) for 15 min with a flow of 15 ml/min. At the end of the perfusion, the capsule of the liver was removed, and the liver was teased gently and cut into small pieces by a scissor. The liver fragments were placed into a 250-μm mesh Nytex screen (Tetko, Elmsford, NY) and washed with 10 to 15 ml of oxygenated calcium-free Joklik medium without EGTA (pH 7.4). Hepatocytes that filtered through the mesh Nytex screen were collected. The bigger liver fragments were placed into Joklik medium containing collagenase and hyaluronidase for 15 min and again filtered through the mesh Nytex screen. The cell mixture was centrifuged at 100 × g for 3 min. The pellet was resuspended into 50 ml of oxygenated calcium-free Joklik medium and refiltered through the mesh Nytex screen. The cell mixture was centrifuged again at 100 × g for 3 min, and the pellet was resuspended in oxygenated calcium-free Joklik medium to give approximately 3 × 10⁸ cells/ml and kept on ice.

The cells displayed well-preserved refringent shape under the microscope as described by Seglen (14) and by us (15). Viability of the cells was >90% as assessed by the trypan blue exclusion test, and their viability and intactness were evaluated further by their responsiveness to agonists. The hepatocytes produced cAMP in response to PTH and displayed a rise in their ICa²⁺ in response to PTH and vasopressin, and the values of [Ca²⁺]i of hepatocytes kept on ice at zero time and after 30, 60, and 90 min were not different (15).

Measurement of Hepatocyte pH₁

An aliquot of 2 ml of the cell suspension was used for the loading of the hepatocytes with 50 μg (2.5 μM) of acetoxymethylene 2',7'-bis (carboxymethyl)-5,6-carboxyfluorescence (Molecular Probes, Eugene, OR). The loading was done in a shaking water bath (37°C) for 40 min. The hepatocytes were then purified with 47% Percoll (Pharmacia LKB Biotechnology, Piscataway, NJ) step gradient (500 g for 2 min in swinging bucket centrifuge). All of the supernate and the Percoll layer were discarded. The pellet was removed and washed again with a calcium-free Joklik medium by centrifugation. The final pellet was suspended in 2 ml of calcium-free Joklik medium and kept on ice.

An aliquot of 100 μl of the cell suspension was added to 1.9 ml of potassium solution (120 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 20 mM HEPES acid, pH 7.4) or sodium solution. The sodium solution was similar to the potassium solution except that it contained 140 mM NaCl and 1 mM KCl. The mixture was placed in a cuvette of a spectrofluorometer Hitachi 2000 (Hitachi Instruments, Inc., Danbury, CT), and was kept under constant magnetic stirring at 37°C. The fluorometer was connected to a personal computer loaded with F-2000 intracellular cation measurement system software version 1.01 (1991) for acquisition of data, calculation of fluorescence excitation/emission ratio (wavelengths: excitation, 500 and 400 nm; emission, 520 nm), and conversion of the data into pH₁ using an external calibration curve performed during each experiment. The basal pH₁ of hepatocytes remained stable over the period of 300 to 500 s.

The external calibration procedure was done with the nigericin (Sigma Chemical Co.) technique as described by Grinstein et al. (16). For this purpose, an aliquot of 100 μl of cells was suspended in 1.9 ml of potassium solution, and 10 μl of nigericin (5 μM) was added. The pH of the medium was altered in a stepwise manner with acid (1N HCl) or base (1N NaOH) in the range of 6.5 to 7.5, the pH of the medium was measured, and fluorescence was recorded after each addition.

Measurement of Buffering Capacity

The buffering capacity was estimated by ammonium chloride pulse technique (17). An aliquot of 100 μl of cell suspension was added to 1.9 ml of choline chloride medium (130 mM choline chloride, 1 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 10 mM HEPES acid, pH 7.4), and the mixture was placed in the cuvette of the fluorometer. The cells were acidified by the addition of 10 μl of nigericin (5 μM), and when pH was approximately 6.5 to 6.7, 50 μl of albumin (100 μg/ml) was added to scavenge the nigericin and to stop the acidification process. Subsequently, 50 μl of (200 mM) NH₄Cl (5 mM final concentration) was added to rapidly alkalinate the cells. This was followed by the addition of 10 μl of 10% Triton-X. The buffering capacity (β) was calculated using the formula of Ross and Boron (17), which was rearranged according to Saleh et al. (18).

β = \frac{Δ[NH_4^+]}{ΔpH_1} \times \frac{10^{pH_1}}{10^{pH_4} \times 10^{pH_5, initial}}

where [NH₄⁺]₀ = concentration of NH₄Cl added (5 mM), ΔpH₁ = pH after addition of NH₄Cl minus pH₁ before addition of NH₄Cl, pH₀ = pH after addition of Triton, and pH initial = pH after acidification with nigericin.

Estimation of Na⁺-H⁺ Exchanger

Cells were acidified to pH 6.5 to 6.7 (6.58 ± 0.015) with nigericin as described above in the procedure for determining buffering capacity. Nigericin was scavenged by albumin. The mixture was centrifuged and the supernatant was discarded, and 100 μl of the cells’ pellet were suspended in 1.9 ml of sodium medium (150 mM NaCl, 1 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 10 mM glucose, and 20 mM HEPES acid, pH 7.4). The speed of recovery from acid load was
calculated from the change of \( \text{pH}_1 \) over 60 s. The activity of the exchanger estimated by hydrogen efflux (\( J_{\text{H}^+} \)) was calculated as follows:

\[
J_{\text{H}^+} = \frac{\Delta \text{pH}_1}{\Delta t} \times \beta
\]

where \( \Delta \text{pH}_1 = \text{pH}_1 - \text{pH}_0 \) at 60 s in the presence of NaCl minus \( \text{pH}_0 \) after acidification with nigericin, \( \Delta t = 60 \) s, and \( \beta \) = buffering capacity.

The kinetics of the \( \text{Na}^+-\text{H}^+ \) exchanger was evaluated by measuring \( J_{\text{H}^+} \) at different concentrations of sodium chloride (25, 50, 75, 100, and 150 mM). The osmolality of the solution was always the same, and this was achieved by choline chloride substituting for NaCl. The effect of \( 10^{-6} \) M PTH- (1-84) on the \( \text{Na}^+-\text{H}^+ \) activity was evaluated after incubation of the hepatocytes with the hormone for 5 min. The \( K_m \) and \( V_{\text{max}} \) of \( \text{Na}^+-\text{H}^+ \) exchanger were calculated using the Lineweaver-Burk plot.

The measurement of calcium in the plasma was made by Perkin-Elmer atomic absorption spectrophotometer model 503 (Perkin-Elmer Corp., Norwalk, CT) and those of plasma creatinine and phosphorus and urine creatinine by an autoanalyzer (Technicon Instrument Corp., Tarrytown, NY). The serum levels of PTH were determined by an INS-PTH immunoassay kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). This assay recognizes the aminoterminal fragment of PTH. The lowest detectable level is 3 pg/ml; the interassay variation is 7.3% and the intra-assay variation is 4%.

Statistical Analyses

Statistical analysis was done by one-way ANOVA and significant differences with Bonferroni-Dunn test for multiple comparison between the groups. Data are presented as mean ± SEM.

Results

Table 1 shows the effects of the various experimental procedures on body weight; plasma concentrations of creatinine, calcium, and phosphorus; blood bicarbonate and pH; and serum levels of PTH and creatinine clearance. There were no significant differences in body weight and in the plasma concentrations of calcium and phosphorus among the five groups of rats. The plasma concentrations of creatinine were significantly (\( P < 0.01 \)) higher, and the creatinine clearances were significantly (\( P < 0.01 \)) lower in CRF, CRF-PTX, and CRF-V rats than in normal and normal-V animals. The blood bicarbonate concentrations and the values of pH were significantly (\( P < 0.01 \)) reduced in CRF animals. There were no significant differences between these parameters among CRF, CRF-PTX, and CRF-V rats. The serum levels of PTH were significantly (\( P < 0.01 \)) higher in CRF and CRF-V rats than in normal, normal-V, and CRF-PTX animals.

The effect of the various experimental procedures on the pH was and the buffering capacity of hepatocytes is shown in Table 2. There were no significant differences in these parameters among the five groups of rats.

CRF was associated with inhibition of \( \text{Na}^+-\text{H}^+ \) exchanger activity of hepatocytes. At all levels of external sodium, the \( J_{\text{H}^+} \) in hepatocytes from CRF animals was significantly lower than in normal rats (Figure 1), indicating that the kinetics of \( 
\text{Na}^+-\text{H}^+ \) exchanger is altered in CRF. Indeed, the \( V_{\text{max}} \) of the \( \text{Na}^+-\text{H}^+ \) exchanger was reduced (CRF, 12.3 ± 0.66 mmol/liter/min versus normal 15.8 ± 0.79 mmol/liter/min, \( P < 0.01 \)) and the \( K_m \) was increased (CRF, 89 ± 3.3 mM versus normal, 71 ± 4.1 mM, \( P < 0.01 \)) (Figure 2). These abnormalities were prevented by prior PTX of the CRF rats or by their treatment with verapamil; the administration of verapamil to normal rats did not alter the kinetics of the \( \text{Na}^+-\text{H}^+ \) exchanger activity (Figures 1 and 2).

Bovine PTH- (1-84) (Lot 10H0661, Sigma Chemical Co.) in a dose of \( 10^{-6} \) M inhibited the \( 
\text{Na}^+-\text{H}^+ \) exchanger activity, measured at 150 mM of sodium chloride, in all groups of animals, but both the absolute decrease in the \( \text{Na}^+-\text{H}^+ \) activity and the percentage inhibition was smaller in the CRF animals than in normal, CRF-PTX, CRF-V, and normal-V rats. There were no significant differences in these parameters in the latter four groups of rats (Table 3).

Discussion

The results of the present study demonstrate that the \( \text{Na}^+-\text{H}^+ \) exchanger activity of hepatocytes is impaired in CRF. This observation is similar to that of Greiber et al. (9), who found that the activity of this exchanger in thymocytes of CRF rats was also reduced. In addition, Rombola et al. (8) reported a decreased activity of the \( \text{Na}^+-\text{H}^+ \) exchanger of T lymphocytes obtained from hemodialysis patients. Our data further show that CRF altered the kinetics of the \( \text{Na}^+-\text{H}^+ \) exchanger in that the \( V_{\text{max}} \) of this transporter was reduced and its \( K_m \) was increased. Our results also provide evidence that this abnormality is, in major part, due to the state of secondary hyper-

Table 1. Body weight, biochemical parameters, and serum PTH levels in all groups of rats at time of euthanizationa

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>Body Weight (g)</th>
<th>Plasma</th>
<th>Blood</th>
<th>Serum PTH (pg/ml)</th>
<th>Creatinine Clearance (( \mu l/min per 100 g ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>26</td>
<td>343 ± 5.3</td>
<td>0.29 ± 0.01</td>
<td>9.6 ± 0.07</td>
<td>6.5 ± 0.26</td>
<td>27 ± 1.0</td>
</tr>
<tr>
<td>CRF</td>
<td>20</td>
<td>351 ± 4.2</td>
<td>1.43 ± 0.13b</td>
<td>9.4 ± 0.12</td>
<td>7.2 ± 0.14</td>
<td>19 ± 0.3</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>11</td>
<td>362 ± 10.9</td>
<td>1.28 ± 0.12b</td>
<td>9.3 ± 0.17</td>
<td>7.3 ± 0.18</td>
<td>18 ± 0.1b</td>
</tr>
<tr>
<td>CRF-V</td>
<td>18</td>
<td>352 ± 6.4</td>
<td>1.34 ± 0.12b</td>
<td>9.4 ± 0.14</td>
<td>7.1 ± 0.17</td>
<td>20 ± 1.0b</td>
</tr>
<tr>
<td>Normal-V</td>
<td>5</td>
<td>350 ± 4.5</td>
<td>0.31 ± 0.03</td>
<td>9.5 ± 0.16</td>
<td>6.6 ± 0.25</td>
<td>26 ± 1.0</td>
</tr>
</tbody>
</table>

*Data are presented as mean ±SEM.

\( a \) P < 0.01 versus normal and normal-V and in the case of serum PTH versus normal, CRF-PTX, and Normal-V.
Table 2. Effect of the various experimental procedures on the pH$_i$ and buffering capacity of hepatocytes

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>pH$_i$ (mM)</th>
<th>Buffering Capacity (mM/pH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25</td>
<td>7.04 ± 0.006</td>
<td>30.8 ± 0.92</td>
</tr>
<tr>
<td>CRF</td>
<td>7</td>
<td>7.03 ± 0.005</td>
<td>30.9 ± 1.37</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>6</td>
<td>7.03 ± 0.010</td>
<td>31.6 ± 1.52</td>
</tr>
<tr>
<td>CRF-V</td>
<td>7</td>
<td>7.04 ± 0.010</td>
<td>31.8 ± 1.60</td>
</tr>
<tr>
<td>Normal-V</td>
<td>5</td>
<td>7.03 ± 0.012</td>
<td>30.6 ± 1.36</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± 1 SEM.

Figure 1. The relationship between the activity of Na$^+$.H$^+$ exchanger (H$^+$ efflux) of hepatocytes and the external sodium concentration in the media. Each datum represents the mean of five to nine studies, and brackets denote ±1 SEM.

Figure 2. The $V_{\text{max}}$ and $K_m$ of the Na$^+$.H$^+$ exchanger in the five groups of animals studied. Each column represents the mean of five to nine studies, and the brackets denote ±1 SEM. *P < 0.01 versus other groups.

parathyroidism of CRF. Indeed, the activity of the Na$^+$.H$^+$ exchanger of hepatocytes from CRF-PTX rats is normal. This notion is in agreement with the data of others who reported that PTH inhibits the Na$^+$.H$^+$ exchanger of renal cells (12,13,19) and normal hepatocytes (11). Furthermore, the activity of the Na$^+$.H$^+$ exchanger of renal brush border vesicles from parathyroidectomized dogs is increased (20). The normalization of the Na$^+$.H$^+$ exchanger in CRF-PTX rats occurred despite no differences between the degree of the acidosis of these animals and those with CRF and intact parathyroid glands.

Although the mechanism(s) through which PTH exerts its effect on the Na$^+$.H$^+$ exchanger of hepatocytes from CRF rats is not defined, the action of the hormone is most likely due to the PTH-mediated elevation in [Ca$^{2+}$]$_i$ in CRF (1), including that of hepatocytes (3). Available data and observations in our study support this proposal. First, the acute exposure of hepatocytes to PTH causes a rise in [Ca$^{2+}$]$_i$, an effect that is mediated through the activation of calcium channels inhibitable by calcium channel blockers such as verapamil (15). This action of the hormone is associated with inhibition of the activity of the Na$^+$.H$^+$ exchanger (11). Second, CRF is associated with an elevation in basal levels of [Ca$^{2+}$]$_i$ of hepatocytes, and prior PTX of CRF rats or their treatment with verapamil prevents the rise in [Ca$^{2+}$]$_i$ (3). Third, our data show that the activity of the Na$^+$.H$^+$ exchanger of hepatocytes in CRF-PTX rats (low PTH blood levels and normal [Ca$^{2+}$]$_i$) (3) and CRF-V animals (high PTH blood levels and normal [Ca$^{2+}$]$_i$) (3) is normal. All of these observations are consistent with the notion that the inhibitory effect of PTH on the Na$^+$.H$^+$ exchanger of hepatocytes of CRF animals is due to the hormone-mediated elevation in their [Ca$^{2+}$]$_i$. 


Table 3. Effect of $10^{-6}$ M of PTH (1–84) on the Na$^\text{+}$-H$^\text{+}$ exchanger activity of hepatocytes in various groups of animals$^a$

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Before PTH</th>
<th>After PTH</th>
<th>$\Delta$</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mmol/L per min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>8</td>
<td>11.1 ± 0.25</td>
<td>7.9 ± 0.31</td>
<td>3.2 ± 0.12</td>
<td>30 ± 1.59</td>
</tr>
<tr>
<td>CRF</td>
<td>9</td>
<td>8.9 ± 0.21$^b$</td>
<td>7.3 ± 0.15</td>
<td>1.6 ± 0.17$^b$</td>
<td>18 ± 1.36$^b$</td>
</tr>
<tr>
<td>CRF-PTX</td>
<td>6</td>
<td>10.2 ± 0.25</td>
<td>7.0 ± 0.30</td>
<td>3.2 ± 0.21</td>
<td>31 ± 2.03</td>
</tr>
<tr>
<td>CRF-V</td>
<td>5</td>
<td>9.8 ± 0.13</td>
<td>6.7 ± 0.35</td>
<td>3.1 ± 0.33</td>
<td>33 ± 3.20</td>
</tr>
<tr>
<td>Normal-V</td>
<td>5</td>
<td>10.6 ± 0.29</td>
<td>7.0 ± 0.21</td>
<td>3.6 ± 0.12</td>
<td>33 ± 0.64</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± 1 SEM.
$^b$ P < 0.01 versus other groups.

It should be mentioned that the elevation in the basal levels of [Ca$^{2+}$] of cells in CRF exerts inhibitory effects on other transport systems as well. In hepatocytes, the elevation of their [Ca$^{2+}$], in CRF inhibits the activity of Ca$^{2+}$ ATPase, Na$^+$-K$^+$ ATPase, and Na$^+$-Ca$^{2+}$ exchanger (3). Similar effects were reported in brain synaptosomes (1), cardiac myocytes (1), and adipocytes (2). Further, in phosphate-depleted rats that have normal renal function and low blood levels of PTH, the basal levels of [Ca$^{2+}$] are elevated in many cells (21). This is associated with reduced activity of Ca$^{2+}$ ATPase, Na$^+$-K$^+$ ATPase, and Na$^+$-Ca$^{2+}$ exchanger (21,22). Restoration of [Ca$^{2+}$], in phosphate-depleted rats to normal by their treatment with a calcium channel blocker is associated with normalization of the activity of these transport systems despite phosphate depletion (21). It is apparent that there are many observations indicating that an elevation in [Ca$^{2+}$] in the presence or absence of CRF and/or excess PTH is associated with impairment in many cellular transport systems of cations.

Our data in hepatocytes, those of Greiber et al. (9) in thymocytes, and those of Rombola et al. (8) in lymphocytes are different from those of Corry et al. (6), who reported an increase in Na$^+$-H$^+$ exchanger activity in red blood cells in CRF. However, Corry et al. (6) used a different methodology from that used in our studies and in those of Greiber et al. (9) and Rombola et al. (8). Cohn et al. (7) also found an increase in Na$^+$-H$^+$ exchanger activity of renal brush border vesicles from dogs with renal failure. The reasons for the differencesbetween the results of these various studies are not evident. They may be related to differences in the degree of renal insufficiency, the severity of secondary hyperparathyroidism, the magnitude of the elevation in [Ca$^{2+}$], of the different cells, differences in methodology, and/or variations in nutrient intake. Indeed, Harris et al. (23) found that high dietary intake of protein increases Na$^+$-H$^+$ exchange of renal microvillus membrane vesicles, and Moe et al. (24) showed that high salt intake decreases Na$^+$-H$^+$ exchanger activity of renal cortical apical membrane vesicles. Finally, it is also possible that the effect of CRF on the Na$^+$-H$^+$ exchange of various cells is not uniform. Moe et al. (10) provided data supporting this notion, at least regarding the effect of acidosis.

The reduction in the $V_{\text{max}}$ of the Na$^+$-H$^+$ exchanger in CRF could be due to a reduced transport rate by each antiport unit and/or a decrease in the number of the antiporters. Our study did not examine this issue. We have reported previously that the elevation of [Ca$^{2+}$] of hepatocytes in CRF caused a reduction in the concentration of the liver mRNA of several proteins, including those of PTH-PTH-related protein, angiotensin II, vasopressin receptors (25), and hepatic lipase (26); these derangements were corrected when the levels of [Ca$^{2+}$], of hepatocytes were restored to normal by PTX of the CRF animals or by their treatment with verapamil. It is possible that the elevation in [Ca$^{2+}$], of hepatocytes in CRF exerts a similar effect on the mRNA of isoform 1 of the Na$^+$-H$^+$ exchanger that is present in the liver (27). Such an effect could result in a reduction in the number of Na$^+$-H$^+$ exchanger units.

Prolonged exposure of normal hepatocytes to PTH in vitro caused a dose-dependent inhibition of their Na$^+$-H$^+$ exchanger (11). The data of the present study again showed that the activity of the Na$^+$-H$^+$ exchanger of hepatocytes from all of the five groups of animals studied was inhibited after their exposure to PTH in vitro. However, the magnitude of the inhibition in hepatocytes from CRF was significantly lower than that observed in hepatocytes from normal, CRF-PTX, CRF-V, and normal-V rats. This finding indicates that in CRF the activity of the Na$^+$-H$^+$ exchanger of hepatocytes is somewhat protected from the effect of a continued rise in blood levels of PTH. This is apparently achieved by the downregulation of the PTH-PTH-related protein receptor. Indeed, the concentration of the mRNA of this receptor in the liver is decreased in CRF (25), but it is normal or near normal in CRF-PTX and CRF-V rats.

The results of the present study showed that pH$_i$ of hepatocytes was not affected by CRF, and the values were normal in all groups of animals studied. This observation is similar to that reported by Greiber et al. (9). Because the preparation of hepatocytes took time and because the measurement of Na$^+$-H$^+$ exchanger activity was evaluated in the absence of the CO$_2$HCO$_3$ environment, as was the case in the in vivo setting, one cannot exclude that impairment in the pH$_i$ regulation of hepatocytes does exist in CRF. The available data on the effect of CRF on pH$_i$ are variable. Lower pH$_i$ values have been reported in red blood cells (28) and peripheral leukocytes (29) from nondiabetic hemodialysis patients, whereas normal values of pH$_i$ were observed in uremia as well (30).
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