# Na$^+$/H$^+$ Exchange in Human Lymphocytes and Platelets in Chronic and Subacute Metabolic Acidosis

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## Abstract

The effect of acid-base disturbances on sodium/proton (Na$^+$/ H$^+$) exchange has been examined in animal models; however, few data are available from human studies. To test the effect of metabolic acidosis on Na$^+$/H$^+$ exchange in man, as well as to examine the relationship between Na$^+$/H$^+$ exchange and cytosolic calcium ([Ca$^{2+}$]$_i$), we measured both variables in patients with decreased renal function with mild metabolic acidosis (pH 7.34±0.06), in normal control subjects (pH 7.41±0.02), and in subjects before (pH 7.40±0.01), and after (pH 7.26±0.04) ammonium chloride (NH$_4$Cl) 15 g for 5 d. Lymphocytes and platelets were loaded with the cytosolic pH (pH$_i$) indicator 2'-7'-bis(carboxyethyl)-5,6-carboxyfluorescein and acidified to pH ~6.6 with propionic acid. To quantitate Na$^+$/H$^+$ exchange, dpH$_i$/d$t$ was determined at 1 min. [Ca$^{2+}$]$_i$ was measured with fura-2. Na$^+$/H$^+$ exchange was significantly increased only in lymphocytes of patients with renal insufficiency. Neither intracellular pH (pH$_i$) nor [Ca$^{2+}$]$_i$ was different from controls. NH$_4$Cl resulted in a significant increase in Na$^+$/H$^+$ exchange in lymphocytes, but not in platelets of normal subjects. Values of pH$_i$ and [Ca$^{2+}$]$_i$ in either cell type remained unaffected. Since metabolic acidosis influenced Na$^+$/ H$^+$ only in lymphocytes, but not in platelets, it is possible that protein synthesis may be involved in increasing Na$^+$/H$^+$ exchange. (*J. Clin. Invest.* 1993. 92:858–865.) Key words: Na$^+$/ H$^+$ antiporter • lymphocytes • platelets • acidosis • renal failure

## Introduction

The Na$^+$/H$^+$ exchanger is an integral membrane protein that mediates a 1:1 electroneutral exchange of extracellular Na$^+$ for intracellular H$^+$ ions (1, 2). All vertebrate cells investigated so far contain such an exchange mechanism which regulates cytosolic pH (pH$_i$) and cell volume, and which is activated by mitogenic stimuli and a variety of hormones (1, 2). In renal epithelial cells, two types of Na$^+$/H$^+$ exchangers exist which are distinguished by their different sensitivities to amiloride and its analogues (3). Here, Na$^+$/H$^+$ exchange is also involved in Na$^+$ and HCO$_3^-$ absorption.

Hypertension and diabetes, which frequently occur concomitantly, are characterized by progressive vascular disease, the pathogenesis of which may involve Na$^+$/H$^+$ exchange by as yet imperfectly defined mechanisms (4). There is a high incidence of both, which results in, or contributes to, the development of chronic renal failure (CRF).[1] Subtle disturbances in acid-base regulation have recently been described in patients prone to salt-sensitive, essential hypertension (5). An enhanced Na$^+$/H$^+$ exchange has been well documented in platelets, leukocytes, red blood cells, and skeletal muscle in essential hypertension, but little is known about the underlying mechanism (for a recent review see reference 6). It is conceivable that the phenomenon develops as a consequence of other hitherto unidentified hormonal or metabolic disturbances. Thus, the definition of factors that modulate Na$^+$/H$^+$ exchange activity in humans in vivo may also contribute to the understanding of this abnormality in disease states such as essential hypertension and diabetes.

While the effects of acid-base disturbances on Na$^+$/H$^+$ exchange in renal tubular cells of experimental animals have been studied, few human data are available. We sought to characterize Na$^+$/H$^+$ exchange and pH$_i$ regulation in vivo in two states of metabolic acidosis in man. We first investigated chronic acidosis in patients with diminished renal function. Subsequently we investigated the effect of subacute, metabolic acidosis on Na$^+$/H$^+$ exchange in lymphocytes and platelets from normal individuals.

## Methods

*Subject groups and conditions.* Due approval and informed consent were obtained from the normal subjects and patients with renal insufficiency before the investigations. The protocol was approved by the University of Erlangen committee for the protection of human subjects according to the declaration of Helsinki.

*Study I: chronic renal acidosis.* Blood samples were obtained from 10 male patients with renal insufficiency, each of whom had a Cimino-Brescia fistula placed in preparation for subsequent hemodialysis. These patients had a variety of chronic renal diseases (glomerulonephritis, type II diabetes mellitus, hypertensive nephrosclerosis, and analgesic nephropathy), but none had type I diabetes mellitus. All were undergoing pharmacological treatment for hypertension. Venous blood specimens were also obtained from 10 healthy volunteer subjects as controls. Creatinine, Na$^+$, K$^+$, Cl$^-$, HCO$_3^-$, Pco$_2$, and pH were measured in serum or plasma as appropriate by automated methods. Plasma renin activity and plasma aldosterone concentration were measured by radioimmunoassay (7).

*Study II: induced metabolic acidosis in normal volunteers.* Eight normal male volunteers were recruited to participate in a second protocol. Arterialized venous blood specimens (8) were obtained for the variables described above. These blood specimens all had an oxygen saturation of > 90%. Thereafter, the subjects ingested 30 500-mg

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1. *Abbreviations used in this paper:* BCECF, 2'-7'-bis(carboxyethyl)-5,6-carboxyfluorescein; CRF, chronic renal failure; EIPA, 5,(*N*-ethyl-*N*-isopropyl)-amiloride; pH$_i$, cytosolic pH.

NH₄Cl capsules in six divided, five-capsule doses for a total of 5 d as described elsewhere (9). On the morning of the fourth and fifth days, arterialized venous blood specimens were again obtained. On day 10 blood was again drawn for recovery observations.

*Preparation of platelet-rich plasma and isolation of lymphocytes.* The blood was anticoagulated by the addition of 20% (vol/vol) of acid citrate dextrose (85 mM trisodium citrate, 65 mM citric acid, and 11 mM glucose). Platelet-rich plasma was prepared by centrifugation of blood at 200 g for 15 min at room temperature. The upper two-thirds of the supernatant was used for preparation of either fura-2 or 2'-7'-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF)–loaded platelets. The remaining blood was resuspended 1:1 with Hepes-buffered RPMI 1640 medium, pH 7.4. Lymphocytes were prepared after centrifugation of blood on a Ficoll gradient (10). Inspection of stained cell smears revealed that lymphocyte preparations were composed of 90% lymphocytes and 10% monocytes.

*Measurement of $pH_i$.* Cytosolic pH was determined using the fluorescent pH indicator BCECF. Pelleted platelets were resuspended in Hepes buffer consisting of 140 mM NaCl, 5 mM KCl, 5 mM KH₂PO₄, 1 mM MgSO₄, 10 mM Hepes (free acid), and 5 mM glucose, pH 6.5 (at 37°C). 10 μM BCECF-AM (final concentration; Molecular Probes, Inc., Eugene, OR) was added, and the cells were incubated for 30 min at 37°C. Thereafter, 1 μM PGI₂ was added and the cells were washed twice in Hepes buffer, pH 6.5, by repeated centrifugation. The final platelet pellet was resuspended in Hepes buffer, pH 7.4, at a concentration of 5 × 10⁹ cells/ml. 100-μl aliquots of these suspensions were transferred to 2 ml of Hepes buffer in a cuvette and prewarmed at 37°C. All measurements were conducted within 1 h after loading. During this time leakage of BCECF did not exceed 10% as assessed by comparison of fluorescence of platelets and corresponding supernatants and was, therefore, neglected.

Lymphocytes were incubated with 10 μM BCECF-AM for 30 min at 37°C in RPMI 1640 medium and washed twice in this medium by repeated centrifugation. Before prewarming to 37°C, aliquots of lymphocytes were briefly spun down in an Eppendorf centrifuge and then resuspended into Hepes buffer before being used for fluorescence measurements (final concentration 1 × 10³ cells/μl). This procedure efficiently removes extraneous dye. Leakage of dye during prewarming was < 10% and was therefore not corrected. The fluorescence of BCECF was recorded under constant stirring using a KONTRON SFM 24 spectrofluorimeter (Kontron, Düsseldorf, FRG) equipped with a thermostatted cuvette holder. Wavelengths for excitation and emission were set to 495 and 530 nm, respectively. Calibration of the BCECF signals in terms of $pH_i$ was performed using the high K⁺/nigericin method (11).

*Determination of Na⁺/H⁺ exchange activity.* Na⁺/H⁺ exchange was activated by addition of various amounts of Na⁺-propionate or K⁺-propionate solution (final concentrations 10–50 mM) from a 1-M stock solution, pH 7.4, exactly as described recently (12). Recovery of $pH_i$ in lymphocytes (Fig. 1 A) was inhibited by > 90% in the absence of extracellular sodium (Fig. 1 B) and by 95% at 10 μM of the specific inhibitor 5-(N-ethyl-N-isopropyl)-amiloride (EIPA; Fig. 1 C). Both findings suggest that $pH_i$ recovery was almost exclusively mediated by Na⁺/H⁺ exchange. Osmotic activation of the antiport by 50 mM propionate appeared unlikely, as a challenge of cells by 50 mM NaCl failed to affect $pH_i$ (Fig. 1 D). Identical observations were made in platelets (not shown).

Initial rates of EIPA-sensitive $pH_i$ recovery were calculated as described in detail recently (12) and are expressed as $dpH_i/min$. The relationship between $pH_i$ and $pH_i$ recovery could best be described by a sigmoidal function as detailed earlier (12). Parameters calculated from fitting experimentally derived data to a sigmoidal function allowed the calculation of apparent $v_{max}$ values (see reference 12 for full detail.)

It is well known that exact characterization of the antiport's kinetic parameters can only be achieved by the nigericin $pH_i$ clamp method (13) or by the NH₄Cl prepulse method. Determination of true $v_{max}$ requires acidification to $pH_i$ 6.0, whereas with propionate acidification beyond $pH_i$ 6.6 (at $pH_o$ 7.4) cannot be achieved. Furthermore, $pH_i$
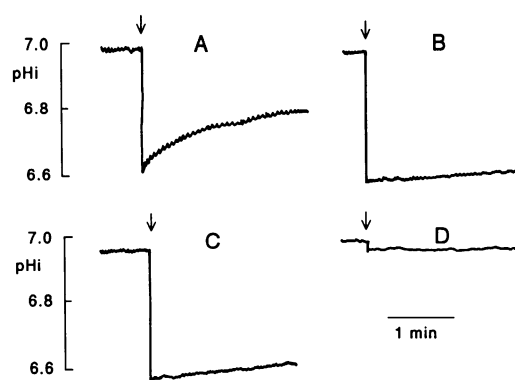


*Figure 1.* Representative fluorescence recordings from BCECF-loaded human lymphocytes. Displayed are typical recordings obtained from BCECF-loaded lymphocytes suspended in Hepes buffer, pH 7.4, at 37°C. (*A*) Effect of addition of Na⁺ propionate (50 mM, final concentration; *arrow*). Lymphocytes were suspended in buffer containing 150 mM Na⁺. (*B*) Lymphocytes were suspended in Na⁺-free buffer (NaCl isoosmotically replaced by choline chloride) and acidified by addition of 50 mM K⁺ propionate (*arrow*). (*C*) Effect of EIPA (10 μM) on $pH_i$ recovery. Lymphocytes suspended in buffer containing 150 mM Na⁺ were acidified by addition of 50 mM Na⁺ propionate (*arrow*). (*D*) Effect of addition of NaCl on $pH_i$. Lymphocytes suspended in Na⁺-containing buffer were challenged with 50 mM NaCl (final concentration; *arrow*). The initial decrease in BCECF fluorescence is a dilution artifact. No further change in fluorescence occurred during 6 min. Similar results were obtained in BCECF-loaded platelets in response to identical experimental maneuvers (not shown).

recovery rates are dampened and the original baseline pH is not re-established due to the continuous influx of propionic acid. A full discussion of the limitations of this method can be found elsewhere (12). However, we wished to examine antiport activity and cytosolic free Ca²⁺ in two cell specimens from one individual at the same occasion, which requires fast experimental procedures. This renders application of the nigericin $pH_i$ clamp method impossible, as this technique is time consuming. It is conceivable that prolonged storage of blood may affect antiport activity and/or $Ca^{2+}_i$. Finally, neither the nigericin $pH_i$ clamp technique nor the NH₄Cl prepulse method can be applied to platelets, as these techniques require one or more centrifugation steps. In platelets this is possible only in the presence of agents that raise cAMP, or at acidic extracellular pH, in order to prevent any preactivation or aggregation of these cells. Since these manipulations might have caused unforeseen effects on antiport activity, we preferred to use acidification by propionate, which enabled us to apply the same procedure to both blood cell types. Finally, all these potential confounders are present to a similar extent in the cells of control subjects, patients with renal failure, and volunteers on whom metabolic acidosis was imposed. This notion is also supported by the evaluation of the immediate effects of propionate addition on $pH_i$ in platelets and lymphocytes under the various (patho-) physiological states investigated. In any event, it can be stated that identical amounts of Na⁺ propionate produced similar acidification in all individuals, whereas only $pH_i$ recovery rates were significantly different (see Results).

*Determination of $[Ca^{2+}]_i$.* Intracellular free calcium was determined after loading platelets with the fluorescent dye fura-2-AM essentially as described earlier (14). Lymphocytes were loaded with 2 μM fura-2-AM for 1 h at room temperature in RPMI 1640 medium. After two washing steps in the same medium cells were resuspended at a density of 1 × 10⁶ cells/ml. Samples were briefly spun down in an Eppendorf centrifuge before they were added to a cuvette containing Hepes buffer. In that way we minimized the contribution of leaked fura-2 to the measured fluorescence. Fluorescence was measured in thermostatted (37°C) cuvettes with continuously stirred buffer at 339

Table I. Plasma Electrolytes and Acid-Base Values of Normal Subjects and Patients with Renal Insufficiency

| | Normal subjects | Patients with renal insufficiency |
|---|---|---|
| Creatinine (mg/dl) | 0.9±0.14 | 6.4±0.71* |
| Na⁺ (mmol/liter) | 140±2 | 139±1 |
| K⁺ (mmol/liter) | 4.4±0.3 | 4.5±0.5 |
| Cl⁻ (mmol/liter) | 103±3 | 104±2 |
| HCO₃⁻ (mmol/liter) | 28±1.3 | 21±3.3* |
| pH | 7.41±0.02 | 7.34±0.06* |
| Pco₂ (mmHg) | 44±2.7 | 37±1.7* |

Values represent means ± SD of 10 normal controls and 10 patients with renal insufficiency. * Significantly different from controls at $P < 0.05$.

nm for excitation and 505 nm for emission, respectively. Calibration of fluorescence in terms of $[Ca^{2+}]_i$ was performed as described by Pollock et al. (15).

*Presentation of data.* Unless stated otherwise, data are expressed as means±SD. Comparison between data was made by unpaired (patients and normal subjects) or paired (subjects before and after $NH_4Cl$-induced metabolic acidosis) two-sided $t$ tests. Differences were assumed to be significant at $P < 0.05$.

## Results

### Study I: chronic renal acidosis

*Clinical parameters and acid-base status of patients with renal insufficiency.* In Table I are shown serum creatinine, $Na^+$, $K^+$, and $Cl^-$, as well as plasma pH, $Pco_2$, and $HCO_3^-$ of normal subjects and patients with CRF. Plasma pH, $Pco_2$, and $HCO_3^-$ values were significantly lower in the patients, while creatinine values were higher, compared with the normal subjects.

$Na^+/H^+$ *exchange and* $[Ca^{2+}]_i$. In Fig. 2 are shown $pH_i$ recovery rates in control and CRF in both lymphocytes and

platelets. The lowest values of $pH_i$ achieved by propionate acidification did not exceed $pH_i$ 6.6; i.e., true $v_{max}$ values, which require acidification to $pH_i$ 6.0, could not be determined by our technique (cf. Methods). Nevertheless, it is evident that lymphocytes from patients (Fig. 2 *B*) displayed distinctly enhanced $pH_i$ recovery rates compared with those from controls (Fig. 2 *A*). The parameters of the fitted lines through the pooled data from all individuals yielded an apparent $v_{max}$ of the $Na^+/H^+$ exchanger of 0.053 $dpH_i/min$ in controls (Fig. 2 *A*) and a value of 0.079 in patients (Fig. 2 *B*). Apparent half-maximum activation occurred at $pH_i$ 6.84 in controls and at $pH_i$ 6.80 in patients, respectively. Hence, the increase in apparent $v_{max}$ in the lymphocytes of patients was not accompanied by an alkaline shift in the $Na^+/H^+$ exchanger activation curve. In contrast, no such difference in $v_{max}$ was noticeable when platelets of controls (Fig. 2 *C*) and patients (Fig. 2 *D*) were compared. The parameters of the fitted sigmoidal function yielded values for apparent $v_{max}$ of 0.086 and 0.096 $dpH_i/min$ in controls and patients, respectively. Thus, although the $Na^+/H^+$ exchanger appeared to be slightly enhanced also in platelets of patients with renal insufficiency, this difference was not statistically significant (see below). Apparent half-maximum activation of the antiport occurred at $pH_i$ 6.86 and 6.84 in controls and patients, these values being again not significantly different.

To display the scatter of apparent $v_{max}$ values in different subjects, individual $pH_i$ recovery rates in lymphocytes of controls and patients are shown in Fig. 3 *A*. In controls, mean $pH_i$ recovery averaged 0.056±0.02 $dpH_i/min$. In CRF, $pH_i$ recovery averaged 0.091±0.013 $dpH_i/min$. This latter value is significantly different from that of controls ($P = 0.0005$). Basal $pH_i$ values in lymphocytes of controls and patients (Fig. 3 *B*) averaged 7.01±0.09 and 6.97±0.08 and were, hence, not significantly different ($P = 0.2$). Fig. 3 *C* displays the immediate change in $pH_i$ from basal ($-\Delta pH_i$) evoked by addition of identical amounts of propionic acid (final concentration 50 mM) in lymphocytes of controls and patients. Under otherwise identical conditions, i.e., similar values of $pH_i$ and $pH_o$, these val-
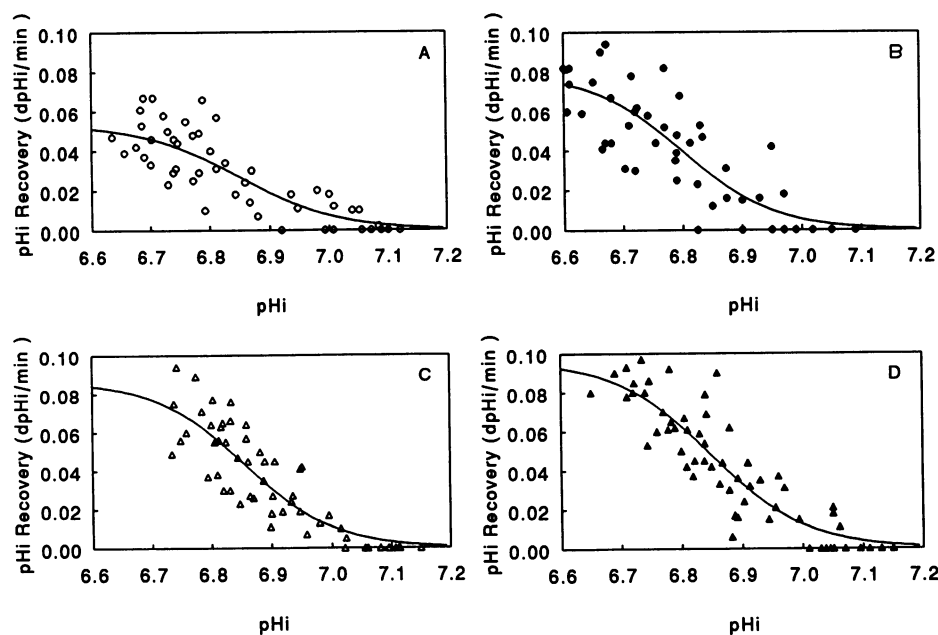


Figure 2. $Na^+/H^+$ exchange activity in lymphocytes and platelets in healthy subjects and patients with CRF. Shown are $pH_i$ recovery rates ($dpH_i/min$) at different values of $pH_i$ achieved by acidification of lymphocytes (*A, B*) and platelets (*C, D*) with $Na^+$-propionate (10–50 mM) in healthy subjects (*A, C*) and patients with renal insufficiency (*B, D*). In each panel pooled data from 10 individuals are displayed. The lines were obtained after fitting the data to a sigmoidal function (see reference 12). ○, lymphocytes; △, platelets; *open symbols*, controls; *closed symbols*, patients.
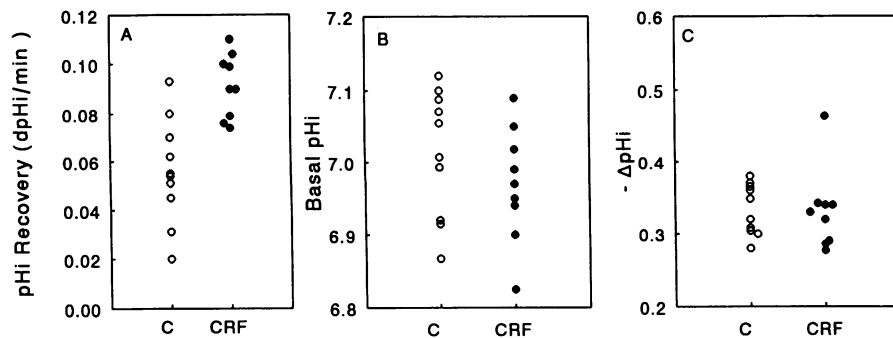
*Figure 3.* pH$_i$ recovery, basal pH$_i$, and estimate of buffer capacity in lymphocytes of controls and patients with CRF. (*A*) Displayed are individual apparent maximum pH$_i$ recovery rates ($d$pH$_i$/min) in 10 controls (*C*) and 10 patients with CRF. Values were obtained by fitting individual pH$_i$ recovery rates at different values of pH$_i$ to a sigmoidal function (see Methods and reference 12 for full details). (*B*) Basal pH$_i$ values in lymphocytes of controls (*C*) and patients (*CRF*). Measurements were made in HCO$_3^-$-free Hepes buffer, pH 7.4, at 37°C. (*C*) Acidification by 50 mM Na$^+$-propionate of lymphocytes of controls (*C*) and patients (*CRF*). Values represent the initial immediate decrease in pH$_i$ ($-\Delta$pH$_i$) after the addition of Na$^+$-propionate. At identical pH$_o$ and similar pH$_i$ (cf. *B*) these values are a reflection of the cytosolic buffer capacity for H$^+$.

ues are a rough reflection of the cytosolic buffer capacity for H$^+$. Basal pH$_i$ in controls and patients decreased by 0.33±0.09 and 0.33±0.05 pH units ($P = 0.9$), respectively. Therefore, the distinct enhancement of Na$^+$/H$^+$ exchange in lymphocytes of patients with renal failure cannot be attributed to an alkaline shift in basal pH$_i$ of the antiport (which would be excepted if the enhancement were due to agonist stimulation of these cells), or to changes in the cytosolic buffer capacity for H$^+$.

Platelets, on the other hand, displayed much higher pH$_i$ recovery rates than lymphocytes, perhaps due to their more favorable surface to volume ratio (Fig. 4 *A*). Mean individual apparent $v_{max}$ values were not different in controls and patients ($P = 0.34$) and averaged 0.082±0.022 and 0.089±0.014 $d$pH$_i$/min, respectively. There was a marginal tendency for more alkaline basal pH$_i$ values in platelets compared with lymphocytes, and means were 7.09±0.04 and 7.07±0.05 in controls and patients, respectively (Fig. 4 *B*). Again, acidification by 50 mM propionate induced an identical fall in pH$_i$ by 0.32±0.04 and 0.33±0.04 in controls and patients, respectively, suggesting no change in the buffer capacity in platelets of CRF patients.

To investigate whether or not the enhanced Na$^+$/H$^+$ exchange in lymphocytes of patients with CRF would coincide with elevated values of [Ca$^{2+}$]$_i$, this parameter was also determined in both blood cell types of controls and patients. In lymphocytes, the mean [Ca$^{2+}$]$_i$ amounted to 74.3±9.4 ($n = 10$) and 71.2±22.4 nM ($n = 10$) in controls and patients, respectively. In platelets the mean [Ca$^{2+}$]$_i$ values were 121±19 ($n$

= 10) and 119±14 nM ($n = 10$) in controls and patients, respectively. Thus, we conclude that the enhancement of Na$^+$/H$^+$ exchange in lymphocytes of patients with renal insufficiency is not associated with a parallel rise in [Ca$^{2+}$]$_i$ in these cells.

## Study II: induced metabolic acidosis in normal volunteers

*Clinical parameters and acid-base status of normal subjects with or without NH$_4$Cl.* This study was performed to investigate whether or not the enhancement of Na$^+$/H$^+$ exchange seen in lymphocytes of patients with CRT was predominantly due to the simultaneously prevailing chronic metabolic acidosis. This state was achieved by inducing short-term (5 d) metabolic acidosis in normal healthy volunteers with NH$_4$Cl. Table II displays values for Na$^+$, K$^+$, Cl$^-$, pH, Pco$_2$, and HCO$_3^-$ before and after administration of NH$_4$Cl for 5 d. Plasma pH, Pco$_2$, and HCO$_3^-$ decreased significantly in response to NH$_4$Cl, whereas Cl$^-$ increased concomitantly. Body weight decreased by 2 kg compared with baseline values. Systolic blood pressure did not change; a borderline increase in diastolic blood pressure (0.05 < $P$ < 0.01) was observed. The plasma renin activity and plasma aldosterone concentrations increased significantly. The 24-h urine Na$^+$ excretion was not different, while urine K$^+$ excretion, urine Cl$^-$ excretion, urine Ca$^{2+}$ excretion, and urine urea excretion were increased compared with baseline values. No effect on the clearance of creatinine was observed.

*Na$^+$/H$^+$ exchange and [Ca$^{2+}$]$_i$ in blood cells of healthy volunteers with or without NH$_4$Cl.* Fig. 5 displays typical activation curves of the antiport in lymphocytes of eight subjects
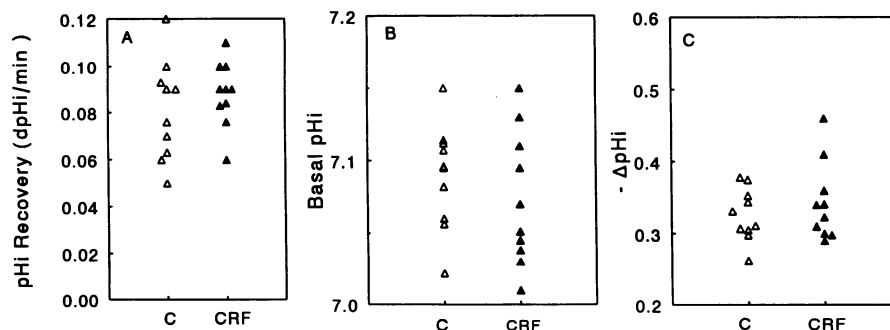


*Figure 4.* pH$_i$ recovery, basal pH$_i$, and estimate of buffer capacity in platelets of controls and patients with CRF. (*A*) Displayed are individual apparent maximum pH$_i$ recovery rates ($d$pH$_i$/min) in platelets of 10 controls (*C*) and of 10 patients with CRF. (*B*) Basal pH$_i$ values in platelets of controls (*C*) and patients (*CRF*) obtained in HCO$_3^-$-free Hepes buffer, pH 7.4, at 37°C. (*C*) Acidification by 50 mM Na$^+$-propionate of platelets of controls (*C*) and patients (*CRF*). Values represent the initial immediate decrease in pH$_i$ ($-\Delta$pH$_i$) after the addition of Na$^+$-propionate. At identical pH$_o$ and similar pH$_i$ (cf. *B*) these values reflect the cytosolic buffer capacity for H$^+$.

*Table II. Effects of NH$_4$Cl on Plasma Acid-Base Values, Body Weight, Blood Pressure, Plasma Renin Activity, Plasma Aldosterone, Creatinine Clearance, Urine Urea, and Urine Electrolyte Excretion in Normal Subjects*

|  | Control | NH$_4$Cl |
|---|---|---|
| Na (mmol/liter) | 140±2 | 137±3 |
| K (mmol/liter) | 4.4±0.4 | 4.4±0.3 |
| Cl (mmol/liter) | 103±3 | 116±4* |
| HCO$_3^-$ (mmol/liter) | 27±0.8 | 13±1.7* |
| pH | 7.4±0.01 | 7.26±0.04* |
| Pco$_2$ (mmHg) | 39±1.7 | 28±0.9* |
| BWT (kg) | 72.2±5.1 | 70.2±5.6* |
| Systolic BP (mmHg) | 126±10 | 128±8 |
| Diastolic BP (mmHg) | 75±4 | 84±7 |
| PRA (ng Ang l/h) | 1.67±0.75 | 13.49±10* |
| PA (ng/100 ml) | 62.17±57 | 386±567* |
| ClCr (ml/min) | 116±11 | 128±27 |
| UureaV (mg/24 h) | 2,359±773 | 3,176±712* |
| UNaV (mmol/24 h) | 160±42 | 169±91 |
| UKV (mmol/24 h) | 67±33 | 138±32* |
| UCIV (mmol/24 h) | 167±33 | 390±73* |
| UCaV (mmol/24 h) | 5.2±0.6 | 14.2±2.01* |

All data are means ± SD from eight individuals before and after NH$_4$Cl treatment. * Significantly different from control at $P < 0.05$. BWT, body weight; BP, blood pressure; PRA, plasma renin activity; PA, plasma aldosterone concentration, ClCr, creatinine clearance; UureaV, urine urea excretion; UNaV, urine Na$^+$ excretion; UKV, urine K$^+$ excretion; UCIV, urine Cl$^-$ excretion; UCaV, urine Ca$^{2+}$ excretion.



*Figure 5.* Effect of NH$_4$Cl-induced metabolic acidosis on Na$^+$/H$^+$ exchange rates in lymphocytes. In each panel are displayed pooled pH$_i$ recovery rates ($d$pH$_i$/min) from lymphocytes of eight individuals. Lines were obtained by fitting the experimentally derived data to a sigmoidal function. (*A*) Control before NH$_4$Cl treatment; (*B*) day 4 of NH$_4$Cl ingestion; (*C*) 5 d after release from NH$_4$Cl treatment.

before, during, and after metabolic acidosis induced by NH$_4$Cl ingestion. On day 1 (control; Fig. 5 *A*), the sigmoidal fit through the data points yielded an apparent $v_{max}$ of the antiport of 0.45 $d$pH$_i$/min and apparent half-maximum activation was attained at pH$_i$ 6.94. On day 4 of NH$_4$Cl treatment (Fig. 5 *B*) these values were 0.084 $d$pH$_i$/min and 6.85, respectively. Lymphocyte Na$^+$/H$^+$ exchange activity normalized on day 10, i.e., 5 d after the release from NH$_4$Cl treatment (Fig. 5 *C*), and apparent $v_{max}$ and pH$_i$ for half-maximum activation were calculated at 0.06 pH$_i$/min and 6.83, respectively. Fig. 6 shows calculated apparent $v_{max}$ values of the lymphocyte Na$^+$/H$^+$ exchanger for all individuals enrolled. It is evident that Na$^+$/H$^+$ exchange activity in lymphocytes increased in all but one subject during NH$_4$Cl ingestion (days 4 and 5) and returned to control values 5 d after the release from treatment (day 10). Before treatment (day 1) $v_{max}$ averaged 0.06±0.02 $d$pH$_i$/min. On day 4 this value increased significantly to 0.10±0.04 $d$pH$_i$/min ($P < 0.025$). On day 5 antiport activity in lymphocytes remained elevated at 0.09±0.03 $d$pH$_i$/min. This value was again significantly different from that of day 1 ($P < 0.05$) but not different from that of day 4. On day 10 apparent $v_{max}$ normalized to 0.06 $d$pH$_i$/min, this value being indistinguishable from that of control, i.e., day 1. No change in lymphocyte basal pH$_i$ was seen before, during, or after NH$_4$Cl-induced acidosis, and values averaged 7.03±0.07, 7.03±0.06, 7.03±0.09, and 7.04±0.05 on days 1, 4, 5, and 10, respectively. Furthermore, enhancement of the lymphocyte antiport seemed not to be due to changes in the buffer capacity for H$^+$, since addition of 50
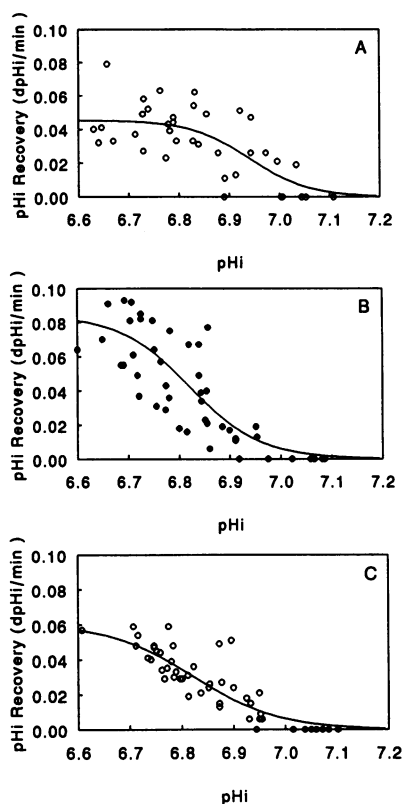
mM propionate caused the same degree of acidification on days 1, 4, 5, and 10 and pH$_i$ decreased by 0.34±0.08, 0.36±0.05, 0.33±0.04, and 0.34±0.09 U, respectively. Hence, NH$_4$Cl-induced metabolic acidosis in healthy volunteers apparently evoked an almost twofold enhancement of Na$^+$/H$^+$ exchange in lymphocytes, comparable to that of patients with CRF, without major changes in the buffer capacity for H$^+$ or an alkaline shift in the antiport's activation curve.

The effect of metabolic acidosis on platelet Na$^+$/H$^+$ exchange activity is displayed in Fig. 7. In control (Fig. 7 *A*) apparent $v_{max}$ was calculated at 0.066 $d$pH$_i$/min and this value did not significantly change on day 4 of NH$_4$Cl treatment (0.076 $d$pH$_i$/min; Fig. 7 *B*). On day 10, apparent $v_{max}$ was 0.71 $d$pH$_i$/min. Individual apparent $v_{max}$ values of the platelet antiport before (day 1), during (days 4, 5), and after (day 10)
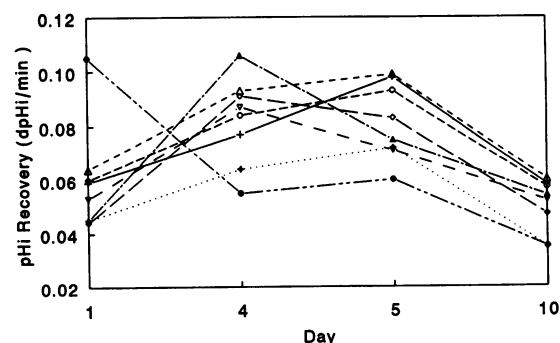


*Figure 6.* Changes in Na$^+$/H$^+$ exchange activity in lymphocytes during NH$_4$Cl-induced metabolic acidosis. Displayed are pH$_i$ recovery rates ($d$pH$_i$/min) in lymphocytes before (day 1), during (days 4 and 5), and 5 d after NH$_4$Cl ingestion (day 10). Each symbol represents one individual.
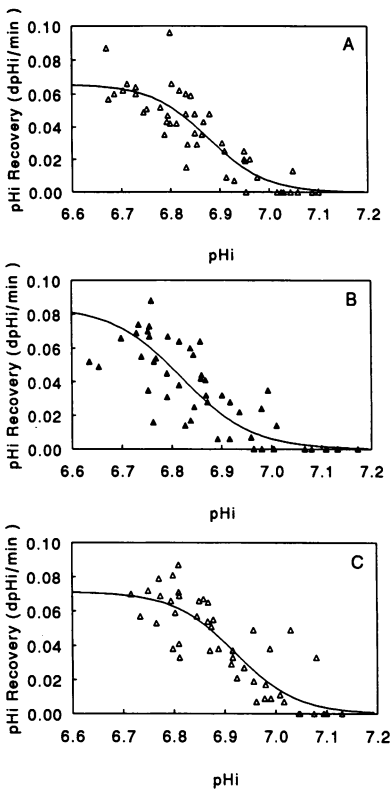
*Figure 7.* Effect of NH$_4$Cl-induced metabolic acidosis on Na$^+$/H$^+$ exchange rates in platelets. In each panel are displayed pooled pH$_i$ recovery rates ($d$pH$_i$/min) from platelets of eight individuals. (*A*) Control before NH$_4$Cl treatment; (*B*) day 4 of NH$_4$Cl ingestion; (*C*) 5 d after release from NH$_4$Cl treatment.



*Figure 8.* Changes in Na$^+$/H$^+$ exchange activity in platelets during NH$_4$Cl-induced metabolic acidosis. Displayed are pH$_i$ recovery rates ($d$pH$_i$/min) in platelets before (day 1), during (days 4 and 5), and 5 d after NH$_4$Cl ingestion (day 10). Each symbol represents one individual.

NH$_4$Cl-induced metabolic acidosis are shown in Fig. 8. $v_{max}$ values were 0.068±0.017, 0.071±0.02, 0.08±0.04, and 0.074±0.014 $d$pH$_i$/min on days 1, 4, 5, and 10, respectively, and hence not significantly different. Basal pH$_i$ and the acidification from basal pH$_i$ by 50 mM propionate were also identical on all days.

We also determined basal values of [Ca$^{2+}$]$_i$ in platelets and lymphocytes at day 5 of NH$_4$Cl treatment and under control conditions. Mean values in lymphocytes and platelets were 73±19 and 122±17 nM, respectively. These values were again not significantly different from those of the untreated control condition (76±17 and 118±15 nM).

## Discussion

*Na$^+$/H$^+$ exchange in lymphocytes and platelets in CRF.* We report here that lymphocyte but not platelet Na$^+$/H$^+$ exchange activity is significantly enhanced by a factor of ~ 1.6 in cells from patients with CRF compared with those from controls (Figs. 2–4). Thus we will first briefly discuss the mechanism(s) potentially mediating this behavior. The most trivial cause of an enhanced pH$_i$ recovery from acidification might be a reduction in the intracellular buffer capacity for H$^+$ in CRF. However, as identical amounts of propionate induced identical initial acidification in platelets and lymphocytes of controls and patients (Figs. 3 and 4), this explanation can largely be ruled out. Furthermore, under our assay conditions (no HCO$_3^-$) the buffer capacity is predominantly determined by the total cytosolic protein concentration. It appears unlikely that an almost twofold enhancement of pH$_i$ recovery in lymphocytes is due to a corresponding 50% reduction in cytosolic protein concentration. In addition, other investigators have also determined un-
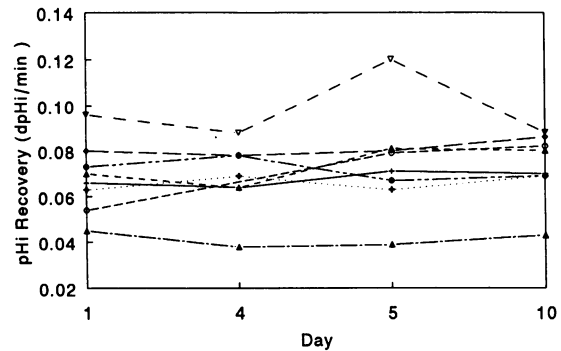
changed (16) or even increased (17) buffer capacities in white blood cells of CRF patients.

Activation of the Na$^+$/H$^+$ exchanger can occur after stimulation of cells by a wide variety of hormones (2). In most cells this enhancement of transport activity involves an alkaline shift of the antiport's setpoint, i.e., the pH value above which the transporter is silent (1). However, despite an enhanced pH$_i$ recovery neither baseline pH$_i$ nor the pH$_i$ value at which half-maximum activation occurred were significantly shifted in lymphocytes of CRF patients. Although circumstantial, this may serve as an argument against prestimulation by circulating agonists as the major cause of the enhancement of Na$^+$/H$^+$ exchange. Furthermore, since average [Ca$^{2+}$]$_i$ was not different in the lymphocytes of controls and patients, an elevation of [Ca$^{2+}$]$_i$ also appears unlikely to mediate the distinct enhancement of Na$^+$/H$^+$ exchange. Therefore, other underlying mechanisms (e.g., an increased amount of antiporter protein) must be taken into consideration (see below). Although speculative, this would also explain why Na$^+$/H$^+$ exchange is not accelerated in platelets of CRF patients, since these cells lack a nucleus and are therefore unable to newly synthesize antiport protein.

One might wonder why basal pH$_i$ was not lowered in platelets and lymphocytes of patients compared with controls, since plasma pH was also significantly reduced in CRF. However, it should be emphasized in this context that our pH$_i$ determinations were conducted at an extracellular pH of 7.4 in HCO$_3^-$-free buffer. It is conceivable that cell pH$_i$ might be acidic in patients under in vivo conditions.

*Na$^+$/H$^+$ exchange in lymphocytes during NH$_4$Cl-induced metabolic acidosis.* The main objective of treating healthy subjects with NH$_4$Cl was to answer the question whether the enhancement of Na$^+$/H$^+$ exchange observed in lymphocytes of CRF patients could be reproduced by simply imposing metabolic acidosis on these subjects. Thus, these otherwise healthy individuals were expected to display some of the abnormalities found in CRF patients, e.g., changes in plasma acid-base status and electrolyte composition and, potentially, similar acidosis-related alterations in blood hormone composition, but were devoid of uremia-related changes in metabolism. In fact, acid-base status and electrolytes (except Cl$^-$) were similar in CRF and during NH$_4$Cl-induced metabolic acidosis (cf. Tables I and II).

The subjects noted some lassitude, but otherwise had no subjective side effects from $NH_4Cl$, which at higher doses may cause quite profound effects primarily related to acidosis (18). Body weight decreased by 2 kg, which we attributed to diuresis and natriuresis. Urine $Na^+$ excretion was not increased on day 5; however, it is likely that $Na^+$ balance was reachieved by that time; the $t_{1/2}$ of $Na^+$ balance is $\sim$ 1.5 d (19). We were surprised that blood pressure did not decrease. The sharp increase in renin and aldosterone values may have contributed to maintain blood pressure. The increased renin secretion may have been related to volume losses as reflected by body weight. The substantial amounts of $Cl^-$ ions delivered to the distal tubule would be expected to decrease rather than increase renin release. Kotchen et al. (20) have emphasized the role of $Cl^-$ delivery to the thick ascending portion of Henle's loop in signaling renal renin release. They found that increased $Cl^-$ delivery reduced renin release. A brisk kaliuresis ensued after $NH_4Cl$ administration. Serum $K^+$ values were not changed, suggesting that cytosolic $K^+$ concentrations may have decreased. Total body potassium depletion is known to increase renin release (21). An increased urea synthesis and calciuresis are recognized features of $NH_4Cl$ administration (18).

$Na^+/H^+$ exchange activity was accelerated in lymphocytes during metabolic acidosis and it appears that this enhancement (Fig. 5 B) was strikingly similar to that observed in CRF patients (Fig. 2 B). The reversibility of this enhancement also appears noteworthy and $Na^+/H^+$ exchange activity normalized in all subjects 5 d after the termination of $NH_4Cl$ treatment (Fig. 5 C). Furthermore, it should be emphasized that $NH_4Cl$ ingestion enhanced the lymphocyte $Na^+/H^+$ exchanger in all individuals examined, albeit to a different degree (Fig. 6). In one individual $pH_i$ recovery was apparently higher before treatment with $NH_4Cl$. However, we believe that this might reflect an experimental artifact rather than a true inverse behavior of antiport activity. The results depicted in Fig. 6 may also serve to underline the validity of the propionate technique for estimation of $Na^+/H^+$ exchange activity despite its well-known and already discussed limitations (cf. Methods). It can be clearly seen that the rates of $pH_i$ recovery are very similar on days 4 and 5 of metabolic acidosis and during control (days 1 and 10), suggesting that our results were quite reproducible. We found again that the enhancement of $Na^+/H^+$ exchange during metabolic acidosis was not accompanied by an alkaline shift of basal $pH_i$ ex vivo, which again may argue against prestimulation of the cells. Furthermore, as the buffer capacity for $H^+$ and $[Ca^{2+}]_i$ remained unaffected during $NH_4Cl$ ingestion, these parameters are unlikely to contribute to the acceleration of the lymphocyte antiporter during metabolic acidosis.

In contrast, platelet $Na^+/H^+$ exchange activity was not significantly accelerated in metabolic acidosis in any of the individuals investigated. This observation was similar to that made in the CRF patients.

Taken together, $Na^+/H^+$ exchange is elevated in lymphocytes but not in platelets of patients with CRF, and the fact that this phenomenon could be reproduced in healthy volunteers after the ingestion of $NH_4Cl$ may suggest that in fact metabolic acidosis, but not other uremia-related abnormalities in CRF, mediate this enhancement.

The major issue is whether the enhancement of $Na^+/H^+$ exchange in blood cells is a direct result of metabolic acidosis in patients with renal failure and $NH_4Cl$-treated volunteers, i.e., a direct consequence of reduced plasma pH (and, potentially, reduced $pH_i$ in vivo), or whether metabolic acidosis causes other hormonal changes that in turn activate the $Na^+/H^+$ exchanger. Some arguments may favor the notion that it is the fall in $pH_i$ that directly activates the $Na^+/H^+$ exchanger, possibly by increasing the amount of exchanger protein in the plasma membrane. CRF is characterized by long-term metabolic acidosis. Krapf et al. (22) have shown that in rats metabolic but not respiratory acidosis induces an increase in antiport mRNA in kidney cortex. Moe et al. (23, 24) were able to confirm that mouse renal cortical tubule cells and an opossum kidney cell line responded to a 24-h in vitro metabolic acidosis with an almost twofold increase in $Na^+/H^+$ exchange activity that was accompanied by a threefold increase in antiport mRNA. These results could be confirmed by feeding rats with a diet that induced metabolic acidosis. Fibroblasts, on the other hand, responded with a slight, albeit significant reduction in antiport activity in response to acidosis, which was paralleled by a reduction of antiport mRNA abundance (24). Although details involving this behavior remain unexplained, the conclusion remains that acid preincubation may enhance antiport synthesis in some cells and tissues.

We are currently investigating whether or not $NH_4Cl$-induced metabolic acidosis increases the steady-state concentration of antiport mRNA in lymphocytes using quantitative polymerase chain reaction. Preliminary results indicate that antiport mRNA is in fact increased in metabolic acidosis by a factor of $\sim$ 1.5 above normal (Siffert, W., unpublished observations). This finding would also explain why metabolic acidosis enhances $Na^+/H^+$ exchanger only in nucleated lymphocytes but not in platelets, as these latter nonnucleated blood components are not capable of protein synthesis.

In summary, we present the first observations in man suggesting that $Na^+/H^+$ exchange is important in the regulation of $pH_i$ under the condition of short-term and chronic metabolic acidosis. They suggest that $pH_i$ is well regulated even in the face of metabolic acidosis. The homeostatic adjustments in $pH_i$ regulation and the regulation of $Na^+/H^+$ exchange in other clinical acid-base disturbances and the effects of therapeutic interventions remain to be elucidated.

## Acknowledgments

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