*Kidney International, Vol. 58 (2000), pp. 229–235*

# Red cell $Na^+/Li^+$ countertransport and $Na^+/H^+$ exchanger isoforms in human proximal tubules

**Leong L. Ng, Paulene A. Quinn, Frease Baker, and Susan J. Carr**

*Department of Medicine, Leicester Royal Infirmary, and Department of Nephrology, Leicester General Hospital, Leicester, England, United Kingdom*

**Red cell $Na^+/Li^+$ countertransport and $Na^+/H^+$ exchanger isoforms in human proximal tubules.**

*Background.* Increased activity of the $Na^+/Li^+$ countertransporter (SLC) is a well-recognized intermediate phenotype of hypertension and diabetic nephropathy and may indicate a predisposition to hypertension. Previous work has attempted to link this membrane transport marker to altered $Na^+$ reabsorption in the proximal tubule. Since the $Na^+/H^+$ exchanger (NHE) isoforms 1 and 3 are expressed in the basolateral and apical membranes of the proximal tubule, respectively, we investigated the relationship between these transport proteins and red cell SLC to examine whether the peripheral blood transport phenotype is associated with altered levels of transport proteins in the proximal tubule.

*Methods.* Proximal tubules were prepared from human nephrectomy specimens. NHE-1 and NHE-3 were detected on Western blots by specific antibodies. Red cell SLC was also measured.

*Results.* Both NHE-1 and NHE-3 proteins were demonstrated, with molecular weights of 97 and 85 kD, respectively. SLC was very strongly correlated with the level of NHE-3 protein ($r = 0.78$, $P < 0.001$) and was negatively related to NHE-1 protein ($r = -0.32$). In multiple regression analysis, only NHE-3 and NHE-1 protein levels were significant predictors of red cell SLC, accounting for up to about 70% of the variance of this parameter.

*Conclusions.* We conclude that red cell SLC may be a marker of increased NHE-3 protein expression in the proximal tubule, which may account for the blunted pressure natriuresis and predisposition to hypertension.

Elevated erythrocyte sodium-lithium countertransport (SLC) activity identifies a group of essential hypertensive patients with a genetic predisposition to hypertension and cardiovascular disease [1–3]. Similarly, elevated SLC activity has been reported in subjects with diabetic nephropathy [4, 5] and in hypertensive patients with IgA nephropathy [6, 7], in whom it was related to a less favorable disease outcome [8, 9]. A genetic association of SLC activity with IgA nephropathy was suggested by the demonstration of elevated standard SLC activity in normotensive relatives of patients with IgA nephropathy [8].

Recent evidence has also demonstrated that this SLC marker predicts future development of both hypertension [10] and diabetic nephropathy [11, 12]. Another transporter that has been linked to both hypertension and diabetic nephropathy is the $Na^+/H^+$ exchanger isoform 1 (NHE-1) [13–15], where increased activity is not associated with increased protein expression [16, 17]. Although there are similarities between these two transporters, recent evidence suggests that SLC may not be mediated by the best characterized NHE isoform, NHE-1, the former being phloretin sensitive and the latter amiloride sensitive [18, 19]. SLC may be thus mediated by a NHE isoform that is not NHE-1 [18].

The NHEs represent a growing family of membrane transporters that use the extracellular to intracellular $Na^+$ gradient to drive $H^+$ efflux from cells [20–22]. Among the essential functions performed by this group of transporters are the regulation of cell volume and pH, a permissive role in cell proliferation, and the *trans*-epithelial transport of $Na^+$ [20–22]. The cloning of the amiloride-sensitive growth factor activatable NHE isoform, called NHE-1 [23], has enabled further isoforms to be characterized. NHE-1 is present in most types of tissue [24] and is likely to have a "housekeeping" role in regulating cell $pH_i$ and volume. NHE-1 is not the only isoform present in $Na^+$ reabsorbing epithelia such as gut and kidney (where it is localized mainly to the basolateral membranes). In such tissues, NHE-3 and NHE-4 have been described [24, 25] in which these isoforms are located on the apical membrane of epithelial cells. Therefore, in the rat, it appears that kidney tissue contains mRNA of all four isoforms, but the abundance varies in the order NHE-3 > NHE-1 >> (NHE-2 and NHE-4) [24].

Long-term control of blood pressure may depend on

**Key words:** sodium/hydrogen exchanger, sodium/lithium countertransport, hypertension, diabetic nephropathy, natriuresis.

Received for publication October 22, 1999
and in revised form January 27, 2000
Accepted for publication February 10, 2000

the pressure-natriuresis mechanism in the kidney, and in hypertension this phenomenon is blunted [26, 27]. The proximal tubule is the major site for $H^+$ secretion, $Na^+$ and $HCO_3^-$reabsorption, and is therefore important for both acid-base and $Na^+$ homeostasis [28]. It has been suggested that proximal tubule $Na^+$ reabsorption, as measured by $Li^+$ clearance, is increased in both hypertensive humans [29–32] and rats [33–35] and could account for the blunted pressure-induced natriuresis. Recent research on modeling the $Na^+$ reabsorption of proximal tubules has suggested that only a 14% increase in apical NHE activity may increase $Na^+$ reabsorption (by 8%), which is sufficient to raise blood pressure [36]. In rat proximal tubules, the increased $Na^+$ reabsorption has been attributed to increased NHE activity [37, 38], and characterization of the NHE isoforms with isoform specific antibodies led to documentation of increased apical membrane NHE-3 expression but no difference in the NHE-1 protein expression [38].

There is some in vivo evidence from humans that renal $Li^+$ clearance (representing proximal tubule $Na^+$ handling) is reduced in patients with hypertension, especially those who had a high red cell SLC [39, 40]. These findings were not always confirmed by other investigators, however, perhaps because of patient selection [41, 42]. As $Li^+$ clearance or $Na^+$ reabsorption by the proximal tubule is the net result of a number of different transporters (apical NHE-3 and basolateral $Na^+$,$K^+$-ATPase and sodium bicarbonate cotransporter), it is not currently known whether the phenotype of increased SLC reflects an altered expression of these transporters. NHE-3 would be the prime candidate for a transporter controlling $Na^+$ reabsorption in the proximal tubule since it mediates $Na^+$ movement across the apical membrane and exhibits increased protein expression in hypertensive rat proximal tubules [38], while NHE-1 on the basolateral membrane mediates a housekeeping role. The basolateral $Na^+$,$K^+$-ATPase generates the *trans*-cellular $Na^+$ gradient, which operates the NHE-3–mediated apical $Na^+$ influx.

In the present investigation, we have therefore determined the levels of NHE isoform proteins in freshly isolated proximal tubule cells from human patients undergoing nephrectomy. Simultaneously, the red cells have been isolated and the SLC activity measured. This enabled an analysis of the relationship between the phenotype of increased SLC and altered expression of proximal tubule NHE isoforms. Our findings suggest a strong correlation between red cell SLC and proximal tubule NHE-3 expression, which could explain the increased $Na^+$ reabsorption in this tubule segment in vivo.

## METHODS

### Materials

Ham's F12 medium and Dulbecco's modified Eagle's medium (DMEM), fetal calf serum (FCS), Percoll, collagenase type IV, hyaluronidase type 1S, soybean trypsin inhibitor, and fluorescamine were supplied by Sigma Chemical Co. (Poole, UK). Protein A Sepharose CL4B was from Pharmacia LKB Biotechnology (Uppsala, Sweden). Protogel acrylamide solution was obtained from National Diagnostics (Atlanta, GA, USA). X-ray film was from Genetic Research Instrumentation Ltd. (Essex, UK). Hybond C extra supported nitrocellulose, horseradish peroxidase-conjugated donkey antirabbit antibody, and enhanced chemiluminescence (ECL) Western blotting reagents were obtained from Amersham International (Amersham, Little Chalfont, Buckinghamshire, UK). Molecular weight markers, TEMED, and ammonium persulfate were from Bio-Rad (Hercules, CA, USA).

### Patients

Seventeen Caucasian patients (8 males) admitted to the urology ward for unilateral nephrectomy were studied. All patients had a unilateral renal tumor. Patients with diabetes or known parenchymal renal disease were excluded from the study. All patients gave informed consent for this study, which was approved by the Leicestershire Health Authority Ethics Committee.

Patients were considered to be hypertensive if they were already receiving antihypertensive medication or if their systolic blood pressure was >160 mm Hg and/or diastolic blood pressure was >95 mm Hg during the hospital admission. Nine patients were receiving one or more antihypertensive medications: two β-blockers, five calcium antagonists, three angiotensin-converting enzyme inhibitors, five diuretics, and one vasodilator. One or more additional medications (1 thyroxine, 2 warfarin, 2 isosorbide mononitrate, 2 ferrous sulfate, 1 tamoxifen, 3 aspirin, 2 statin) were taken by five patients. Eight subjects were normotensive (blood pressure of less than 140/90 mm Hg).

Patients were interviewed to ascertain the presence of a family history of hypertension and cardiovascular disease in first-degree relatives. A blood sample was then taken for SLC assay.

The patients underwent a nephrectomy, and kidney cortex from the opposite pole of the kidney that was not invaded by tumor was dissected away from the specimen. The slices of cortex were kept in ice-cool Ham's F12/DMEM (1:1) medium containing 2 mmol/L glutamine, 10 mmol/L pyruvate, and antibiotics until extraction of the proximal tubules.

### Preparation of human proximal tubules

The slices of human renal cortex were washed free of blood with ice-cold preoxygenated DMEM/Ham's F12 medium. The method for preparing proximal tubule segments was modified from Gesek, Wolff, and Strandhoy [43] and had been demonstrated to be effective in purifi-

cation of rat proximal tubules [38]. Briefly, the cortical tissue was finely minced and transferred to siliconized flasks containing an enzyme solution saturated with 95% $O_2$, 5% $CO_2$ (DMEM/Ham's F12 with added collagenase type IV 1 g/L, hyaluronidase type 1S 1 g/L, soybean trypsin inhibitor 0.5 g/L, glutamine 2 mmol/L, pyruvate 10 mmol/L, pH 7.4, with HEPES/NaOH). The tissue was gently agitated in a 37°C incubator over 90 minutes, and digestion was usually complete by this period. The supernatant containing crude tubule segments was removed from the siliconized flasks at 10-minute intervals and pelleted by brief centrifugation ($60 \times g$ for 30 s). Residual enzyme was washed off the pellets with oxygenated ice-cold DMEM medium containing 10% FCS. At the end of the digestion, the crude tubule fragments were layered onto a preformed 43% Percoll gradient. The tubes were centrifuged at $1100 \times g$ for 20 minutes in a cold centrifuge. The proximal tubule segments were recovered at a density of 1.076 to 1.088 g/mL and pelleted. The tubules were washed free of Percoll with ice-cold DMEM/Ham's F12 medium and then pelleted. Microscopy at this stage showed tubules with typical proximal tubule morphology, with granular cytoplasm and a brush border.

### Determination of NHE-1 and NHE-3 protein abundance by Western blotting

The freshly isolated proximal tubule segments were suspended in homogenization buffer consisting of (in mmol/L) ethylenediaminetetraacetic acid (EDTA) 5, phenylmethylsulfonyl fluoride 1, phenanthroline 1, iodoacetamide 1 (in μg/mL), pepstatin 1, leupeptin 2, in phosphate-buffered saline. The tubule suspension was solubilized by adding Laemmli gel sample buffer [125 mmol/L Tris, pH 6.8, 4% sodium dodecyl sulfate (SDS), 20% glycerol, 0.004% bromophenol blue], was sonicated briefly, and was then boiled for five minutes. Protein concentrations were determined with a Bio-Rad detergent compatible protein assay kit and were also checked by the fluorescamine protein assay [38]. The proteins (100 μg) of extracts from each preparation were resolved on 7.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gels followed by electrotransfer onto supported nitrocellulose. These nitrocellulose membranes were blocked overnight with 10% low-fat milk powder (Marvel, Premier Brands UK, Ltd., Wirral, Merseyside, UK) in 20 mmol/L Tris, pH 7.4, 137 mmol/L NaCl, and 0.1% Tween 20 (TBS-Tween).

Western blotting of these membranes was performed by the addition of 1 μg/mL of G252 antibody or 2 μg/mL of G110 antibody in 5% Marvel in TBS-Tween for two hours. The antibody G252 is specific for NHE-1 protein and was raised against a fusion protein consisting of β-galactosidase and amino acids 650 to 815 of the human NHE-1 sequence [16, 17]. Antibody G110 is specific for NHE-3 protein and was raised against a fusion protein consisting of glutathione-S-transferase and amino acids 528 to 636 of the rat NHE-3 protein (which shows extensive homology to the human sequence) [38]. After extensive washes in TBS-Tween, blots were developed with horseradish peroxidase-linked donkey antirabbit second antibody (1:1500 dilution) with ECL detection according to manufacturer's instructions (Amersham). Bands corresponding to NHE-1 (approximately 97 kD molecular weight) and NHE-3 (approximately 85 kD) were quantitated using a Bio-Rad densitometer and expressed as arbitrary units. These analyses were performed with the investigator blinded to the status of the patient's SLC values.

### Red cell $Na^+/Li^+$ countertransport assay

The method used was similar to that described by Canessa et al [1], with modifications as previously described [44]. Venous blood was taken into tubes containing lithium-heparin and centrifuged for five minutes at $2500 \times g$. Cells (2 mL) were suspended in lithium loading solution (8 mL; 140 mmol/L LiCl, 10 mmol/L glucose, 10 mmol/L lithium carbonate, 10 mmol/L Tris acetate, pH 7.4; 5% carbon dioxide/95% oxygen was bubbled through the solution until lithium carbonate dissolved) for two hours at 37°C. The erythrocytes were then washed twice with isotonic $MgCl_2$ (285 mOsm/kg) and twice with choline medium (139 mmol/L choline chloride, 1 mmol/L $MgCl_2$, 10 mmol/L glucose, 10 mmol/L Tris MOPS, pH 7.4, 290 mOsm/kg). The cells were resuspended and incubated in choline medium for five minutes at 37°C.

Cells (0.2 mL) were incubated in either 1.2 mL choline medium containing $10^{-3}$ mol/L ouabain (sodium free) or 1.2 mL sodium-containing medium (145 mmol/L NaCl, 1 mmol/L $MgCl_2$, 10 mmol/L glucose, 10 mmol/L Tris MOPS, pH 7.4, 290 mOsm/kg) in the presence of $10^{-3}$ mol/L ouabain for 40, 80, and 120 minutes at 37°C. The samples were sedimented at $2000 \times g$ for five minutes, and 200 μL of supernatant were withdrawn. The supernatant was added to 2 mL CsCl (1.65 mmol/L) prior to measurement of lithium content using an Instrumentation Laboratories IL943 flame photometer. The packed cell volume of the initial blood sample was measured using a microhematocrit. The SLC activity was determined from the difference in lithium efflux from erythrocytes in the sodium containing and sodium-free media. The coefficient of variation of repeated assays was 9.8%.

### Statistics

Results are expressed as means ± SEM, and ranges are also reported. The degree of association between SLC and each of the continuous variables log NHE-3 protein, NHE-1 protein, age, creatinine, and blood pressures was quantitated using Pearson's rank correlation

**Table 1.** Clinical details, red cell and proximal tubule measurements of the patients in the study

| | Mean | SEM | Range |
|---|---|---|---|
| Number *male* | 17 (8) | | |
| Age *years* | 59.7 | 2.1 | 44–72 |
| Hypertension | 9 | | |
| Treatment | | | |
| Beta blocker | 2 | | |
| Calcium antagonist | 5 | | |
| ACE inhibitors | 3 | | |
| Diuretics | 5 | | |
| Vasodilators | 1 | | |
| Family history of hypertension | 4 | | |
| Creatinine *μmol/L* | 93.6 | 5.6 | 56–139 |
| Systolic blood pressure *mm Hg* | 150 | 4.5 | 115–180 |
| Diastolic blood pressure *mm Hg* | 87 | 2.3 | 59–100 |
| Red cell measurements | | | |
| $Na^+/Li^+$ countertransport *mmol* $L^{-1}$ $h^{-1}$ | 0.347 | 0.035 | 0.08–0.69 |
| Proximal tubule measurements | | | |
| NHE-1 protein *arbitrary units* | 1.17 | 0.19 | 0.31–2.81 |
| NHE-3 protein *arbitrary units* | 1.86 | 0.37 | 0.53–6.73 |

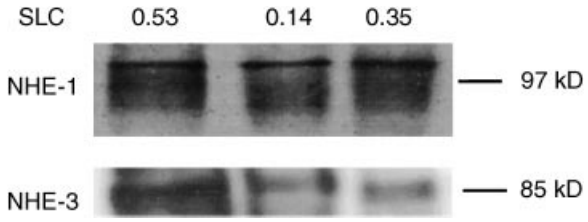


**Fig. 1. Western blot of protein extracts from freshly isolated proximal tubule segments from patients with varying $Na^+/Li^+$ countertransport (SLC) values (denoted above the lanes in mmol Li $L^{-1}$ $h^{-1}$).** Equal amounts of protein (100 μg) were loaded per lane. The $Na^+/H^+$ exchanger (NHE-1) specific antibody (G252) revealed immunoreactivity at around 97 kD. The NHE-3–specific antibody (G110) revealed the presence of NHE-3 protein at around 85 kD.

coefficient. Predictive models for the response variable (SLC) were developed using multiple linear regression analysis, stepwise logistic regression analysis, and best subsets analysis. All statistical analyses were carried out using the software package Minitab (Minitab Inc., Coventry, UK). Two-tailed *P* values less than 0.05 were considered significant.

## RESULTS

The demographic details of the patients in the study are reported in Table 1. The SLC of the red cells ranged from 0.08 to 0.69 mmol/L/h, with a mean of 0.35 mmol/L/h. All of the proximal tubule extracts from the kidney biopsies expressed both NHE-3 and NHE-1 protein, and an example of a Western blot for these proteins is shown in Figure 1. NHE-3 protein was detected as an 85 kD protein. NHE-1 protein ranged in molecular weight from 92 to 110 kD, with a mean of 97 kD. These molecular weights of human proximal tubule NHE-1 and NHE-3 proteins are similar to those reported previously in rat proximal tubules [38]. The densities of these NHE-1 and NHE-3 protein bands were scanned, and arbitrary units are reported (Table 1). The only variable that did not exhibit a Gaussian distribution was NHE-3 protein content, and this was therefore normalized by log transformation before further analysis.

In Figure 1, proximal tubule extracts from patients with high and low red cell SLC values had similarly high and low NHE-3 protein densities. On regression analysis of the whole population, a very significant correlation was documented between log NHE-3 protein content of the proximal tubule extracts and the SLC in the red cells ($r = 0.78$, $P < 0.001$; Fig. 2). In contrast, although NHE-1 protein content was inversely correlated to SLC, this was not significant ($r = -0.32$; Fig. 2). There was no correlation between NHE-1 and log NHE-3 protein contents of the tubule extracts ($r = -0.03$). Log NHE-3 protein content was also directly correlated to the systolic blood pressure ($r = 0.50$, $P < 0.04$) but not to the diastolic blood pressure ($r = -0.05$). There were no significant correlations between NHE-1 content and systolic ($r = 0.12$) or diastolic ($r = 0.06$) blood pressures.

On multiple-regression analysis in the whole population using NHE-1 and NHE-3 protein contents, age, and creatinine as continuous variables and family history of hypertension and presence of hypertension as categorical variables in the prediction of red cell SLC, the only variables independently associated with red cell SLC was log NHE-3 protein content (regression coefficient 0.434, $P < 0.001$) and NHE-1 protein content (regression coefficient −0.068, $P < 0.03$). Likewise, in a stepwise logistic model, the only variables entered for prediction of SLC were (*1*) log NHE-3 protein content ($r^2 = 59.8\%$) and (*2*) NHE-1 protein content ($r^2 = 71.9\%$). Using a best subset analysis, log NHE-3 protein alone ($r^2 = 57.1\%$) was a better predictor of SLC than any other single variable. The predictive value of the model was improved slightly by consideration of combinations of variables, all of which included log NHE-3 protein content: log NHE-3 protein content + NHE-1 protein content ($r^2 = 67.9\%$); log NHE-3 protein content + family history of hypertension ($r^2 = 61.6\%$); log NHE-3 protein content + NHE-1 protein content + family history of hypertension ($r^2 = 71.9\%$). Thus, in all models, proximal tubule NHE-3 protein content accounted for a very substantial proportion of the total variance in red cell SLC.

## DISCUSSION

Although SLC has long been recognized as a marker for predisposition for hypertension [1, 10] and other renal diseases such as diabetic nephropathy [2, 3, 11, 12], the actual transport mechanism that it represents in red cells has never been elucidated. However, it has been

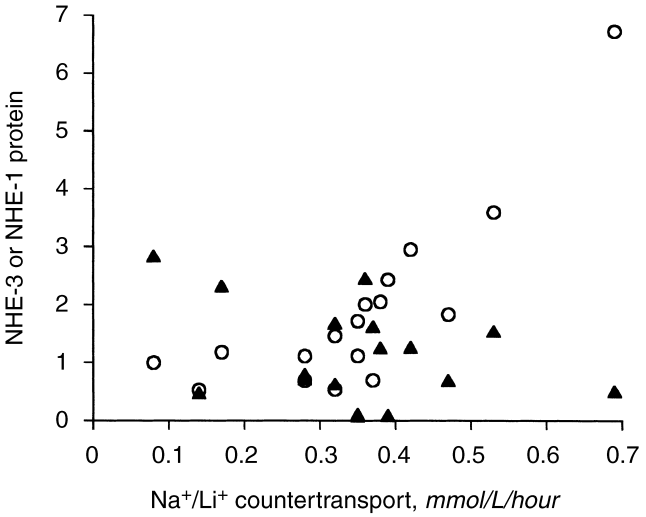


**Fig. 2. Scatterplot illustrating the relationship between red cell SLC values and the density of NHE-1 (▲) or NHE-3 (○) protein (in arbitrary units).** Pearson's correlation coefficients between SLC and NHE-3 protein were very significant ($r = 0.78$, $P < 0.001$).

postulated that it may represent a mode of operation of the NHE-1. This has, however, been refuted by pharmacological studies on SLC and NHE activity in red cells [18, 19]. For example, SLC is phloretin sensitive and amiloride insensitive, whereas NHE-1 fluxes are phloretin insensitive and amiloride sensitive. In addition, NHE-1 is activated by osmotic shrinking, but SLC is not affected. Although there is evidence for the presence of NHE-1 in red cells, amiloride-insensitive isoforms such as NHE-3 protein are absent [45]. This led to proposals that a novel NHE isoform was involved [18].

Others have postulated that SLC may be related to the reabsorption of $Na^+$ in the proximal tubule, this parameter being determined indirectly by $Li^+$ clearance [39, 40]. Weder reported an inverse relationship between SLC and $Li^+$ clearance, implying that there was increased proximal tubule $Na^+$ reabsorption [39]. However, this finding was not confirmed by other groups [41, 42]. This may not be surprising since the reabsorption of $Na^+$ in the proximal tubule is complex and depends on a number of different transport mechanisms.

Recent evidence indicates the absorption of $Na^+$ and $HCO_3^-$ by the proximal tubules is mediated to a large extent by operation of NHEs [28]. The apical localization of NHE-3 [46, 47] in particular is suggestive of a primary role in $Na^+$ absorption, with the basolateral $Na^+,K^+$-ATPase responsible for creating the *trans*-apical membrane $Na^+$ gradient. NHE-1 is localized on the baso-lateral membrane [38] and has been suggested to perform an intracellular pH "housekeeping" regulatory role rather than one directly involved with $Na^+$ absorption. In hypertension, although there is evidence that NHE-1, the isoform that is most frequently studied, exhibits increased activity in proximal tubules [37, 38], there was no evidence for increased NHE-1 protein expression [38]. Thus, the findings in the present study of a slight negative but insignificant correlation between SLC and NHE-1 protein is not surprising. However, when a NHE isoform is overexpressed on the brush border of kidney tubules, such as in the NHE-1 transgenic mouse, salt-sensitive hypertension results from the increased renal $Na^+$ reabsorption [48]. Similarly, in the hypertensive rat, there is evidence for overexpression of NHE-3 at the apical membrane of the proximal tubule [38]. Our present findings of increased NHE-3 protein in human proximal tubule extracts from those patients with high SLC concur with these findings in the hypertensive rat and reinforce the red cell SLC as a phenotypic marker for increased proximal tubule $Na^+$ reabsorption through the apical membrane NHE-3 protein. The reason for this association is not apparent at the moment. Although NHE-3 shares the property of amiloride insensitivity with SLC, there are no data on its sensitivity to phloretin. In addition, NHE-3 activity is inhibited by cell shrinkage, but SLC is unaffected. Furthermore, the absence of NHE-3 protein in red cells [45] makes it unlikely that this protein mediates SLC, and may imply that similar cellular regulatory control mechanisms (for example, accessory proteins) exist for the proximal tubule NHE-3 and the novel NHE isoform [18] that could mediate SLC. There is evidence of a role for accessory proteins (called NHE regulatory factor, or NHE-RF) in the control of NHE-3 activity [49, 50], especially in the effect of protein kinase A-mediated inhibition of NHE-3 activity. The possibility that variance in SLC could be mediated by differences in NHE-RF expression in both red cells and tubule cells remains to be examined. Nevertheless, our present findings of increased NHE-3 protein expression could explain the reports of reduced renal $Li^+$ clearance in those patients with high red cell SLC previously documented by others [39, 40]. Furthermore, the addition of family history of hypertension and NHE-1 protein levels to the NHE-3 protein levels improved the prediction of red cell SLC.

Our present findings that NHE-3 is a homogeneous 85 kD protein concur with other reports of the size of this protein in rat tissue [46, 47, 51] in which the size has been estimated to range between 80 and 87 kD, and in rat proximal tubules in which the size was estimated at 85 kD [38]. NHE-3 is not N-glycosylated, hence its homogeneous molecular mass. In contrast, the molecular weight heterogeneity of human proximal tubule NHE-1 immunoreactivity may be due to N-linked glycosylation [16, 17, 21, 22].

A limitation of our study is the relatively small number of patients caused by the relative paucity of suitable cases. In addition, the presence of the renal tumor may have influenced both red cell and proximal tubule trans-

porters, but it is likely that they would have been affected in a similar fashion. In addition, functional transport studies were not possible, as the enzymatic digestion of renal slices could have affected the membrane permeability of the tubule cells.

In summary, to our knowledge this is the first report of NHE isoforms in the proximal tubule of humans. A strong correlation between red cell SLC and proximal tubule NHE-3 protein expression was documented, which could explain previous findings of an association between a phenotypic marker of hypertension (SLC) and proximal tubule $Na^+$ handling. In combination with environmental factors such as salt intake, these findings may explain the robustness of SLC as a predictive marker for future blood pressure and diabetic renal disease.

*Reprint requests to Leong L. Ng, M.D., Department of Medicine and Therapeutics, Level 4, Clinical Sciences Building, Leicester Royal Infirmary, Leicester, LE2 7LX, United Kingdom.*
*E-mail: lln1@le.ac.uk*

## REFERENCES

1. Canessa M, Adragna N, Solomon HS, Connolly TM, Tosteson DC: Increased sodium-lithium countertransport in red cells of patients with essential hypertension. *N Engl J Med* 302:772–776, 1980
2. Clegg G, Morgan DB, Davidson C: The heterogeneity of essential, hypertension: Relation between lithium efflux and sodium content of erythrocytes and family history of hypertension. *Lancet* 2:891–894, 1982
3. Carr SJ, Thomas TH, Wilkinson R: Erythrocyte sodium-lithium countertransport in primary and renal hypertension: Relation to family history. *Eur J Clin Invest* 19:101–106, 1989
4. Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti G: Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 318:146–150, 1988
5. Krolewski AS, Canessa M, Warram JH, Laffel LM, Christlieb AR, Knowler WC, Rand LI: Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318:140–145, 1988
6. Boero R, Degli Esposti E, Fabbri A, Guarena C, Forneris G, Quarello F, Fusaroli M, Piccoli G: Increased sodium-lithium countertransport activity in red cells of IgA nephropathy patients. *Kidney Int* 40:1118–1122, 1991
7. Boero R, Fabbri A, Degli Esposti E, Guareno C, Forneris G, Lucatello A, Sturani A, Quarrello F, Fusaroli Piccoli G: Sodium-lithium countertransport activity in red blood cells of patients with IgA nephropathy. *Am J Kidney Dis* 21(5 Suppl 2):61–65, 1993
8. Fabbri A, Boero R, Degli-Eposti E, Guarena C, Lucatello A, Sturani A, Piccoli G, Fusaroli M: Aggregation of erythrocyte sodium-lithium countertransport activity in families of patients with IgA nephropathy. *Clin Sci* 83:241–245, 1992
9. Kontessis PS, Friedman R, Tariq T, Moro F, Williams DG, Hartley RB, Viberti GC: Sodium-lithium countertransport activity as a determinant of deterioration of glomerular filtration rate in IgA nephropathy. *Exp Nephrol* 2:176–181, 1994
10. Strazzullo P, Siani A, Cappuccio FP, Trevisan M, Ragone E, Russo L, Iacone R, Farinaro E: Red blood cell sodium-lithium countertransport and risk of future hypertension: The Olivetti Prospective Heart Study. *Hypertension* 31:1284–1289, 1998
11. Fujita J, Tsuda K, Seno M, Obayashi H, Fukui I, Seino Y: Erythrocyte sodium-lithium countertransport activity as a marker of predisposition to hypertension and diabetic nephropathy in NIDDM. *Diabetes Care* 17:977–982, 1994
12. Monciotti CG, Semplicini A, Morocutti A, Maioli M, Cipollina MR, Barzon I, Palaro C, Brocco E, Trevisan M, Fioretto P, Crepaldi G, Nosadini R: Elevated sodium-lithium countertransport activity in erythrocytes is predictive of the development of microalbuminuria in IDDM. *Diabetologia* 40:654–661, 1997
13. Rosskopf D, Dusing R, Siffert W: Membrane sodium-proton exchange and primary hypertension. *Hypertension* 21:607–617, 1993
14. Ng LL, Fennell DA, Dudley C: Kinetics of the human leukocyte $Na^+$-$H^+$ antiport in essential hypertension. *J Hypertens* 8:533–537, 1990
15. Ng LL, Simmons D, Frighi V, Garrido MC, Bomford J, Hockaday TDR: Leukocyte $Na^+/H^+$ antiport activity in type 1 insulin dependent diabetic patients with nephropathy. *Diabetologia* 33:371–377, 1990
16. Ng LL, Davies JE, Siczkowski M, Sweeney FP, Quinn PA, Krolewski B, Krolewski AS: Abnormal $Na^+/H^+$ antiporter phenotype and turnover of immortalized lymphoblasts from type 1 diabetic patients with nephropathy. *J Clin Invest* 93:2750–2757, 1994
17. Ng LL, Sweeney FP, Siczkowski M, Davies JE, Quinn PA, Krolewski B, Krolewski AS: $Na^+/H^+$ antiporter phenotype, abundance and phosphorylation of immortalized lymphoblasts from humans with hypertension. *Hypertension* 25:971–977, 1995
18. Zerbini G, Maestroni A, Mangili R, Pozza G: Amiloride-insensitive $Na^+$-$H^+$ exchange: A candidate mediator of erythrocyte $Na^+$-$Li^+$ countertransport. *J Am Soc Nephrol* 9:2203–2211, 1998
19. Orlov SN, Kuznetsov SR, Pokudin NI, Tremblay J, Hamet P: Can we use erythrocytes for the study of the activity of the ubiquitous $Na^+/H^+$ exchanger (NHE-1) in essential hypertension? *Am J Hypertens* 11:774–783, 1998
20. Mahnensmith RL, Aronson PS: The plasma membrane sodium-hydrogen exchanger and its role in physiological and pathophysiological processes. *Circ Res* 56:773–788, 1985
21. Noel J, Pouyssegur J: Hormonal regulation, pharmacology, and membrane sorting of vertebrate $Na^+/H^+$ exchanger isoforms. *Am J Physiol* 268:C283–C296, 1995
22. Yun CHC, Tse C-M, Nath SK, Levine SA, Brant SR, Donowitz M: Mammalian $Na^+/H^+$exchanger gene family: Structure and function studies. *Am J Physiol* 269:G1–G11, 1995
23. Sardet C, Franchi A, Pouyssegur J: Molecular cloning, primary structure, and expression of the human growth factor-activatable $Na^+/H^+$ antiporter. *Cell* 56:271–280, 1989
24. Orlowski J, Kandasamy RA, Shull GE: Molecular cloning of putative members of the Na/H exchanger gene family. *J Biol Chem* 267:9331–9339, 1992
25. Tse CM, Brant SR, Walker S, Pouyssegur J, Donowitz M: Cloning and sequencing of a rabbit cDNA encoding an intestinal and kidney-specific $Na^+/H^+$exchanger isoform (NHE-3). *J Biol Chem* 267:9340–9346, 1992
26. Guyton AD, Manning RD Jr, Hall JE, Norman R, Young DB, Pan YJ: The pathogenic role of the kidney. *J Cardiovasc Pharmacol* 6:S151–S161, 1984
27. Roman RJ, Cowley AW: Abnormal pressure-diuresis-natriuresis response in spontaneously hypertensive rats. *Am J Physiol* 248: F199–F205, 1985
28. Alpern RJ: Cell mechanisms of proximal tubule acidification. *Physiol Rev* 70:79–114, 1990
29. Semplicini A, Canessa M, Mozzato MG, Ceolotto G, Marzola M, Buzzaccarini F, Casolino P, Pessina AC: Red blood cell $Na^+/H^+$ and $Li^+/Na^+$ exchange in patients with essential hypertension. *Am J Hypertens* 2:903–908, 1989
30. Mbanya JC, Thomas TH, Taylor R, Alberti KG, Wilkinson R: Increased proximal tubular sodium reabsorption in hypertensive patients with type 2 diabetes. *Diabet Med* 6:614–620, 1989
31. Bruun NE, Rehling M, Skott P, Giese J: Enhanced fractional sodium reabsorption in the ischaemic kidney revisited with lithium as a probe. *Scand J Clin Lab Invest* 50:579–585, 1990
32. Burnier M, Biollaz J, Magnin JL, Bidlingmeyer M, Brunner HR: Renal sodium handling in patients with untreated hypertension and white coat hypertension. *Hypertension* 23:496–502, 1994
33. Roos JC, Kirchner KA, Abernethy JD, Langford HG: Differential effect of salt loading on sodium and lithium excretion in Dahl salt-resistant and -sensitive rats. *Hypertension* 6:420–424, 1984
34. Firth JD, Raine AE, Ledingham JG: Sodium and lithium handling in the isolated hypertensive rat kidney. *Clin Sci* 76:335–341, 1989

35. Beach RE: Renal nerve-mediated proximal tubule solute reabsorption contributes to hypertension in spontaneously hypertensive rats. *Clin Exp Hypertens* 14:685–697, 1992
36. Orosz DE, Hopfer U: Pathophysiological consequences of changes in the coupling ratio of Na,K-ATPase for renal sodium reabsorption and its implications for hypertension. *Hypertension* 27:219–227, 1996
37. Morduchowicz GA, Sheikh-Hamad D, Jo OD, Nord EP, Lee DBN, Yanagawa N: Increased Na/H antiport activity in the renal brush border membrane of SHR. *Kidney Int* 36:576–581, 1989
38. Kelly MP, Quinn PA, Davies JE, Ng LL: Activity and expression of $Na^+/H^+$ exchanger isoforms 1 and 3 in kidney proximal tubules of hypertensive rats. *Circ Res* 80:853–860, 1997
39. Weder AB: Red-cell lithium-sodium countertransport and renal lithium clearance in hypertension. *N Engl J Med* 314:198–201, 1986
40. Nosadini R, Semplicini A, Fioretto P, Lusiani L, Trevisan R, Donadon V, Zanette G, Nicolosi GL, Dall'Aglio V, Zanuttini D, Viberti GC: Sodium-lithium countertransport and cardiorenal abnormalities in essential hypertension. *Hypertension* 18:191–198, 1991
41. Strazzullo P, Cappuccio FP, Trevisan M, Iacoviello L, Iacone R, Jossa F, Giorgione N, Farinaro E, Mancini M: Erythrocyte sodium/lithium countertransport and renal lithium clearance in a random sample of untreated middle-aged men. *Clin Sci* 77:337–342, 1989
42. Maling TJ, Siebers RW: Sodium-lithium countertransport is not a marker of proximal tubular sodium clearance. *J Cardiovasc Pharmacol* 16(Suppl 7):S50–S51, 1990
43. Gesek FA, Wolff DW, Strandhoy JW: Improved separation method for rat proximal and distal renal tubules. *Am J Physiol* 253:F358–F365, 1987
44. Carr SJ, Sikand K, Moore D, Norman RI: Altered membrane microviscosity in essential hypertension: Relationship with family history of hypertension and sodium-lithium countertransport activity. *J Hypertens* 13:139–146, 1995
45. Rutherford PA, Pizzonia JH, Biemesderfer D, Abu-Alfa A, Reilly R, Aronson PS: Expression of $Na(^+)$-$H^+$ exchanger isoforms NHE1 and NHE3 in kidney and blood cells of rabbit and rat. *Exp Nephrol* 5:490–497, 1997
46. Biemesderfer D, Pizzonia J, Abu-Alfa A, Exner M, Reilly R, Igarashi P, Aronson PS: NHE3: A $Na^+/H^+$ exchanger isoform of renal brush border. *Am J Physiol* 265:F736–F742, 1993
47. Amemiya M, Loffing J, Lotscher M, Kaissling B, Alpern RJ, Moe OW: Expression of NHE-3 in the apical membrane of rat renal proximal tubule and thick ascending limb. *Kidney Int* 48:1206–1215, 1995
48. Kuro M, Hanaoka K, Hiroi Y, Noguchi T, Fujimori Y, Takewaki S, Hayasaka M, Katoh H, Miyagishi A, Nagai R, Yazaki Y, Nabeshima Y: Salt-sensitive hypertension in transgenic mice overexpressing $Na^+$ proton exchanger. *Circ Res* 76:148–153, 1995
49. Weinman EJ, Steplock D, Wang Y, Shenolikar S: Characterization of a protein cofactor that mediates protein kinase A regulation of the renal brush border membrane $Na(^+)$-$H^+$ exchanger. *J Clin Invest* 95:2143–2149, 1995
50. Weinman EJ, Steplock D, Tate K, Hall RA, Spurney RF, Shenolikar S: Structure-function of recombinant Na/H exchanger regulatory factor (NHE-RF). *J Clin Invest* 101:2199–2206, 1998
51. Bookstein C, Depaoli AM, Xie Y, Niu P, Musch MW, Rao MC, Chang EB: $Na^+/H^+$ exchangers NHE1 and NHE3 of rat intestine: Expression and localization. *J Clin Invest* 93:106–113, 1994