Albumin Regulates the Na\(^{+}/H^{+}\) Exchanger 3 in OKP Cells

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Abstract. Albumin filtered by the glomerulus is reabsorbed in the proximal tubule. We have shown previously that proteinuria stimulates the proximal tubular Na\(^{+}/H^{+}\) exchanger 3 (NHE3) in rats. Activation of NHE3 may be a pathophysiologically important factor in the development of renal salt and water retention observed in the nephrotic syndrome. For examining whether albumin is a specific inducer of proximal tubular Na\(^{+}/H^{+}\) exchange and to determine the molecular mechanisms by which it regulates Na\(^{+}/H^{+}\) exchange, the effect of albumin on NHE3 in opossum kidney cells was studied. Albumin activated Na\(^{+}/H^{+}\) exchange in a time- and dose-dependent manner up to 100% in 48 h. In the early phase of stimulation (2 to 12 h), NHE3 activity was increased without changes in NHE3 protein and mRNA. At 24 h, increased NHE3 activity was accompanied by increase in cell surface NHE3 protein. The increase in surface NHE3 was associated with increased bidirectional trafficking of NHE3 protein between intracellular compartments and the cell surface. At 48 h, total cell NHE3 protein abundance and mRNA were increased as well. Whereas NHE3 translation was increased, NHE3 protein half-life remained unchanged. The effects of albumin on NHE3 protein abundance were modified by hydrocortisone in a complicated pattern. These results indicate that albumin directly regulates proximal tubular NHE3 at multiple levels.

Albuminuria is a common manifestation of renal disease. Glomerular damage results in variable amounts of urinary protein loss and renal salt and water retention (1). Permeability of an intact glomerulus ensures retention of most of the serum proteins in the glomerular capillary (2). However, up to 5 g of protein per day may be filtered by the glomeruli even under normal conditions, which then are reabsorbed by the renal tubule (3,4). In a diseased kidney, substantial amounts of protein (mainly albumin) are filtered through the damaged glomeruli and into the urinary space and increasing quantities of the filtered protein are reabsorbed by the renal tubule to minimize renal protein loss (5). Other than a hallmark of glomerular disease, proteinuria may be an independent factor that induces and perpetuates renal damage (6). One theory is that enhanced tubular protein reabsorption triggers inflammation and fibrosis by induction of several cytokines and growth-regulating factors such as TGF-\(\beta\) (7). The reabsorption of albumin by the proximal tubule is achieved predominantly by endocytosis (3,4). Several recent studies have suggested an interrelation of transcellular albumin transport by endocytosis and acidification of lysosomes (8,9) through endosomal Na\(^{+}/H^{+}\) exchange (10,11). Besides proteinuria, the nephrotic syndrome is accompanied by various degrees of salt and water retention and represents a major clinical problem in the treatment of patients with nephrosis (12,13). One mechanism of salt retention is systemic interstitial volume sequestration as a result of hypoalbuminemia. This is unlikely to be sufficient as congenital analbuminemia is not accompanied by disturbances in extracellular fluid volume (14). An alternative but not mutually exclusive explanation is that primary renal salt retention per se may contribute substantially to systemic volume expansion in nephrotic syndrome (12,13). Regulation of sodium transport in the nephrotic state has been demonstrated to occur in the collecting duct through activation of the Na/K-ATPase (15,16). However, we have shown recently that proximal tubule Na\(^{+}/H^{+}\) exchanger 3 (NHE3) is activated in rats with puromycin aminonucleoside (PAN)-induced proteinuria (17). This finding suggests that the proximal tubular Na\(^{+}/H^{+}\) exchange not only may be a regulator of transtubular protein reabsorption through endosomal acidification but also may be affected by tubular protein concentration and contributes to transcellular sodium and volume reabsorption. The increase in proximal tubular Na\(^{+}/H^{+}\) exchange may be secondary to either hemodynamic factors or nonprotein substances that are lost in the glomerular ultrafiltrate. The direct effects of albumin have not yet been tested.

In the mammalian proximal tubule, >60% of the Na\(^{+}\) absorption is mediated by apical brush border membrane
Na\(^+/\)/H\(^+\) exchange. Of the eight isoforms known to date, NHE3 and NHE8 are the only NHE isoforms definitively shown to be expressed in the brush border membrane of the renal proximal tubule (18,19). NHE3 mediates proximal tubule transcellular NaCl absorption via coupled transport with chloride/base exchange (20,21) as well as paracellular NaCl transport by lowering luminal [HCO\(_3^-\)] and elevating luminal [Cl\(^-\)] (22). The importance of NHE3 in sustaining extracellular fluid volume is evident by the hypovolemia and hypotension seen in NHE3 null mice (23). To specify further the role of proteins on proximal tubular NHE3 and to study the mechanisms by which its activity is regulated, we examined the direct effects of albumin on NHE3 in OKP cells, an opossum kidney cell line with proximal tubular characteristics. Because hydrocortisone (HC) has been shown to exert a permissive effect for the acid- and insulin-induced activation of Na\(^+/\)/H\(^+\) exchange (24,25), we also examined for glucocorticoid dependence of albumin-induced activation.

**Materials and Methods**

**Materials and Supplies**

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted as follows: acetoxymethyl derivative of 2\('\)-bis(2-carboxyethyl)-5-(and-6)-carboxyfluorescein from Molecular Probes (Eugene, OR); NHS-ss-biotin and immobilized streptavidin from Pierce Chemical Co. (Rockford, IL), and culture media from Life Technologies BRL (Grand Island, NY).

**Cell Culture**

OKP cells (26) were cultured in high glucose (450 mg/dl) DMEM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 \(\mu\)g/ml). Before study, confluent cells were rendered quiescent by incubation in serum-free media (1:1 mixture of low glucose [100 mg/dl] DMEM and Ham’s F12 ± 10\(^-6\) M HC) for 24 to 48 h. BSA, fraction V, from Fluka (Buchs, St. Gallen, Switzerland), was applied for the stated period of time before the assays. The albumin preparation is of high purity grade, processed by the manufacturer using absorptive charcoal and extensive dialysis to reduce contamination with low molecular substances. For further increasing purity, albumin was dialyzed again before use in pilot experiments. However, as the results were comparable irrespective of pretreatment, albumin as provided by the manufacturer was used for the bulk of experiments.

**Measurement of Intracellular pH and Na\(^+/\)/H\(^+\) Exchange Activity**

Continuous measurement of cytoplasmic pH (pH\(_i\)) was performed using the intracellularly trapped pH-sensitive dye BCECF, as described previously (27). Cells were loaded with 10 \(\mu\)M acetoxymethyl ester of BCECF (35 min at 37°C), and pH\(_i\) was estimated from the ratio of fluorescence (\(\lambda_{ex}: 500 \text{ and } 450 \text{ nm, } \lambda_{em} 530 \text{ nm}\)) in a computer-controlled spectrofluorometer (RF-5000; Shimadzu Corporation, Kyoto, Japan). The intracellular BCECF excitation fluorescence ratio was calibrated using K\(\)gericin as described (28). Na\(^+/\)/H\(^+\) exchange activity was assayed as the initial rate of Na\(^+\)-dependent pH\(_i\) increase (dpH/dt) after intracellular acidification (Nigericin H\(^+/\)K\(^+\) exchange) in the absence of CO\(_2\)/HCO\(_3^-\). Comparisons were always made between cells of the same passage studied on the same day, and results are reported as percentage change from the dpH/dt of the relative controls. Intracellular buffer capacity was measured by pulsing with 20 mM NH\(_4\)Cl. Buffer capacity \(\beta\) was then calculated according to the formula \(\beta = [\text{NH}_4\text{Cl}]/\Delta\text{pH}\). Results for control and albumin-treated cells were not significantly different (\(\beta = 24.2 \text{ versus } 26.1 \text{ mM, respectively}\)).

**NHE3 Antigen**

Cells were rinsed with ice-cold PBS three times and dounce-homogenized in isotonic Tris-buffered saline (150 mM NaCl, 50 mM Tris-HCl [pH 7.5], 5 mM EDTA) containing proteinase inhibitors (100 \(\mu\)g/ml PMSF, 4 \(\mu\)g/ml aprotinin, 4 \(\mu\)g/ml leupeptin). After nuclei removal (13,000 \(\times\) g, 4°C, 5 min; Eppendorf 5415C, Hamburg, Germany), membranes were pelletted (109,000 \(\times\) g, 4°C, 20 min; Sorvall RC M 120EX, rotor S120 AT2-0130, DuPont-Sorval, Wilmington, DE) and resuspended in Tris-buffered saline, and total protein content was determined by the method of Bradford. Fifteen micrograms of protein was diluted 1:5 in 5× SDS loading buffer (1 mM Tris-HCl [pH 6.8], 1% SDS, 10% glycerol, 1% 2-mercaptoethanol), size-fractionated by SDS-PAGE (7.5% gel), and electrophoretically transferred to nitrocellulose. After blocking (5% nonfat milk, 0.05% Tween-20 in PBS; 1 h), membranes were probed in the same buffer with a polyclonal anti-opposum NHE3 antibody (antiseraum #5683, generated against a maltose binding protein/NHE3aa 484-839 fusion protein) at a dilution of 1:300 (27). Blots were washed in 0.05% Tween-20 in PBS one time for 15 min and two times for 5 min, incubated with a 1:10,000 dilution of peroxidase-labeled sheep antirabbit IgG, washed as above, and then visualized by enhanced chemiluminescence. NHE3 protein abundance was quantified by densitometry (BioCapt software version 72.02s for Windows, Vilbert Lourmat, France; and Scion Image Beta 3b, 1998, Scion Corporation, Frederick, MD). Similarly, sodium phosphate co-transporter type II antigen in OKP cells (NaPi-4) was measured and quantified using an antipossum NaPi-4 antibody (generated against the carboxy-terminal 12-amino acid sequence of NaPi-4; CGVLSQHNAARTL; provided by Dr. E.D. Lederer, Department of Medicine, University of Louisville, KY).

To measure plasma membrane NHE3, we used a surface biotinylation assay (29). Monolayers were rinsed with ice-cold PBS-Ca-Mg (PBS with 0.1 mM CaCl\(_2\), 1.0 mM MgCl\(_2\)) three times. Membrane proteins were then biotinylated by incubation of cells in 1.5 mg/ml NHS-ss-biotin in 10 mM triethanolamine (pH 7.4), 2 mM CaCl\(_2\), and 150 mM NaCl for 90 min at 4°C. After labeling, plates were washed with 6 ml of quenching buffer (PBS-Ca-Mg, with 100 mM glycine) for 20 min at 4°C × 2. Cells were then lysed in RIPA buffer (150 mM NaCl, 50 mM Tris-HCl [pH 7.4], 5.0 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 100 \(\mu\)g/ml PMSF, 5 \(\mu\)g/ml aprotinin, and 5 \(\mu\)g/ml leupeptin), extracts were rocked for 30 min at 4°C and centrifuged at 12,000 \(\times\) g at 2°C for 10 min, and the supernatant was diluted to 3 mg/ml with RIPA buffer. Biotinylated proteins were then affinity-precipitated with streptavidin-conjugated agarose, released by \(\beta\)-mercaptoethanol, and subjected to immunoblotting with anti-NHE3 antisera as above.

**NHE3 Protein Trafficking**

Measurement of NHE3 endocytosis was performed as described previously (30). OKP cells were treated with either albumin or vehicle for 48 h, surface labeled with NHS-SS-biotin and quenched as described above, and then warmed to 37°C to allow endocytosis to occur over 30 min. Surface biotin was cleaved with the small cell-impertinent reducing agent Tris(2-carboxyethyl)phosphine hydrochloride (TCEP; 100 mM in 50 mM Tris pH 7.4). The freshly endocytosed proteins bearing biotin were protected from TCEP cleavage. Cells
were then solubilized in RIPA, and biotinylated proteins were retrieved and assayed for NHE3 as described above. Exocytic insertion of NHE3 was measured as described previously (31). Cells were rinsed with PBS-Ca-Mg × 3 at room temperature. OK cells were treated with albumin or vehicle for 48 h. The apical surface was then exposed to 1.5 mg/ml sulfo-NHS-acetate in 0.1 M sodium phosphate (pH 7.5), to saturate NHS reactive sites on the cell surface, and 0.15 M NaCl for 2 h at 4°C. After quenching for 20 min as described above, cells were warmed to 37°C to permit trafficking. Cells were then surface-labeled with 1.5 mg/ml sulfo-NHS-SS-biotin and lysed with RIPA buffer. The biotinylated fraction, which represents newly inserted surface proteins, was precipitated with streptavidin-coupled agarose, and the precipitate was subjected to SDS-PAGE and blotting with anti-NHE3 antibodies, as above.

The reinsertion assay was modified from that described by Ehlers (32). The theoretical basis is shown in Figure 1. Confluent quiescent OKP cells were treated with either albumin or vehicle for 24 h before the start of the experiment. Cells were biotinylated, rinsed, and quenched at 4°C exactly as described above. Cells were then warmed to 37°C in serum-free cell culture medium with or without 5 mg/ml albumin for 1 h to allow protein trafficking. Plates were then rinsed with ice-cold TBS × 3, and the surface biotin was cleaved with 50 mM glutathione-SH for two rounds (4°C × 15 min each) of cleavage. One set of plates was lysed at this stage, and biotinylated proteins were retrieved with streptavidin precipitation as described above. This represents the total endocytosed fraction over 1 h (fraction 1 in Figure 1). A second set of plates were subjected to a second round of warming in culture medium containing either 5 mg/ml albumin or vehicle to permit trafficking. Reinserted biotinylated proteins were cleaved again as described above with glutathione-SH. The remaining biotinylated proteins were affinity-precipitated from a RIPA lysate. This represents the endocytosed fraction that was not reinserted (fraction 2 in Figure 1). The difference between fractions 1 and 2 yields the NHE3 that was endocytosed and then reinserted (Figure 1).

**NHE3 Translation and Protein Half-Life**

A pulse-chase assay was used to measure NHE3 translational rate. Confluent serum-deprived OKP cells were incubated in either albumin (5 mg/ml) or vehicle for 48 h. In the last 9 h of this period, the following protocol was instituted. Cells were incubated in methionine-cysteine-deficient medium for 1 h, pulsed with 0.2 to 0.3 mCi/ml 35S-labeled methionine-cysteine mixture (New England Nuclear Research Products, Boston, MA) for 6 h, and chased with regular methionine-cysteine-containing medium for 2 h. All media used in this 9-h period contained either albumin or vehicle for the experimental and control groups, respectively. At the end of this 9-h period, OK cells were lysed with RIPA and NHE3 was immunoprecipitated with antiserum 5683 and resolved by SDS-PAGE, and the signal was imaged and quantified by phosphorimaging (Storm 860, Molecular Dynamics). For measurement of NHE3 half-life, a similar pulse-chase protocol was used as described above except that cell lysis was performed at different times: End of the 9-h period was designated t = 0 h and then at t = 14, 20, and 25 h afterward.

**NHE3 Transcript**

RNA was extracted using RNeasy (QIAGEN, Valencia, CA). Fifteen micrograms of total RNA was size-fractionated by agarose-formaldehyde gel electrophoresis and transferred to nylon membranes. The radiolabeled NHE3 probe was synthesized from a full-length OKP NHE3 cDNA (33), and the 18S probe was synthesized from a 752-base Splhl/BamHI fragment of the mouse 18S rRNA (No. 63178; American Type Culture Collection, Rockville, MD) by the random hexamer method. Prehybridization, hybridization, and washing were performed as described previously (27). Filters were exposed to film overnight at −70°C, and labeling was quantified by densitometry. Changes in NHE3 abundance were normalized for changes in 18S rRNA abundance.

**Statistical Analysis**

Statistical analysis was performed using unpaired t test, unless stated otherwise; “n” refers to the number of plates studied.

**Results**

**Albumin Activates Na+/H+ Exchanger Activity**

Figure 2 summarizes the functional data. At both 1 mg/ml and 5 mg/ml, albumin increased Na+/H+ exchange activity. As shown for 1 mg/ml, the effect became apparent at 6 h of incubation (+50% versus control; P = 0.02) and persisted at 24 h (+40%; P = 0.018) and 48 h (+44%; P = 0.019). Albumin at 5 mg/ml had a comparable effect at 24 h to 1 mg/ml (+47% versus control; P = 0.025) but induced a bigger increase in activity at 48 h (+97% versus control; P = 0.0013). We have previously shown that the activation of NHE3 in response to chronic acid incubation requires the presence of HC (24). We examined the albumin effect in the presence or absence of HC during the periods of serum deprivation (48 h before albumin) and albumin treatment (24 and 48 h, respectively). As shown previously (24), HC (10−6 M) per se significantly activates NHE3 by approximately twofold at 24 h (Figure 3). Combined treatment with albumin and HC resulted in a further increase in activity of 48 and 58% with 1 and 5 mg/ml, respectively, compared with HC alone. The percentage increase in NHE activity induced by albumin is approximately the same in the presence or absence of HC. HC seems to exert...
an additive rather than a synergistic effect on albumin-induced activation of the NHE3 activity.

**Albumin Increases Total and Cell Surface NHE3 Protein Abundance**

Changes in NHE3 activity can be associated with changes in total cellular NHE3 protein and/or changes in surface plasma membrane NHE3 protein. Albumin increased NHE3 total protein abundance by 30 and 37% after 48 h of incubation at the concentration of 1 (P = 0.04) and 5 mg/ml (P = 0.05), respectively (Figure 4). Despite changes in NHE3 activity, no significant effect can be detected at earlier time points for either concentration. The interaction between albumin and HC on total cellular NHE3 seems to depend on the concentration of albumin. The addition of HC did not amplify the magnitude of the albumin-induced increase in total cellular NHE3 at 1 mg/ml albumin (not shown), but the stimulation by 5 mg/ml albumin was higher in the presence (77% increase; P < 0.01, not shown) compared with absence of HC. In contrast, no relevant change in total cellular NaPi-4 antigen, the type II sodium-phosphate co-transporter in OKP cells, was detected after 48 h of incubation with 5 mg/ml albumin (control, 100%; albumin, 96 ± 4%; NS, n = 8).

We next examined the effect of albumin on NHE3 surface protein abundance. At 5 mg/ml, albumin caused an increase in NHE3 surface protein of 96% at 48 h of incubation (P < 0.0001; Figure 5, A and B), which is more than the 37% increase in total NHE3 protein abundance (Figure 4). At 1 mg/ml, albumin has no detectable effect on NHE3 surface protein abundance. Surprising is that the interaction between albumin and HC for surface NHE3 is different from that observed for total cellular NHE3.

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**Figure 2.** Effect of albumin on Na\(^{+}/H\(^{+}\) exchange activity. OK cells were grown to confluence and serum deprived for 24 to 48 h. Cells were then kept in serum-deprived medium and treated with 1 mg/ml albumin for 2 h (n = 4), 6 h (n = 6), 12 h (n = 9), 24 h (n = 9), and 48 h (n = 21), respectively, or 5 mg/ml albumin for 24 h (n = 10) and 48 h (n = 16), respectively. Na\(^{+}/H\(^{+}\) exchange activity was measured fluorimetrically under V\(_{\text{max}}\) conditions as Na\(^{+}\)-dependent cell pH recovery and is expressed as percentage of dpHi/dt (versus time point 0). Bars represent mean ± SEM. *P < 0.05 versus control (0 time); #P < 0.05 versus albumin, 1 mg/ml at the same 48-h time point.

**Figure 3.** Effect of albumin ± hydrocortisone (HC), 10\(^{-6}\) M, on Na\(^{+}/H\(^{+}\) exchanger activity. Cells were grown to confluence and serum deprived for 24 to 48 h in the presence or absence of 10\(^{-6}\) M HC. Cells were then kept in serum-deprived medium ± HC and treated ± albumin (1 or 5 mg/ml, for 24 h). Na\(^{+}/H\(^{+}\) exchange activity was measured fluorimetrically under V\(_{\text{max}}\) conditions as Na\(^{+}\)-dependent cell pH recovery and is expressed as percentage of dpHi/dt (versus time point 0). Each bar represents mean ± SEM for nine experiments. *P < 0.05; ANOVA.

**Figure 4.** Effect of albumin on Na\(^{+}/H\(^{+}\) exchanger 3 (NHE3) protein abundance. Cells were grown to confluence and serum deprived for 24 to 48 h. Cells were then kept in serum-deprived medium and treated ± albumin. Equal quantities of total cell membranes were prepared for immunoblot with anti-opossum NHE3 antisera. (A) Representative immunoblot. (B) Summary of data. Bars represent mean ± SEM of a number of experiments: Albumin 1 mg/ml, for 24 h (n = 11) or 48 h (n = 24); 5 mg/ml, for 24 h (n = 9) or 48 h (n = 16). *P < 0.05 versus control; unpaired t test.
observed with total NHE3. Whereas 1 mg/ml of albumin did not increase surface NHE3, in the background of HC, albumin induced a significant increase of 61% (\(P = 0.0063\)) and 116% (\(P < 0.0002\)) in NHE3 surface protein abundance at 24 and 48 h, respectively (Figure 5B). At 5 mg/ml albumin in the presence of HC, albumin increased NHE3 surface fraction by 64% at 48 h (\(P = 0.038\)), which is not higher than the albumin-induced increase in surface NHE3 in the absence of HC (Figure 5C).

**Regulation of NHE3 Trafficking by Albumin**

The increase in surface NHE3 in response to albumin exposure can be caused by changes in exocytotic insertion or endocytotic retrieval. To determine the mechanisms that regulate increase in NHE3 surface protein content, we studied the effect of albumin on rates of NHE3 endocytosis, exocytosis, and reinsertion. Treatment of cells with 5 mg/ml albumin for 48 h increased exocytosis of NHE3 protein by 116 ± 26% (\(n = 5\); Figure 6A). Endocytosis, however, was increased by 80 ± 23% (\(n = 4\)) compared with control cells (Figure 6B). As the assay performed as outlined above does not distinguish between trafficking of de novo synthesized protein from the endoplasmic reticulum versus recycling of protein between subapical storage compartments and the cell surface, the percentage of NHE3 protein reinsertion was determined by a modified biotinylation assay (described in Materials and Meth-

**Figure 5.** Effect of albumin on cell surface NHE3 protein. Cells were grown to confluence and serum deprived for 24 to 48 h. Cells were then kept in serum-deprived medium and treated ± albumin. Surface proteins (biotin-accessible) from equal amount of cell lysates were immunoblotted for NHE3. (A) Representative blot. (B) Summary data. Bars represents mean ± SEM: Albumin (1 mg/ml) without HC: 24 h (\(n = 4\)) or 48 h (\(n = 4\)); albumin (1 mg/ml) with \(10^{-6}\) M HC: 24 h (\(n = 4\)) or 48 h (\(n = 4\)), respectively. *\(P < 0.05\) versus control, unpaired \(t\) test. (C) Summary data. Bars represents mean ± SEM: Albumin (5 mg/ml) without HC: 24 h (\(n = 6\)) or 48 h (\(n = 4\)); albumin (5 mg/ml) with \(10^{-6}\) M HC: 24 h (\(n = 8\)) or 48 h (\(n = 4\)). *\(P < 0.05\) versus control.

**Figure 6.** Effect of albumin on trafficking of NHE3. Confluent cells were serum deprived for 48 h and then treated ± albumin (5 mg/ml) for another 48 h and then subjected to the exocytosis, endocytosis, and reinsertion assays as described in Materials and Methods. (A) Exocytosis. (B) Endocytosis and reinsertion. Inset shows one typical experiment. Bars represents mean ± SEM of four independent experiments.
There are examples in which changes in NHE3 activity are due to increased NHE3 translation or prolongation of NHE3 protein half-life. Figure 7A shows that NHE3 translational rate was increased 97% after 48 h of albumin incubation. In contrast to NHE3 protein translation, total cellular NHE3 protein half-life was not altered by treatment with albumin (Figure 7B). The increase in NHE3 translation may be due to a translational activation or simply to an increase in the pool of NHE3 transcript.

Discussion

Proteinuria has been implicated both in clinical and in animal studies to be an important factor in the progression of renal damage partially mediated via an inflammatory reaction leading to tissue scarring and functional impairment (6,7). Trans-tubular albumin reabsorption is mainly achieved by lysosomal uptake (8,9), which depends partially on endosomal acidification by NHE3 (10,11). Experiments in PAN-induced nephrotic syndromes have shown an increase in proximal tubular Na\(^+\)/H\(^+\) exchanger activity (17). These findings suggest that proximal tubular Na\(^+\)/H\(^+\) exchange is induced by albumin possibly in response to increased demand in tubular protein reabsorption, and the increase in apical membrane Na\(^+\)/H\(^+\) exchanger may contribute to renal sodium and fluid volume retention. Previous studies have shown that serum (10% FCS, approximately 4 mg/ml albumin) removal increases NHE3 activity (34). However, one cannot equate serum to albumin as serum likely contains many factors other than albumin that can potentially regulate cell differentiation and NHE3 expression in epithelial cells. The present studies in OKP cells support the notion that albumin directly stimulates NHE3.

This study in a cell culture model highlights several points. First, Na\(^+\)/H\(^+\) exchanger function increases after 6 h of incubation with albumin before any detectable changes in surface NHE3 protein. This is unlikely to be due to differential sensitivity of the assays as the surface biotinylation method can detect as low as approximately 25% changes in surface NHE3. There are examples in which changes in NHE3 activity are dissociated from surface NHE3 protein (35–39). Our previous study with the PAN nephrosis model also suggests that NHE3 activity is increased per brush border membrane NHE3 antigen, an effect that may be due to changes in the megalin-bound versus free NHE3 pool (17). The apical uptake of albumin into the proximal tubule is shown to be coupled to megalin and cubulin (40–42), as well as other albumin-binding proteins located in the proximal tubule (7). Biemesderfer et al. (43) demonstrated that cortical brush border NHE3 exists in two different pools—a 21 S, megalin-associated, inactive form and a 9.6 S active form present in brush border microvilli unassociated with megalin—and have postulated that partitioning of NHE3 between these two pools can potentially regulate NHE3 activity. Data from proteinuric rats showing an increase in cortical brush border NHE3 immunofluorescence with an antibody that preferentially detects the megalin-free apical fraction of NHE3 (17) but a generalized decrease in total apical membrane NHE3 are compatible with the hypothesis of Biemesderfer. However, the effect of albumin does not affect all sodium-coupled brush border membrane transporters equally, as the protein abundance of the OKP type II sodium phosphate co-transporter was unchanged by albumin incubation.

Second, after 24 h of incubation with albumin, an increase in surface NHE3 is detectable but increased total NHE3 protein and NHE3 mRNA are not observed until after 48 h. Because the magnitude of increase in surface NHE3 (96%) exceeds and precedes that of total NHE3 (37%), albumin must alter trafficking of NHE3 protein. Indeed, NHE3 exocytosis is increased by approximately 115% and endocytosis stimulated by approximately 80%. However, of the endocytosed NHE3, almost all of it is reinserted back into the cell surface with several-fold increase in recycling rate. This change in insertion and retrieval kinetics results in increased steady-state surface NHE3 without any expansion of the total cellular pool.

Third, after 48 h of albumin incubation, total cellular NHE3 and mRNA are increased. Although the coupling of changes in insertion and retrieval kinetics can increase surface NHE3, this mechanism is shortly supplemented with an additional mode by which total cellular NHE3 is increased. The increase in NHE3 translation is likely accountable by the increase in NHE3 transcript. There are multiple examples of regulation of NHE3 at the mRNA and protein levels (44–47). The mechanism by which albumin increases NHE3 mRNA and protein remains to be determined. These mechanisms are schematically summarized in Figure 9.

Fourth, there is evidence of interaction between albumin and glucocorticoid on NHE3. Because of previous findings of glucocorticoid dependence of regulation of NHE3 by acid incubation and insulin (24,25), we examined whether such a permissive effect exists for albumin. The findings are complex. The modifying effect of HC on regulation of NHE3 by albumin is different for activity, surface protein versus cellular protein, and dosage of albumin used. HC seemed to have a simple additive effect with albumin on NHE3 activity and total NHE3 protein. For surface protein, it seems that at 1 mg/ml albumin, the presence of HC is absolutely required to increase surface NHE3 protein, whereas at 5 mg/ml, supplementation with HC did not seem to make a difference. The complexity of these findings does not permit the construction of a simple paradigm to account for how glucocorticoids interact with albumin.

Although the increase in surface NHE3 (96%) is larger than the increase in total NHE3 (37%), there is approximately 4 times more intracellular than cell surface NHE3 in OK cells...
Figure 9. Proposed model for albumin effect on NHE3 in the proximal tubule. Cell surface NHE3 can be increased by increased exocytotic insertion of newly synthesized NHE3 or reinsertion of endocytosed NHE3. Most of NHE3 retrieved by endocytosis is not destined for degradation but rather recycled back to the membrane. Increased total cellular pool of NHE3 is effected by increased NHE3 translation from increased NHE3 transcript but no change in NHE3 protein degradation.

Figure 8. Effect of albumin on NHE3 transcript. Cells were grown to confluence and serum deprived for 24 to 48 h. Cells were then kept in serum-deprived medium and treated ± albumin. HC (10^{-6} M) was included or omitted from the culture medium. NHE3 transcript was quantified in total cellular RNA by RNA blot. (A) Representative RNA. (B) Summary of data. Bars represent mean ± SEM. Albumin (5 mg/ml) without HC, n = 4, albumin (5 mg/ml) with 10^{-6} M HC, n = 4.

Figure 7. (A) Effect of albumin on NHE3 translation. Confluent serum-deprived cells were incubated with vehicle or albumin (5 mg/ml) for 48 h. Cells were pulsed with 35S-methionine-cysteine as described in Materials and Methods. NHE3 was immunoprecipitated from the cell lysate and resolved by SDS-PAGE, and radiolabeled NHE3 was imaged and quantified by phosphorimaging. Insert shows a representative experiment. Bars and error bars represent mean ± SEM of four experiments. *P < 0.05. (B) Effect of albumin on NHE3 protein half-life. Confluent serum-deprived cells were pulsed with 35S-methionine-cysteine and chased with unlabeled medium as described in Materials and Methods. At the specified times, cells were lysed and the radiolabeled NHE3 was immunoprecipitated, resolved by SDS-PAGE, and imaged and quantified by phosphorimaging. One representative experiment is shown. A total of three independent experiments showed similar results.
(31), which means that the absolute increase in intracellular NHE3 will be much more than cell surface NHE3. The increase in endocytosis of NHE3 with albumin incubation is compatible with the hypothesis that the megalin/NHE3 complex serves as a mediator of albumin endocytosis and processing (40, 41). Recycling of NHE3 from the endosome back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48). After processing of albumin, NHE3 is recycled back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48). After processing of albumin, NHE3 is recycled back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48). After processing of albumin, NHE3 is recycled back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48). After processing of albumin, NHE3 is recycled back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48). After processing of albumin, NHE3 is recycled back to the cell surface has been described in other cell culture models (48). After processing of albumin, NHE3 is recycled back to the apical membrane instead of targeted for degradation peripherally (48).

If proteinuria per se inflicts damage and contributes to progression of renal disease, then to understand tubular toxicity of albumin, one needs to understand the mechanism of its processing. Further therapeutic measures may include antagonists of NHE3 and the megalin/NHE3 complex to reduce volume expansion as well as reduction of tubular toxicity from protein overload.

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