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Glucocorticoids acutely increase cell surface Na^+/H^+ exchanger-3 (NHE3) by activation of NHE3 exocytosis

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Abstract

Glucocorticoids have important effects on renal function, including the modulation of renal acidification by the major proximal tubular Na^+/H^+ exchanger, NHE3. While the chronic effect of glucocorticoids is considered to be primarily at the transcriptional level, with increases in NHE3 mRNA and protein expression driving increased transport activity, the mechanisms by which glucocorticoids activate NHE3 in an acute setting have not been investigated. Previous studies have shown that a glucocorticoid-stimulated increase in NHE3 activity can occur before any detectable change in NHE3 mRNA. The present study examines the acute effects of glucocorticoids on NHE3 using opossum kidney (OKP) cells as a cell model. In OKP cells, total NHE3 protein abundance was not changed by 3 h of treatment with dexamethasone (10^{-6} M). However, the biotin-accessible fraction representing NHE3 at the apical membrane as well as Na^+/H^+ exchange activity measured fluorimetrically using the pH-sensitive dye BCECF-AM were significantly increased. These effects were not prevented by the protein synthesis inhibitor cycloheximide. NHE3 insertion (biotinylatable NHE3 after sulfo-NHS-acetate blockade) was stimulated by dexamethasone incubation, with or without cycloheximide. The rate of NHE3 endocytic retrieval, assessed either by the avidin protection assay (early endocytosis) or by the sodium 2-mercaptoethane sulfonate (MesNa) cleavage assay (early and late endocytosis), was not affected by dexamethasone. These findings suggest that trafficking plays a key role in the acute stimulation of NHE3 by glucocorticoids, with exocytosis being the major contributor to the glucocorticoid-induced rapid increase in cell surface NHE3 protein abundance and Na^+/H^+ exchange activity.

Keywords

OKP; protein trafficking; nongenomic; posttranslational; dexamethasone

Glucocorticoids play an important role in renal acidification. Glucocorticoids increase baseline ammonium and net acid excretion (34,35,61,62) as well as provide permissive action for the kidney to adapt to an acid load (37). Decreased net acid secretion has been described in the adrenal-deficient state (24,37,55,62). In the neonate, the increase in renal acidification is temporally commensurate with a systemic surge in glucocorticoids

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(11,32,46,57). Maturation in proximal acidification is partially impaired by glucocorticoid depletion (29,30) and can be rescued or induced by glucocorticoid administration (10–12,30).

Na^+/H^+ exchange in mammals is encoded by nine known genes that are paralogs, each with characteristic tissue and cellular distributions (51,52). The NHE3 isoform is specific for NaCl- and $NaHCO_3$-transporting epithelia (53). In the kidney, NHE3 is expressed in the proximal tubule apical membrane (3,14) and accounts for a major portion of Na^+/H^+ exchange activity (63), although other NHE isoforms may also contribute (18,28,41,59).

Glucocorticoids have been shown to increase Na^+/H^+ exchange activity when given to intact animals (6,26,37,38). Their direct effect has been shown by addition to renal tubules in suspension (9,13) as well as cultured cells (8). Several studies have described glucocorticoids inducing parallel increases in NHE3 transcript, total cellular protein, and Na^+/H^+ exchange activity in renal and gastrointestinal tissue and cells (2,7–9,16,44). Glucocorticoids have also been shown to directly stimulate NHE3 transcription (7). These findings are compatible with the overall genomic action of glucocorticoids.

However, not all findings fit with this apparently straightforward paradigm. There is suggestive evidence that the activation of NHE3 by glucocorticoids is not through a simple effect of increasing NHE3 gene transcription. In suspensions of rabbit proximal tubules, the V_{max} of Na^+/H^+ exchange was increased as early as 1 h of glucocorticoids (13), and the increase in Na^+/H^+ exchange after 3–4 h was not associated with changes in NHE3 mRNA abundance (9,65). This dissociation may be due to activation of another NHE isoform accounting for the early increase in exchange activity, or glucocorticoids may activate NHE3 via alternative mechanisms.

There are numerous examples of regulation of NHE3 via trafficking (47,48), involving regulation of both NHE3 endocytosis (21,33) and exocytosis (54,64) in epithelial cells. Some of these agonists have been demonstrated to act via more than one mechanism in a multiphasic fashion (25,33,39). NHE1, a close paralog to NHE3, has been shown in vascular smooth muscle cells to be regulated in a biphasic fashion, with the acute phase being insensitive to actinomycin D and cycloheximide, not associated with changes in NHE1 mRNA, but inhibited by colchicines (50). The present study uses opossum kidney (OKP) cells as a cell culture model of the renal proximal tubule to examine how glucocorticoid acutely activates NHE3 in a manner that is independent of changes in NHE3 transcript and total cellular NHE3 protein.

EXPERIMENTAL PROCEDURES

Materials and supplies

All chemicals were obtained from Sigma (St. Louis, MO), except where otherwise noted, and except for the following: culture media (Invitrogen, Carlsbad, CA); penicillin and streptomycin (Whittaker Bioproducts, Walkersville, MD); BCECF-AM (Molecular Probes, Eugene, OR); EZ-Link sulfo-NHS-SS-biotin, EZ-Link biocytin, sulfo-NHS-acetate, immunopure avidin, and immunopure immobilized streptavidin (Pierce, Rockford, IL); nylon membranes (Hybond-N+), horseradish-peroxidase labeled anti-mouse IgG and enhanced chemiluminescence detection kit (Amersham Biosciences, Piscataway, NJ); and polyvinylidene difluoride membranes (Immobilon, Millipore, Billerica, MA).

Cell culture

OKP cells (20) cultured at 37°C in a 95% air-5% CO_2 atmosphere were passaged in high-glucose (450 mg/dl) DMEM supplemented with 10% fetal bovine serum, penicillin (100 U/

ml), and streptomycin (100 g/ml). Before study, confluent cells were rendered quiescent by incubation in a serum-free 1:1 mixture of low-glucose (100 mg/dl) DMEM and Ham's F-12 for 24–48 h. Dexamethasone 10^{-6} M (10^{-3} M stock solution in ethanol) or vehicle was applied for 3 h before the assays. Cycloheximide 10^{-5} M (10^{-2} M stock solution in dimethylsulfoxide) or vehicle was applied for 4 h (1 h before and 3 h during the incubation with dexamethasone).

NHE3 activity assay

NHE3 activity was measured fluorimetrically using the intracellularly trapped pH-sensitive dye BCECF as described previously (49). Cells grown on glass coverslips were loaded with 10 μM BCECF-AM (30 min at 37°C), and intracellular pH (pH_i) was estimated from the ratio of fluorescence ($\lambda_{excitation}$: 500 and 450 nm, $\lambda_{emission}$: 530 nm) in a computer-controlled spectrofluorometer (QM-8/2003, Photon Technology International, London, Ontario). The 500/450-nm fluorescence ratio was calibrated to pH_i using K^+/nigericin as described (1). Na^+/H^+ exchange activity was assayed as the initial rate of the Na^+-dependent pH_i increase after an acid load using nigericin in the absence of CO_2/HCO_3^-, and results are reported as dpH_i/dt. Comparisons were always made between cells of the same passage studied on the same day. Intracellular buffer capacity (β) was measured by pulsing cells with 20 mM NH_4Cl, according to the formula $\beta = [NH_4Cl]/\Delta pH_i$. The β values for control and dexamethasone-treated cells (with or without cycloheximide) were not significantly different.

NHE3 transcript

RNA was extracted using TRIzol Reagent (Invitrogen), and 15 μg of total RNA were size fractionated by agarose-formaldehyde gel electrophoresis, transferred to nylon membranes by upward capillary transfer, and cross-linked under ultraviolet light. The radiolabeled NHE3 probe was synthesized from full-length OKP NHE3 cDNA (4) and an 18S probe from a 752-base *Sph*I/*Bam*HI fragment of mouse 18S rRNA (no. 63178, American Type Culture Collection, Manassas, VA) by the random-sequence hexanucleotides method (Rediprime II DNA Labeling System, Amersham Biosciences; [α-^{32}P] deoxycytidine 5′-triphosphate, PerkinElmer, Wellesley, MA). Membranes were prehybridized for 1 h at 68°C in Quick-Hyb hybridization solution (Stratagene, La Jolla, CA) containing 0.2 mg/ml salmon sperm DNA, hybridized in the same solution containing the radiolabeled NHE3 probe for 2 h at 68°C, and washed in 2× SSC (sodium chloride-sodium citrate buffer; 1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) with 0.1% SDS three times for 15 min at room temperature, in 0.2× SSC with 0.1% SDS two times for 15 min at 68°C, and finally in 2× SSC for 5 min at room temperature. Membranes were exposed to film overnight at −80°C. Membranes were then stripped by being washed twice for 15 min in boiling 0.1× SSC with 0.1% SDS, prehybridized, hybridized with the 18S radiolabeled probe for 1 h, washed, and visualized as above. Labeling was quantified by densitometry (Scion Image Beta 4.0.2, 2000, Scion, Frederick, MD), and changes in NHE3 mRNA abundance were normalized for changes in 18S rRNA abundance.

NHE3 antigen

Confluent cells in 100-mm plates were rinsed three times with ice-cold isotonic wash buffer (in mM: 137 NaCl, 2.7 KCl, 10 Na_2HPO_4, 2 KH_2PO_4, 1 $MgCl_2$, and 0.1 $CaCl_2$, pH 7.4) and homogenized in RIPA buffer [150 mM NaCl, 50 mM Tris · HCl (pH 7.4), 5 mM EDTA, 1% Triton X-100, 0.5% deoxycholate, and 0.1% SDS] containing fresh protease inhibitors (1 complete mini-EDTA-free protease inhibitor cocktail tablet/10 ml; Roche Applied Science, Indianapolis, IN). The lysates were cleared by centrifugation (14,000 *g*, 4°C, 30 min; Denville 260D, Denville Scientific, Metuchen, NJ), and protein content was determined by

the method of Bradford. Identical amounts of protein (30 μg) were diluted in 2.5× loading buffer [2.5 mM Tris · HCl (pH 6.8), 2.5% SDS, 2.5% β-mercaptoethanol, 25% glycerol], heated for 2 min at 95°C, size-fractionated by SDS-PAGE (8% gel), and electrophoretically transferred to polyvinylidene difluoride membranes. After blocking (5% nonfat milk, 0.05% Tween 20 in PBS for 2 h), the membranes were probed overnight at 4°C with the 3H3 monoclonal mouse anti-opossum NHE3 antiserum, which has been characterized to specifically label NHE3 by immunoblotting (27). The membranes were then washed (5 × 10 min) in PBS containing 0.05% Tween 20, incubated with a horseradish peroxidase-labeled anti-mouse secondary antibody for 1 h, washed as above, and visualized by enhanced chemiluminescence. Membranes were allowed to dry, then reactivated in methanol and stripped [30 min at 50°C in 0.125 M Tris · HCl (pH 6.7) with 4% SDS and 0.016% β-mercaptoethanol], blocked, reprobed with a monoclonal anti-β-actin antibody (Sigma) overnight at 4°C, washed, incubated with the secondary antibody, washed, and visualized as above. NHE3 and β-actin protein abundances were quantified by densitometry, with β-actin serving as a loading control.

Cell surface NHE3 antigen

Confluent quiescent cells in 100-mm plates were rinsed with ice-cold PBS, and surface proteins were biotinylated by incubating cells with 1.5 mg/ml sulfo-NHS-SS-Biotin in 10 mM triethanolamine (pH 7.4), 2 mM $CaCl_2$, and 150 mM NaCl for 90 min with horizontal motion at 4°C. After labeling, plates were washed twice with 6 ml of quenching buffer (PBS containing 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, and 100 mM glycine) for 20 min at 4°C. Cells were then lysed in RIPA buffer with protease inhibitors as above, lysates were cleared by centrifugation, and the supernatants were diluted to 2.5 mg/ml of protein with RIPA buffer. Cell lysates of equivalent amounts of protein were equilibrated overnight with streptavidin-agarose beads at 4°C. Beads were washed sequentially with *solutions A* [50 mM Tris·HCl (pH 7.4), 100 mM NaCl, and 5 mM EDTA], *B* [50 mM Tris·HCl (pH 7.4) and 500 mM NaCl], and *C* (50 mM Tris·HCl, pH 7.4). Biotinylated proteins were released by heating to 95°C with 2.5× loading buffer and subjected to immunoblotting with anti-NHE3 antisera as above.

NHE3 exocytic insertion

Confluent quiescent OKP cells were rinsed with PBS as above and the apical surface was exposed to 1.5 mg/ml sulfo-NHS-acetate in 0.1 M sodium phosphate (pH 7.5) and 0.15 M NaCl (3 times 40 min at 4°C) to saturate NHS-reactive sites on the cell surface (54). After quenching for 20 min (see above for quench conditions), cells were warmed to 37°C for 3 h to permit protein 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 affinity-precipitated with streptavidin-coupled agarose, and the precipitate was subjected to SDS-PAGE and blotting with anti-NHE3 antibody as above. Controls were performed with omission of the 37°C step and any signal so obtained denotes incomplete saturation of surface-reactive sites with sulfo-NHS-acetate. Typically, this represents less than 8% of the 37°C signal.

NHE3 endocytic internalization

Measurement of NHE3 endocytosis was performed with sodium 2-mercaptoethane sulfonate (MesNa) or avidin protection assays as described previously (33) with minor modifications. OKP cells were surface-labeled with sulfo-NHS-SS-biotin and quenched as described above. Cells were then warmed to 37°C for 3 h in the presence of 10^{-6} M dexamethasone or vehicle to allow protein trafficking to occur. Surface biotin was either cleaved with the small cell-impermeant reducing agent MesNa (50 mM in 50 mM Tris, pH 7.4), or, alternatively, surface biotin was saturated with avidin (50 mg/ml in PBS) and washed with biocytin (50

mg/ml in PBS). The biotin bound to freshly endocytosed proteins is protected from either MesNa cleavage or avidin saturation. Cells were then solubilized in RIPA, and biotinylated proteins were retrieved with streptavidin-agarose affinity precipitation and assayed for NHE3 antigen as described above. The assay using MesNa cleavage measures only late endocytosis, because the small MesNa molecules can easily access the constricted necks of nascent clathrin-coated pits with its contents still in communication with the aqueous exterior. Because of the much larger size of avidin, this reagent is excluded from entering the constricted neck of even the early coated pits; thus the assay using avidin protection measures both early and late endocytosis.

Statistics

Statistical analysis was performed using ANOVA and Student's *t*-test.

RESULTS

Dexamethasone acutely increases Na^+/H^+ exchange activity in OKP cells

Figure 1 shows that acute treatment of OKP cells for 3 h with 10^{-6} M dexamethasone stimulated Na^+/H^+ exchange activity. Inhibition of protein synthesis with cycloheximide (10^{-5} M) administered for 4 h (1 h before and 3 h during the incubation with dexamethasone) did not inhibit this effect. This dose of cycloheximide effectively blocks de novo protein synthesis in OKP cells as determined by [^{3}H]leucine incorporation (5). These findings suggest that the mechanism of stimulation of NHE3 by corticosteroids may have at least two phases, with the early phase being independent of protein synthesis.

Acute treatment with dexamethasone does not change NHE3 transcript and total NHE3 protein abundance

To examine whether the acute increase in NHE3 activity is associated with increased transcription, we measured NHE3 mRNA abundance after 3 h of treatment with vehicle or dexamethasone, in the absence of cycloheximide. To exclude the possibility of an early, rapid surge in mRNA levels, we also measured NHE3 transcript after 1 h of dexamethasone; another measurement after 12 h served as a positive control. As shown in Fig. 2*A*, NHE3 mRNA abundance was markedly increased after 12 h, but was not changed after 1 or 3 h of treatment.

We also measured total cellular NHE3 protein abundance in OKP cells under identical experimental conditions as in Fig. 1. As shown in Fig. 2*B*, total NHE3 was not changed by any of these treatments, indicating that the early stimulatory effect of dexamethasone on NHE3 activity is not due to an increase in the existing cellular pool of NHE3 protein.

Dexamethasone acutely increases apical membrane NHE3 independently of de novo protein synthesis

Because changes in apical membrane NHE3 protein can account for the increased NHE3 activity, one possible effect of dexamethasone could be due to changes in trafficking between surface and intracellular NHE3. Figure 3 shows that treating OKP cells with dexamethasone for 3 h increases the amount of NHE3 antigen present on the apical membrane, and inhibition of de novo protein synthesis with cycloheximide does not alter this effect.

Dexamethasone alters NHE3 trafficking

The findings thus far support the model that there is a shift of NHE3 from its intracellular pool to the surface membrane. This shift can be caused by increased exocytic insertion,

decreased endocytic retrieval, or by a synergistic combination of these two changes. As shown in Fig. 4, dexamethasone increased exocytic insertion of NHE3 into the apical membrane of OKP cells, and cycloheximide treatment did not influence this effect.

To examine whether modifications in NHE3 endocytosis also play a role in the acute effects of dexamethasone in OKP cells, we measured early and late endocytosis as described in EXPERIMENTAL PROCEDURES. As shown in Fig. 5, the amount of NHE3 protein undergoing endocytic retrieval was not changed by incubation with dexamethasone or vehicle.

DISCUSSION

In a chronic setting, glucocorticoids have been reported to increase NHE3 activity, total cellular protein, and mRNA abundance in epithelial cells from the ileum (16,66), renal proximal tubules (9,44), and OKP cells (2,7,8). Glucocorticoid response elements are present in the promoter region of the NHE3 gene (36), and a direct stimulatory effect of glucocorticoids on NHE3 transcription has been shown in OKP cells (7).

However, the initial, rapid induction of NHE3 activity by glucocorticoids cannot be accounted for only by changes in NHE3 transcription because of the extremely rapid effect and a dissociation of changes in NHE3 activity and mRNA abundance (9,13,65). In the present study, 3 h of dexamethasone led to an increase in apical membrane NHE3 protein, without altering NHE3 mRNA levels, and independent of de novo protein synthesis. Stimulation of NHE3 exocytic insertion was shown to be the major contributor to the dexamethasone-induced increase in cell surface NHE3 and, presumably, to the early increase in Na^+/H^+ exchange activity.

There was an apparent discrepancy between the magnitude of changes in NHE3 activity and apical NHE3 protein (Figs. 1 and 3). This is in accordance with previous studies that have shown that changes in NHE3 activity can occur without parallel changes in apical membrane NHE3 protein levels, e.g., inhibition by cAMP in OKP (60) and AP-1 (58) cells, inhibition by PTH (22), and stimulation by insulin (39) in OKP cells. In addition to their effects on transcription and trafficking, glucocorticoids may act on one or more of the other mechanisms that have been implicated in the regulation of NHE3 activity, such as binding to regulatory or scaffolding proteins, interaction with the cytoskeleton, formation of transporter complexes, and phosphorylation state (31,67). Our findings are compatible with, but do not prove the existence of, additional levels of regulation.

Rapid regulation of NHE3 by a variety of biological or pharmacological substances has been shown to involve changes in the amount of NHE3 present at the plasma membrane, achieved through changes in regulated exocytosis and/or endocytosis (21,23,33,47,48,54,64). NHE3 exocytosis was activated without changes in endocytosis by acid incubation (64) and endothelin (54) in OKP cells. Lysophosphatidic acid also activates NHE3 exocytosis in these cells (17,43), and angiotensin II activates NHE3 exocytosis in another model of cultured proximal tubular cells (23). In heterologous cells, phosphatidylinositol 3-kinase (PI3-kinase) is required for NHE3 exocytic insertion (40) and overexpression and activation of PI3-kinase and Akt are sufficient to induce NHE3 exocytosis (42). Serum and glucocorticoid-inducible kinase-1 have been proposed to be part of this cascade (65).

The present study does not contradict the established paradigm of predominantly genomic effects of corticosteroids on NHE3 but rather proposes a biphasic activation model, according to which the genomic effects are preceded by a rapid mobilization of preexisting NHE3 protein from endosomes to the plasma membrane. A similar biphasic model has been proposed for acid-induced activation of NHE3 (64). The proximal tubule is faced with

handling an enormous amount of Na^+, Cl^-, HCO_3^-, and water from the filtrate compared with the amount excreted in the urine, and acute fine-tuning is as critical as chronic adaptation. It makes intuitive sense that there should be multiphasic mechanisms for NHE3 regulation.

Rapid actions of steroids have been known for more than six decades (56). This has been especially well studied in neuroendocrine cells. Of particular note is the finding that numerous steroids can induce exocytosis, such as testosterone in lactotrophs (19), estrogen in chromaffin cells (45), $1,25(OH)_2$ vitamin D_3 in osteoblasts (68), and 20-hydroxyecdysone in *Drosophila* salivary glands (15).

In summary, dexamethasone acutely stimulates Na^+/H^+ exchange activity and increases NHE3 protein abundance on the plasma membrane of OKP cells without changing total cellular NHE3 protein. Both of these events were independent of de novo protein synthesis. The increase in apical membrane NHE3 was shown to be due, at least in part, to stimulation of exocytic insertion of the protein, whereas the rate of NHE3 endocytic retrieval was not changed. These findings suggest that while transcriptional activation may largely be held accountable for the long-term glucocorticoid effects on NHE3, trafficking plays a key role in the rapid stimulation of NHE3 by glucocorticoids.

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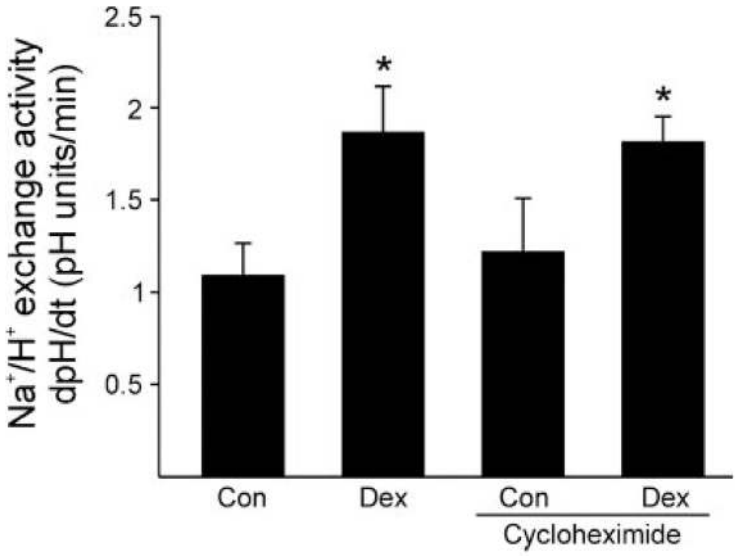

Fig. 1.

Dexamethasone (Dex) acutely increases Na^+/H^+ exchanger-3 (NHE3) activity. Confluent quiescent opossum kidney (OKP) cells were treated with vehicle (Con), dexamethasone (10^{-6} M × 3 h), cycloheximide (10^{-5} M × 4 h), or combined dexamethasone and cycloheximide. NHE3 activity was measured under V_{max} conditions as Na^+-dependent cell pH recovery (dpH_i/dt) after acidification, where pH_i is intracellular pH. Bars and error bars denote means and SE, respectively. *n*, No. of independent measurements; vehicle, $n = 8$; dexamethasone, $n = 7$; cycloheximide, $n = 10$; cycloheximide + dexamethasone, $n = 11$. $^*P < 0.05$ compared with vehicle by ANOVA.

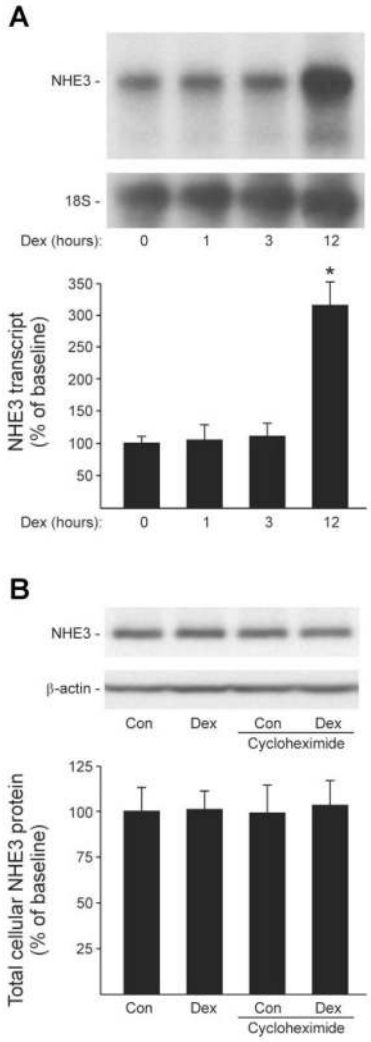

Fig. 2.

Acute treatment with dexamethasone does not alter NHE3 mRNA and total protein abundance. *A*: confluent quiescent OKP cells were treated with vehicle or dexamethasone (10^{-6} M) for 1, 3, or 12 h, and NHE3 mRNA was measured by RNA blotting. For each experiment, the densitometric values of NHE3 mRNA were normalized to 18S rRNA. *B*: confluent quiescent OKP cells were treated with vehicle, dexamethasone (10^{-6} M × 3 h), cycloheximide (10^{-5} M × 4 h), or combined dexamethasone and cycloheximide. NHE3 antigen in whole cell lysates was measured by immunoblot, and β-actin was used as a loading control (Con, vehicle). In *A* and *B*, representative RNA and protein blots (*top*) and the summaries of results from 3 independent experiments (*bottom*) are shown as bars (means) and error bars (SD). *$P < 0.001$ by Student's *t*-test.

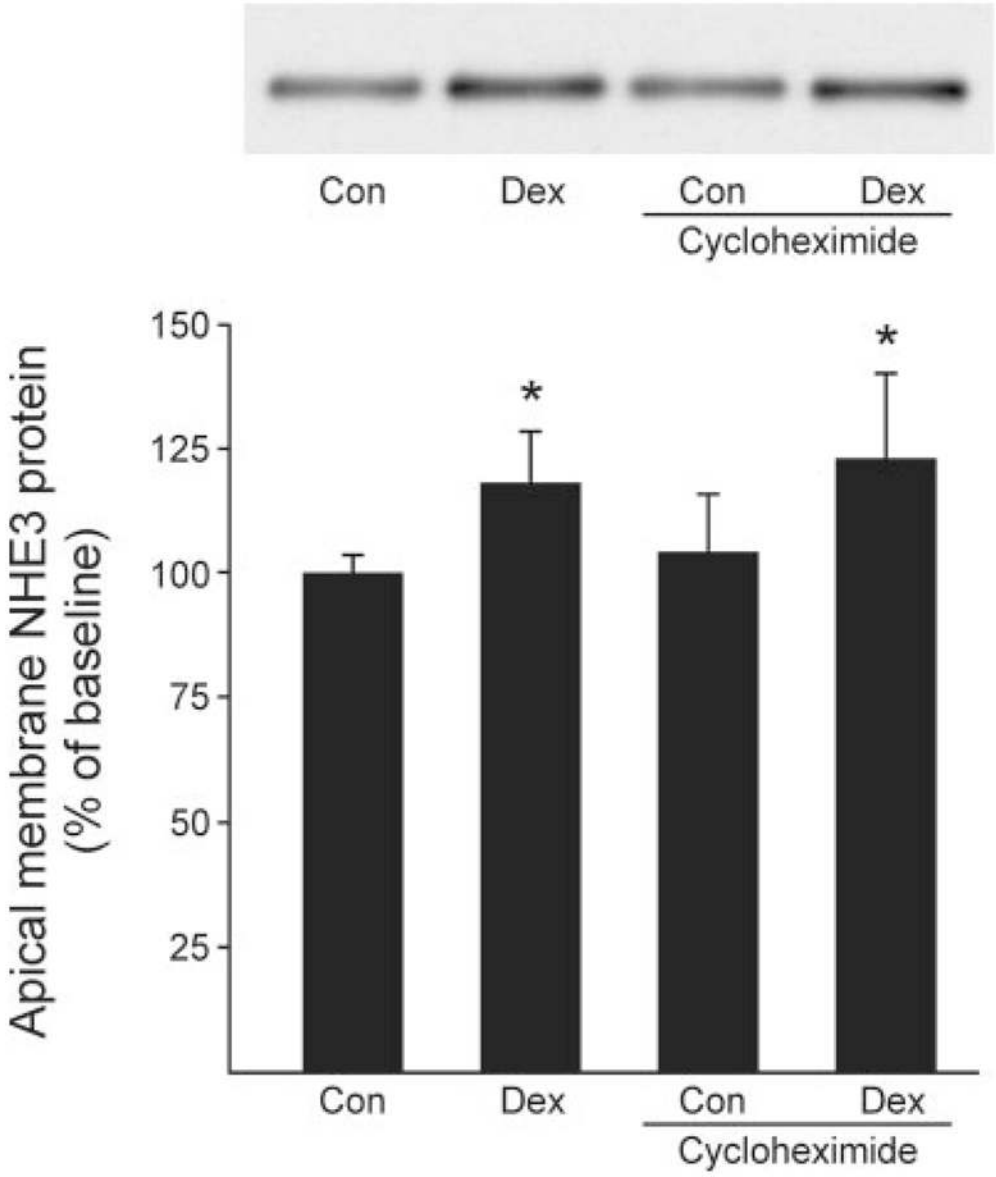

Fig. 3.

Dexamethasone increases cell surface NHE3. Confluent quiescent OKP cells were treated with vehicle, dexamethasone (10^{-6} M × 3 h), cycloheximide (10^{-5} M × 4 h), or combined dexamethasone and cycloheximide. Surface NHE3 antigen was quantified as biotin-accessible and streptavidin-precipitated NHE3 from whole cell lysates measured by immunoblot. *Top*: representative immunoblot. *Bottom*: summary of results from 5 different experiments is shown as bars (means) and error bars (SD). *$P < 0.05$ by unpaired Student's *t*-test.

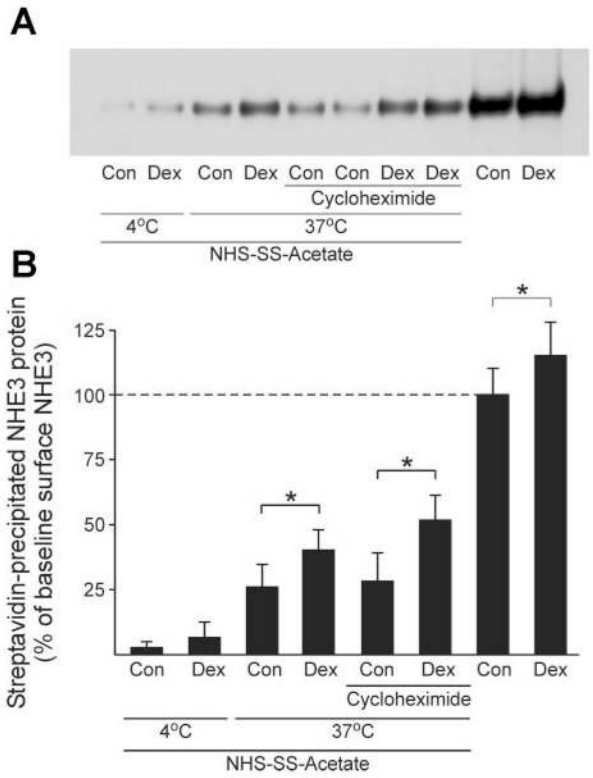

Fig. 4.

Dexamethasone increases exocytic insertion of NHE3 independently of de novo protein synthesis. *A*: typical immunoblot. *B*: summary of results from 6 independent experiments shown as bars (means) and error bars (SD). Cells were pretreated with or without 10^{-5} M cycloheximide, labeled with sulfo-NHS-acetate, and then incubated with 10^{-6} M dexamethasone or vehicle for 3 h at 37°C. Cells kept at 4°C or not labeled with sulfo-NHS-acetate were used as additional controls. Cell surface biotinylation was then performed as described in EXPERIMENTAL PROCEDURES. For each experiment, the densitometric values were normalized to the control that had not been labeled with sulfo-NHS-acetate. *$P < 0.05$ by unpaired Student's *t*-test.

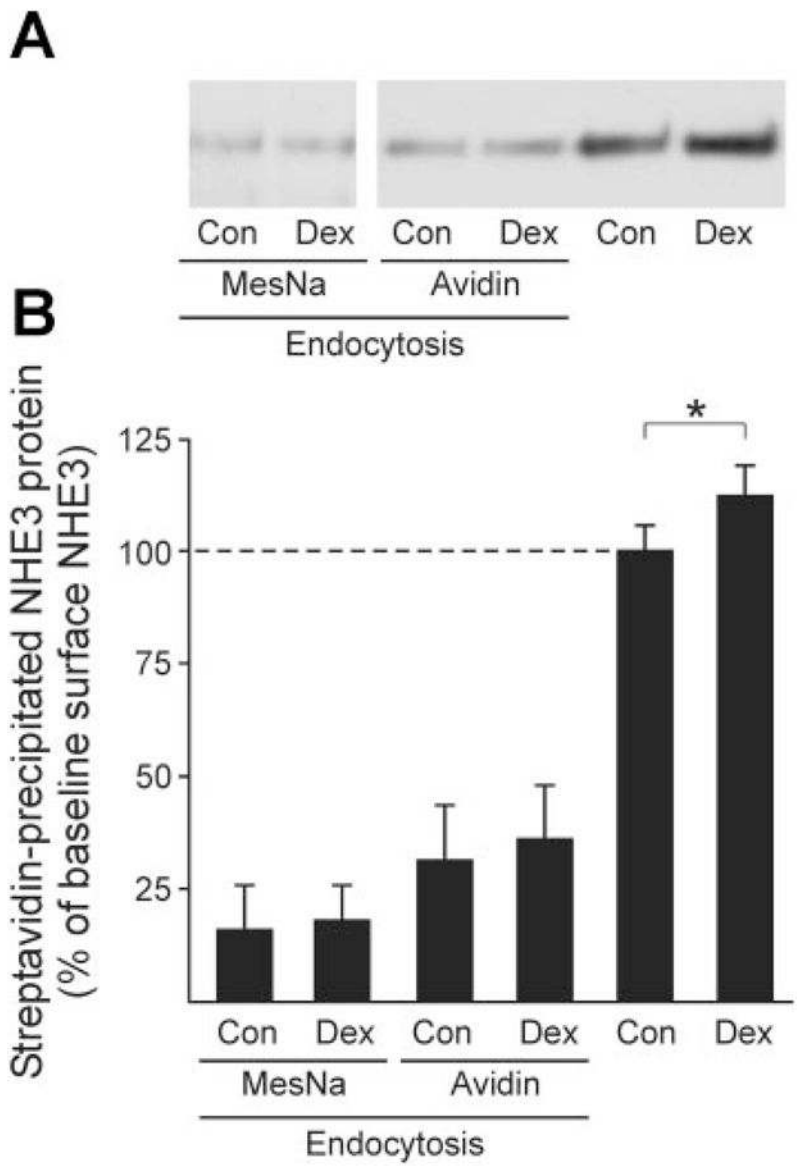

Fig. 5.

Dexamethasone does not alter NHE3 endocytosis. *A*: typical immunoblots. *B*: summary of results from 3 independent experiments (bars are means, error bars are SD). After surface labeling with sulfo-NHS-SS-biotin, confluent quiescent OKP cells were warmed to 37°C in the presence of dexamethasone (10^{-6} M × 3 h) or vehicle. NHE3 endocytosis was measured as the fraction of biotinylated NHE3 protected from either reductive cleavage by sodium 2-mercaptoethane sulfonate (MesNa) or saturation by avidin, as described in EXPERIMENTAL PROCEDURES. For each experiment, the densitometric values were normalized to the control that had not been treated with either MesNa or avidin. *$P < 0.05$ by unpaired Student's *t*-test.