Hormonal Regulation of Sodium/Sulfate Co-Transport in Renal Epithelial Cells (44551)

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Abstract. **Serum sulfate concentrations are elevated in infants, young children, and pregnant women due, at least in part, to increased renal sulfate reabsorption. Little is known about the effects of hormones, particularly those involved in growth, development, and pregnancy, on renal sulfate reabsorption. The objective of this investigation was to examine the effects of growth hormone (GH), insulin-like growth factor 1 (IGF-1), progesterone (PG), and 17β-estradiol (EST) on renal sodium/sulfate co-transport. ^{35}S-sulfate uptake was determined in Madin-Darby canine kidney (MDCK)/NaSi-1 cells (MDCK cells that have been stably transfected with rat sodium/sulfate co-transporter (NaSi-1) cDNA) and in opossum kidney (OK) cells. NaSi-1 mRNA was determined by RT-PCR and protein levels by ELISA. GH (0.1 n*M*) significantly increased the sodium/sulfate co-transport in MDCK/NaSi-1 cells up to 35%. IGF-1 induced a concentration-related stimulation of the sodium/sulfate co-transport with a maximal response observed at 1000 n*M* (59% increase). Sodium-dependent sulfate uptake was significantly increased when cells were preincubated with 10 n*M* PG, 10 n*M* EST, or 10 n*M* PG/10 n*M* EST up to 41%, 46%, or 39%, respectively. OK cells exhibited endogenous sodium-dependent sulfate transport; significantly increased sodium/sulfate co-transport was also observed in OK cells that were preincubated with GH, IGF-1, and PG/EST, although not with EST alone. The NaSi-1 mRNA and NaSi-1 protein levels were significantly increased in MDCK/NaSi-1 cells treated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST compared with control. These results suggest that the increased renal sulfate reabsorption that occurs in neonates, young and pregnant humans, and animals could be mediated by the increased steady-state levels of NaSi-1 mRNA produced by the higher plasma concentrations of GH, IGF-1, or PG/EST.**

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Inorganic sulfate is an important physiological anion that is used in metabolic processes producing biological sulfate conjugates, which are crucial for human growth and development (1–3). The increased serum sulfate concentrations in infants, young children, and pregnant women (4–7) are due, at least in part, to an enhanced sulfate renal reabsorption (8) that represents the predominant mechanism involved in sulfate homeostasis (9). Sulfate renal reabsorption occurs predominantly in the proximal tubule (10). Inorganic sulfate is actively transported through the brush border membrane (BBM) by a sodium-dependent process (11, 12). The cDNA involved in renal sodium/sulfate co-transport at BBM (NaSi-1) has been identified by Markovich *et al.* (13, 14). Sulfate exits from the proximal tubular cells across the basolateral membrane (BLM) *via* an anion exchange mechanism that is electroneutral and saturable (15, 16).

Little is known regarding the hormonal regulation of sulfate homeostasis. Sulfate serum concentrations are elevated in infants and young children (mean serum concentration of 0.47 m*M* in newborns compared with 0.33 m*M* in children over 3 years and adults) (7), and studies in animals have demonstrated that there is increased sodium/sulfate co-transport in the proximal tubule of the kidneys (8). The role of hormones involved in growth and development, such as growth hormone (GH) and insulin-like growth factor–1 (IGF-1) is not known. Renal sulfate reabsorption has been

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reported to be increased in a patient with acromegaly and gigantism (17) and in dogs following GH administration (18), suggesting a role in sulfate regulation. The GH-stimulated increase in sulfate renal reabsorption may be mediated by IGF-1 because the changes in renal plasma flow and glomerular filtration rate (GFR) after GH administration parallel the increase in plasma concentrations of IGF-1 (19). Additionally, sulfate uptake by cartilage of *Xenopus laevis* tadpoles is significantly stimulated by IGF-1 and IGF-2 but not by GH, suggesting that the cartilage growth-promoting activity of GH is mediated by insulin-like growth factors (20).

Serum sulfate concentrations are also elevated in pregnant women (mean serum concentration of 0.43 m*M* during the third trimester vs 0.32 m*M* in adult controls) (4–6), and the renal reabsorption of sulfate is increased in pregnant women (21) and animals (9). 17β-Estradiol (EST) and progesterone (PG) serum concentrations increase with increasing gestational period during pregnancy (22), but their influence on sulfate renal reabsorption has not been examined. Serum sulfate concentrations and renal fractional reabsorption of sulfate are decreased in postmenopausal women, although altered sulfate homeostasis in menopause does not seem to be reversed by estrogen supplementation (23).

The objective of the present study was to investigate the effects of GH, IGF-1, PG, and/or EST on sodium/sulfate co-transport in Madin-Darby canine kidney (MDCK) cells that have been stably transfected with NaSi-1 cDNA (24) and in opossum kidney (OK) cells that exhibit endogenous sodium/sulfate co-transport.

Materials and Methods

Materials. Dexamethasone, 17β-estradiol, and progesterone were obtained from Sigma Chemical Co. (St. Louis, MO). Mouse anti-rabbit IgG conjugated with horseradish peroxidase was purchased from Sigma Immunochemicals (St. Louis, MO). Recombinant human insulin-like growth factor–1 (IGF-1) and human growth hormone were kindly donated from Genentech, Inc. (San Francisco, CA). $^{35}SO_4^{2-}$ (as Na_2SO_4, 1050–1600 Ci/mmol) was obtained from New England Nuclear Research Products (DuPont Company, Boston, MA). Biodegradable counting scintillant was supplied from Amersham Co. (Arlington Heights, IL). Commassie blue dye reagent concentrate and bovine plasma γ-globulin protein standard were purchased from Bio-Rad (Richmond, CA). Dulbecco's modified Eagle's medium, fetal bovine serum, TRIzol reagent, and trypsin were obtained from Gibco BRL (Buffalo, NY). RNase inhibitor (RNasin) and SuperScript were supplied from Promega (Madison, WI). *Ul*Tma polymerase was obtained from Perkin Elmer (Branchburg, NJ). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was supplied from Molecular Probes (Eugene, OR).

Cell Culture Conditions. MDCK/NaSi-1 cells (MDCK cells transfected with rat NaSi-1 cDNA under the control of a dexamethasone-inducible promoter (24)) were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum, 1% nonessential amino acids, 22 m*M* $NaHCO_3$, 2 m*M* L-glutamine, 50 IU/ml penicillin, and 50 μg/ml streptomycin in a humidified atmosphere of 5% CO_2/95% air at 37°C. MDCK/NaSi-1 cells were induced by incubating with 1 μ*M* dexamethasone for 16 hr (24) before hormone treatment and were used up to 15 passages for the studies. OK cells (American Type Cell Culture, Rockville MD) were maintained in DMEM F-12 medium containing 2 m*M* L-glutamine, 10% fetal bovine serum, and 50 IU/ml penicillin and 50 μg/ml streptomycin in a humidified atmosphere of 5% CO_2/95% air at 37°C (25).

Hormone Treatment of MDCK/NaSi-1 and OK Cells. MDCK/NaSi-1 cells were preincubated with GH (dissolved in water) at concentrations of 10^{-8}–10^{-12} *M* and IGF-1 (diluted in 0.9% NaCl solution) at concentrations of 10^{-6}–10^{-9} *M* that represents the range of physiological to pharmacological concentrations of these hormones (17, 18, 20). PG and/or EST (dissolved in ethanol) at concentrations of 10^{-6}–10^{-10} *M*, the range of physiological concentrations during the menstrual cycle (estrous cycle) and late pregnancy in humans and guinea pigs (22, 26, 27), were added to cells in culture. MDCK/NaSi-1 cells were preincubated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST at various times to examine the time course of the hormonal effect on sulfate uptake. Similar concentrations and incubation times were used for the OK cell studies. All hormones were preincubated with the cells in serum-free medium. The effect of the hormones on sodium-dependent and sodium-independent sulfate uptake was determined in MDCK/NaSi-1 cells in the presence and absence of dexamethasone (which is used to induce NaSi-1 expression).

Sulfate Uptake Studies. Inorganic sulfate uptake was examined in MDCK/NaSi-1 and OK cells grown to confluency on culture dishes (35 mm), as previously described (24, 25). Uptake studies were done at room temperature in a buffered solution (137 m*M* NaCl, 5.3 m*M* KCl, 2.8 m*M* $CaCl_2$, 1.2 m*M* $MgCl_2$, 10 m*M* HEPES/Tris, pH 7.4) containing 0.5 m*M* K_2SO_4 and tracer amounts of radiolabeled sulfate (2 μCi/ml). For studies done in the absence of sodium, NaCl was replaced by an equimolar amount of N-methyl-D-glucamine/HCl. At the end of the incubation period, the uptake buffer was removed by suction, and the cells were washed rapidly three times with ice-cold stop solution consisting of 137 m*M* NaCl and 10 m*M* Tris/HCl (pH 7.4). Cells were then lyzed with 1% Triton X-100 (for MDCK/NaSi-1 cells) or 0.5% Triton X-100 (for OK cells) for 1 hr. Radioactivity and protein concentrations were determined by liquid scintillation counting and the Coomassie blue binding method (28), respectively.

Cell RNA Preparation. Total RNA was extracted from MDCK/NaSi-1 cells using TRIzol reagent according to the manufacturer's protocol. Final RNA concentrations in

samples were determined by measuring the optical density at 260 nm.

The primers were derived from the NaSi-1 cDNA identified by Markovich *et al.* (14) and were designed to produce a 700 base-pair DNA (native DNA), as described by Sagawa *et al.* (29). A deletion standard cDNA (600 base pairs) was prepared by depleting 100 base pairs of native DNA located in the middle of the sequence. The cRNA *in vitro* transcribed from the depletion cDNA was added as an external standard to the RT-PCR mixture, and coamplified with sample RNA to correct for amplification efficiency.

For the reverse transcriptase reaction, the reactant containing 10 ng total RNA extracted from MDCK/NaSi-1 cells, 600 fg deletion standard cRNA, 5 m*M* DTT, and 0.1 m*M* dNTP were mixed in buffer containing 50 m*M* Tris/HCl (pH 8.3), 75 m*M* KCl, and 3 m*M* $MgCl_2$, and were denatured at 75°C for 5 min before two units of RNase inhibitor, 10 units of SuperScript, and 0.5 μ*M* primers were added. The total reaction volume was 20 μl, and the reaction was carried out at 42°C for 45 min. After the reverse transcriptase reaction, additional reactants for PCR (10 m*M* Tris (pH 9.3), 0.4 μ*M* primers, 40 n*M* dNTP and 3 U/100 μl *UlTma* polymerase) were added to the same tubes. After first heating at 95°C for 1 min, 25 cycles were run as follows: 95°C for 1 min, 65°C for 1 min, and 72°C for 1 min. The final extension was at 72°C for 7 min, and samples were kept at 4°C.

Crude Cell Membrane Preparation for ELISA. Crude membrane fractions were prepared from MDCK/NaSi-1 cells to determine the protein expression levels in the cell using a modification of the procedure of Biber *et al.* (30). The cells were scraped in brush border membrane (BBM) buffer (300 m*M* mannitol, 20 m*M* HEPES/Tris, pH 7.4) and centrifuged at 31,000*g* for 15 min at 4°C. The pellet was resuspended in BBM buffer and homogenized with a polytron homogenizer (Type PTA-10S, setting 5, Kinematica, Switzerland) for 2 min at 4°C. The resulting homogenate was centrifuged at 130,000*g* for 30 min at 4°C. The final pellet was resuspended in 2.5% Triton X-100 in phosphate buffered saline solution (PBS) to gently extract proteins. Protein concentrations were determined by the method of Bradford (28). All samples were stored at –80°C until assayed.

Sandwich Type ELISA Procedure. The NaSi-1 polyclonal and monoclonal antibodies were raised against rabbits and mice, respectively (31). An ELISA was performed, as previously described by Sagawa *et al.* (31). Briefly, assay plates (polystyrene flatbottom microtiter plates, Nunc, Denmark) were coated with the NaSi-1 monoclonal antibody (10 μg/ml), then incubated with 5% Blotto/PBS overnight at 4°C to block nonspecific adsorption. Wells were washed and incubated with samples or sample buffer only (negative control) at 4°C overnight. The wells were incubated with NaSi-1 antiserum or preimmune serum (1:600 diluted in 0.3% BSA/PBS), then incubated with horseradish peroxidase conjugated mouse anti-rabbit IgG. After washing, freshly prepared substrate solution (0.5 mg/ml o-phenylenediamine dihydrochloride, 0.045% H_2O_2) was added. The reaction was stopped with 2 *M* sulfuric acid, and the optical density at 490 nm was measured using a Microkinetics Reader (Bio-Tek Instruments, Winooski, VT). The amounts of NaSi-1 protein in MDCK/NaSi-1 cells were calculated using a standard curve obtained by a serial dilution of the NaSi-1 standard protein (6.58–164 fmoles).

Measurement of Membrane Motional Order. The membrane fluidity of intact MDCK/NaSi-1 cells preincubated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST was determined by measuring the fluorescence polarization of DPH. MDCK/NaSi-1 cells were diluted with 2 ml of PBS (pH 7.4), and 5 μl of 1 mg/ml DPH in tetrahydrofuran were added to the cells. Fluorescence polarization measurements were conducted using an SLM Aminco (SLM Aminco, Urbana, IL) 8000 spectrofluorometer with film polarizers (FP110) at temperatures of 25°C and 37°C with excitation wavelength of 355 nm and the emission wavelength of 430 nm (32). The correction for light scattering was performed by control experiments performed without the added probe (33).

Statistical Analysis. All results are presented as the mean ± SD, unless otherwise indicated. The data were compared by one-way ANOVA followed by a Tukey's test among more than two groups and by an unpaired Student's *t* test between two groups. The differences were considered to be statistically significant when the *P*-value was less than 0.05.

Results

Characterization of Sulfate Uptake into MDCK/NaSi-1 Cells. Sulfate (0.5 m*M*) uptake into MDCK/NaSi-1 cells was determined at various time points. Sodium-dependent sulfate uptake into MDCK/NaSi-1 cells increased linearly with time, up to 20 min of incubation, and reached a plateau after 60 min, indicating equilibrium. Sodium/sulfate co-transport in the cells was saturable over a wide range of sulfate concentrations (0.1 m*M*–6 m*M*) (Fig. 1). The V_{max} and K_m for sodium-dependent sulfate uptake in MDCK/NaSi cells were 12.0 ± 0.9 nmol/mg protein/5 min and 464 ± 55.9 μ*M*, respectively (n = 3). In all studies, sulfate uptake in MDCK/NaSi-1 cells was significantly increased in the presence of sodium compared with that in the absence of sodium, suggesting the sodium dependence of the transport process. Preincubation of cells with 1 μ*M* dexamethasone produced a 27.5-fold increase in the sodium-dependent transport of sulfate (0.5 m*M*), from 0.19 ± 0.10 nmol/mg protein/5 min to 5.22 ± 0.54 nmol/mg protein/5 min, mean ± SD (n = 11–12).

Characterization of Sulfate Uptake in OK Cells. Sodium-dependent sulfate (0.5 m*M*) uptake into OK cells increased linearly with time, up to 20 min of incubation. OK cells exhibited a high degree of sodium-independent sulfate transport and a smaller degree of sodium-dependent sulfate uptake (mean values of 3.76 nmol/mg protein/5 min and

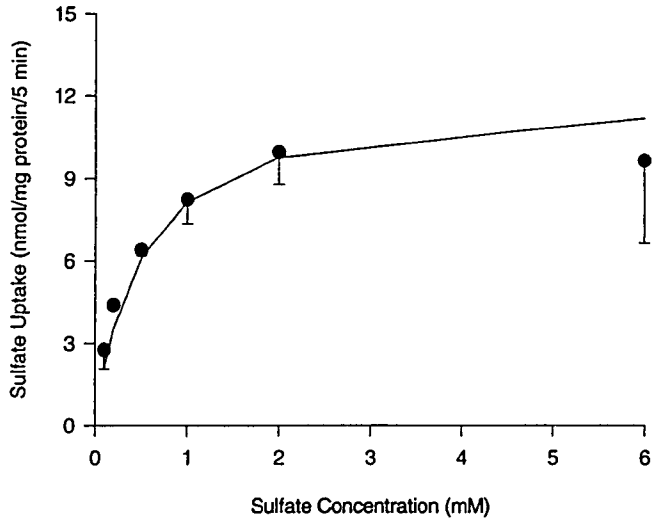

Figure 1. Concentration-dependent uptake of sulfate into MDCK/NaSi-1 cells. Sodium-dependent sulfate uptake was calculated as the difference between sulfate uptake rates at 5 min determined with and without sodium. The data were fitted to the Michaelis-Menten equation using nonlinear regression analysis. Each data point is the mean ± SD from three separate experiments, with triplicate determinations of uptake in each preparation.

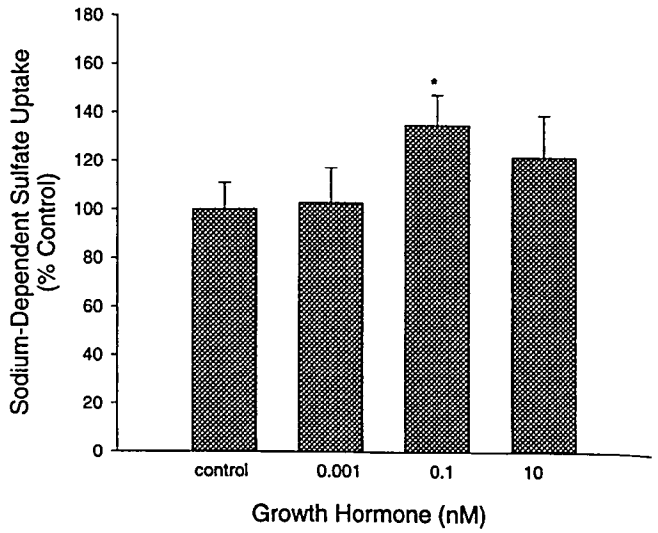

Figure 3. Concentration-dependent effect of GH on sodium-dependent sulfate uptake in MDCK/NaSi-1 cells. The data represent the difference between sulfate uptake rates at 5 min determined in the presence and absence of sodium. Cells were preincubated with varying concentrations of GH for 24 hr. The data are the mean ± SD from four separate preparations, with triplicate determinations of uptake in each preparation. $^*P < 0.05$ compared with control.

1.43 nmol/mg protein/5 min), compared with the MDCK/NaSi-1 cells (Fig. 2).

Effect of GH or IGF-1 on Sodium/Sulfate Transport into MDCK/NaSi-1 Cells. Maximal effects of GH on sodium/sulfate co-transport were observed after 24 hr whereas that for IGF-1 was observed at 5 hr (data not shown) in MDCK/NaSi-1 cells. These were the preincubation conditions used in subsequent studies. GH increased sodium/sulfate co-transport activity in MDCK/NaSi-1 cells, with a maximal response observed at 0.1 n*M* GH (Fig. 3). A concentration-dependent increase of sodium-dependent sulfate transport was seen upon preincubation with IGF-1 (Fig. 4). IGF-1, at a concentration of 1000 n*M*, produced the greatest stimulation of the sodium/sulfate co-transport process of the concentrations examined in this investigation, whereas a significant increase in the process was also seen at 100 n*M* IGF-1. GH and IGF-1 had no effect on sodium-independent sulfate transport (data not shown). Preincubation of MDCK/NaSi-1 cells with 1000 n*M* IGF-1 in the absence of dexamethasone did not alter the sodium-dependent transport of sulfate compared with control (untreated) values (Fig. 5). Preincubation with 0.1 n*M* GH (which produced the maximal response on sulfate uptake),

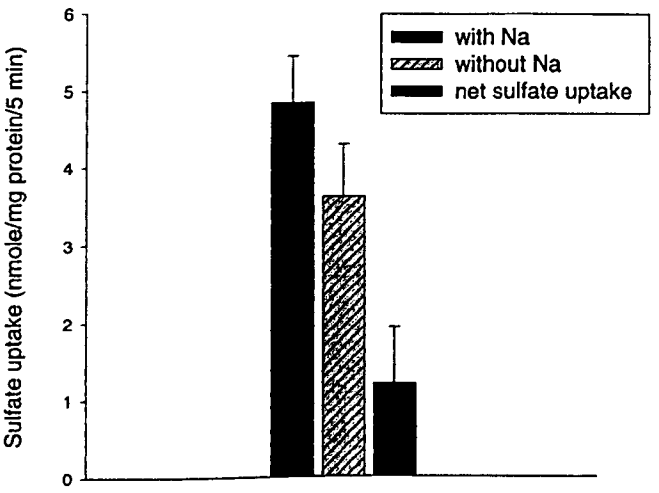

Figure 2. Sulfate uptake into OK cells. Sulfate uptake was determined in OK cells in the presence and absence of sodium (an equimolar amount of N-methyl-D-glucamine/HCl was substituted for NaCl) at a sulfate concentration of 0.5 m*M*. The data represent the mean ± SD, $n = 9$. Uptake in the presence of sodium was significantly greater ($P < 0.001$) than in the absence of sodium.

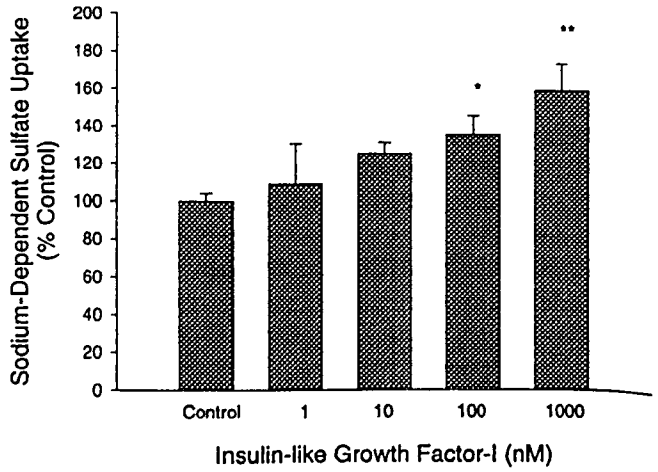

Figure 4. Concentration-dependent effect of IGF-1 on sodium-dependent sulfate uptake in MDCK/NaSi-1 cells. Sodium/sulfate co-transport was determined from the difference between uptake rates at 5 min measured in the presence and absence of sodium. Cells were preincubated with varying concentrations of IGF-1 for 5 hr. The data are the mean ± SD from three separate experiments in which triplicate determinations were obtained. $^*P < 0.05$, $^{**}P < 0.01$ compared with control.

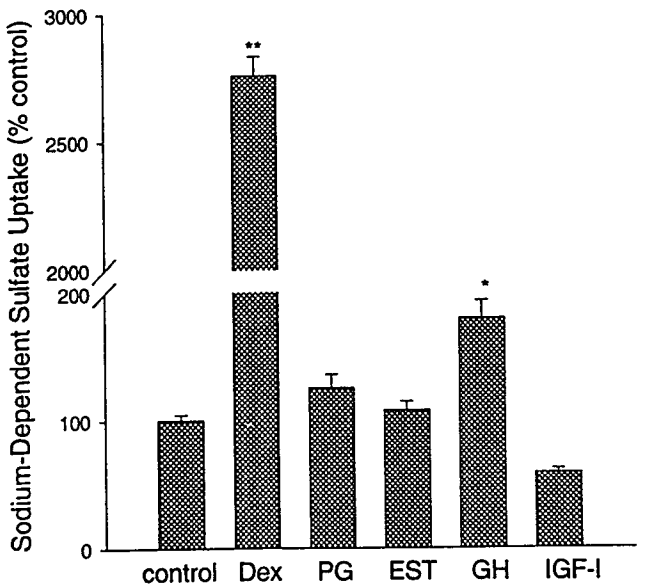

Figure 5. Effect of PG, EST, GH, and IGF-1 on sodium-dependent sulfate uptake in MDCK/NaSi cells in the absence of dexamethasone-induced expression of NaSi-1. MDCK/NaSi-1 cells were preincubated with PG (10 n*M*), EST (10 n*M*), or GH (0.1 n*M*) for 24 hr, with IGF-1 (1000 n*M*) for 5 hr or with dexamethasone (1 μ*M*) for 16 hr. These concentrations of hormones and times of preincubation have been shown to produce the maximal effects on sodium/sulfate co-transport. The data represent the mean ± SE of $n = 4$–12 determinations. $^*P < 0.05$, $^{**}P < 0.001$ compared with control.

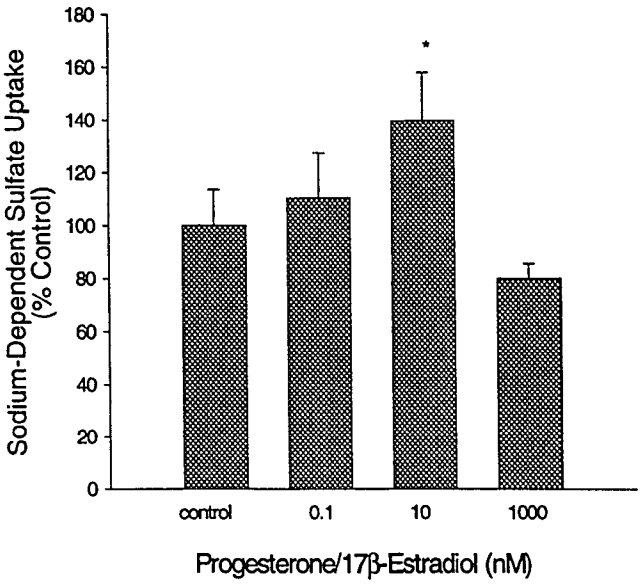

Figure 6. Concentration-dependent effect of PG/EST on sodium-dependent sulfate uptake in MDCK/NaSi-1 cells. Sodium-dependent sulfate uptake was calculated as the difference between sulfate uptake rates at 5 min determined with and without sodium. The data are the mean ± SD from four separate experiments, with triplicate determinations of sulfate uptake in each experiment. $^*P < 0.05$ compared with control.

in the absence of dexamethasone, increased the sodium-dependent transport of sulfate by 79.9% (Fig. 5); however, this is small compared with the 2660% increase seen after dexamethasone treatment.

Effects of PG and/or EST on Sodium/Sulfate Co-Transport in MDCK/NaSi-1 Cells. Cells were preincubated with PG and/or EST for 24 hr since maximal effects on sodium-dependent sulfate transport were observed after preincubation for 24 hr (data not shown). PG, EST, or PG/EST increased sulfate uptake in a concentration-dependent manner up to 10 n*M*; further increases in the female sex hormone concentrations decreased the sulfate uptake in MDCK/NaSi-1 cells (Fig. 6, Table I). Sodium/sulfate co-transport was significantly enhanced when cells were preincubated with 10 n*M* PG, 10 n*M* EST, or 10 n*M* PG/10 n*M* EST up to 41%, 36%, or 40%, respectively. PG and EST had no effect on sodium-independent sulfate transport (data not shown). Preincubation of MDCK/NaSi-1 cells with 10 n*M* PG or EST in the absence of dexamethasone did not alter the sodium-dependent transport of sulfate compared with control (untreated) values (Fig. 5).

Hormonal Effects in OK Cells. The time course for hormonal effects was studied in OK cells and found to be similar to those observed in MDCK/NaSi-1 cells; therefore, the same incubation times were used. Various concentrations of hormones were examined, and those producing the maximal effects are shown in Figure 7. IGF-1, GH, and PG all significantly increased the sodium-dependent uptake of sulfate in OK cells. EST had no significant effect on sulfate uptake at concentrations of 0.1, 10, and 1000 n*M*. However, PG plus EST at concentrations of 10^{-1} n*M* each significantly increased the uptake of sulfate.

NaSi-1 mRNA and NaSi-1 Protein Levels. The NaSi-1 mRNA level was significantly increased when MDCK/NaSi-1 cells were preincubated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, 10 n*M* EST, or 10 n*M* PG/10 n*M* EST compared with control (untreated) cells (Fig. 8). The NaSi-1 protein abundance was also significantly higher in the cells treated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST than in the control cells (Fig. 9).

Membrane Fluidity. The membrane fluidity of MDCK/NaSi-1 cells preincubated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST was significantly decreased compared with control (untreated) cells at 25°C and 37°C (Table II).

Discussion

Sulfate homeostasis is maintained predominantly by the renal reabsorption of sulfate in the kidney proximal tubules. The administration of low or high sulfate diets alters the V_{max} for sodium/sulfate co-transport at rat renal BBM (29, 34). This is associated with corresponding changes in NaSi-1 mRNA and protein expression (29). Triiodothyronine (T_3) significantly stimulates sodium-dependent sulfate transport activity in renal BBM (35) whereas glucocorticoid treatment decreases the V_{max} for this process (36). Experimentally induced hypothyroidism in rats results in a decreased V_{max} for sodium/sulfate co-transport and a decrease in NaSi-1 mRNA and protein (37). Vitamin D-deficient rats exhibit decreased plasma sulfate levels, decreased sodium/sulfate co-transport activity in re-

Table I. Concentration-Dependent Effects of Progesterone and/or 17 β-Estradiol on Sodium-Dependent Sulfate Uptake into MDCK/NaSi-1 Cells

	Sulfate Uptake (% control)		
Concentration	Progesterone	17 β-Estradiol	Progesterone/ 17 β-estradiol
0.1 n*M*	105.0 ± 11.9	103.8 ± 8.9	110.3 ± 17.0
10 n*m*	141.2 ± 14.7[a]	146.4 ± 19.5[a]	139.2 ± 18.1[a]
1000 n*M*	129.4 ± 17.9	126.1 ± 4.9	79.6 ± 5.8

Note. Values are presented as a percentage of control and expressed as mean ± SD of three to four separate experiments in which triplicate determinations were obtained. Sodium-dependent sulfate uptake was calculated as the difference between sulfate uptake rates at 5 min determined in the presence and absence of sodium.
[a] $P < 0.05$ compared with control.

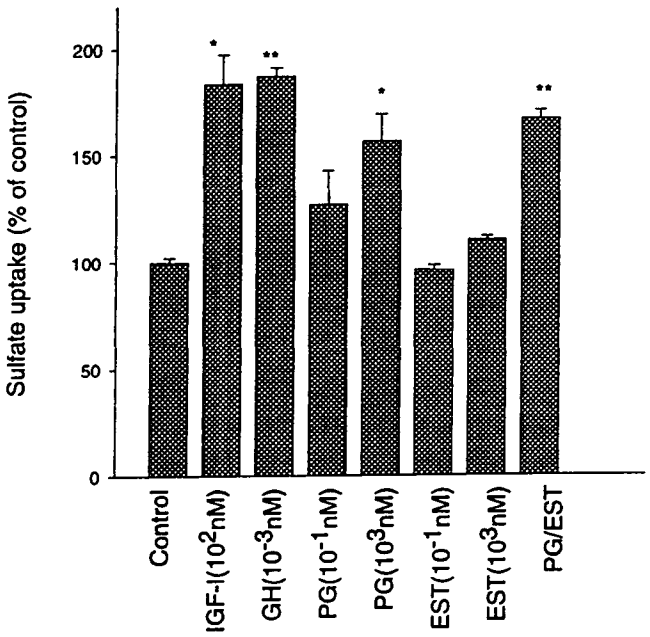

Figure 7. Effect of GH, IGF-1, PG, and EST on sodium-dependent sulfate uptake in OK cells. OK cells were preincubated with GH (10^{-3} n*M*), PG (10^{-1} and 10^{3} n*M*), EST (10^{-1} and 10^{3} n*M*) and PG/EST (10^{-1} n*M* of each) for 24 hr or IGF-1 (10^{2} n*M*) for 5 hr. The data represents the mean ± SE for the sodium-dependent sulfate uptake determined at 5 min in two to four separate experiments, with triplicate determinations of sulfate uptake in each experiment. $^{*}P < 0.05$, $^{**}P < 0.001$ compared with control.

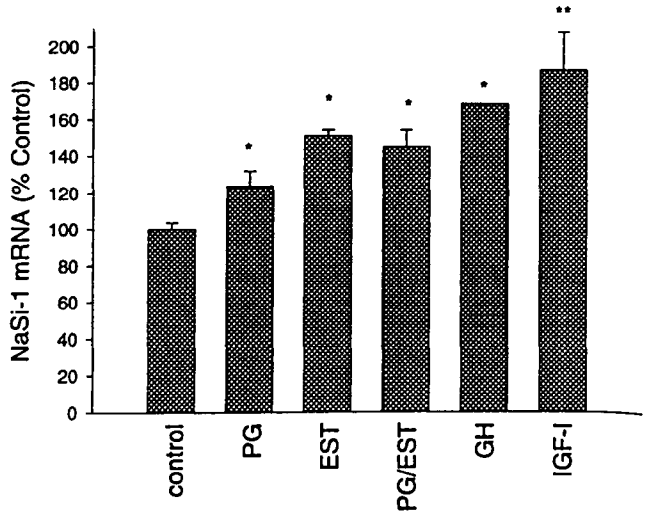

Figure 8. NaSi-1 mRNA levels in MDCK/NaSi-1 cells preincubated with GH, IGF-1, PG, and/or EST. The data are the mean ± SD of duplicate determinations of two RNA preparations of the cells. MDCK/NaSi-1 cells were preincubated with 0.1 n*M* GH, 10 n*M* PG, 10 n*M* EST, or 10 n*M* PG/10 n*M* EST for 24 hr or with 100 n*M* IGF-1 for 5 hr. The mRNA values were compared as RT-PCR products that were expressed as the volume ratio of co-amplified NaSi-1 DNA and deletion DNA and normalized by the amount of total RNA. $^{*}P < 0.001$, $^{**}P < 0.0005$ compared with control.

nal BBM as well as decreased levels of renal NaSi-1 mRNA and protein compared with control rats (38). These studies suggest that sulfate homeostasis is regulated by alterations in the steady-state NaSi-1 mRNA expression in the renal proximal tubules.

The use of cultured renal epithelial cells is widely accepted as a powerful tool to investigate the regulatory mechanisms for the functional alterations of transport systems such as sodium/phosphate co-transport and sodium/glucose co-transport (30). Regulation of sulfate homeostasis is mediated by altered sodium/sulfate co-transport at the BBM. The present study investigated the effects of hormones, those involved in growth, development and pregnancy, on sodium-dependent sulfate transport in OK cells and on sodium-dependent transport and NaSi-1 mRNA and protein expression in MDCK/NaSi-1 cells. MDCK cells show negligible or very little sodium-dependent sulfate or phosphate transport (24). GH, IGF-1, EST, or PG may be responsible for the increased sulfate reabsorption in young and pregnant mammals.

In MDCK/NaSi-1 cells, sulfate uptake was significantly increased in the presence of sodium compared with that in the absence of sodium, indicating the sodium dependence of the transport process, and that the dexamethasone-inducible sodium/sulfate co-transport activity was predominantly expressed at the apical membrane (BBM) of the cells (24). Sodium-dependent sulfate uptake was saturable with a V_{max} of 12.0 ± 0.9 nmol/mg protein/5 min and K_m of 464 ± 55.9 μ*M*. The K_m value for sodium/sulfate co-transport in MDCK/NaSi-1 cells was similar to the values for this transport process in BBM vesicles isolated from the kidney cortex of rats (39) and guinea pigs (8).

GH and IGF-1 increased sodium/sulfate co-transport activity in OK cells, which exhibited endogenous sodium/sulfate co-transport activity, and in the transfected renal cell

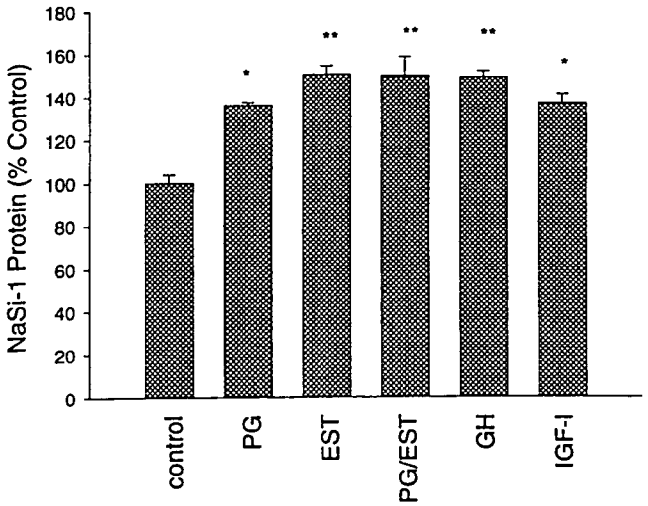

Figure 9. NaSi-1 protein levels in MDCK/NaSi-1 cells preincubated with GH, IGF-1, PG, and/or EST. The data are the mean ± SD of four measurements from each of two crude membrane preparations of the cells. MDCK/NaSi-1 cells were preincubated with 0.1 n*M* GH, 10 n*M* PG, 10 n*M* EST, or 10 n*M* PG/10 n*M* EST for 24 hr or with 100 n*M* IGF-1 for 5 hr. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with control.

Table II. Fluorescence Polarization of DPH in MDCK/NaSi-1 Cells Preincubated with GH, PG, and/or EST as a Function of Temperature[a]

	25°C	37°C
Control	0.324 ± 0.011	0.239 ± 0.007
GH	0.360 ± 0.009[a]	0.300 ± 0.005[a]
IGF-I	0.355 ± 0.007[a]	0.289 ± 0.009[a]
PG	0.347 ± 0.009[b]	0.307 ± 0.011[a]
EST	0.350 ± 0.002[a]	0.305 ± 0.009[a]
PG/EST	0.360 ± 0.009[a]	0.294 ± 0.006[a]

Note. Values are presented as mean ± SD from four to five measurements. The fluorescence polarization of DPH, which is inversely related to membrane fluidity, was determined as a function of temperature in MDCK/NaSi-1 cells preincubated with 0.1 n*M* GH, 100 n*M* IGF-I, 10 n*M* PG, and/or 10 n*M* EST as well as in control (untreated) cells. All the hormones significantly increased the fluorescence polarization of DPH in intact MDCK/NaSi-1 cells at both temperatures, compared with the value in control cells. The data were compared by ANOVA followed by a Tukey's test among six groups at the same temperature.

[a] $P < 0.001$ compared with control value.

[b] $P < 0.01$ compared with control value.

line, MDCK/NaSi-1. The maximal effect of GH in MDCK/NaSi-1 cells occurred at a concentration of 0.1 n*M* whereas IGF-1 stimulated sodium-dependent sulfate uptake into the cells in a concentration-dependent manner. The mRNA level of NaSi-1 was significantly enhanced by 69% and 87% in the MDCK/NaSi-1 cells preincubated with GH and IGF-1, respectively. The NaSi-1 protein level was also increased by 49% and 36% in GH-treated and IGF-1–treated cells, respectively, which corresponds to the increase in sodium/sulfate co-transport activity observed in the cells treated with these hormones. In the absence of dexamethasone pretreatment of MDCK/NaSi-I cells, IGF-1 had no effect on sodium/sulfate uptake, indicating that it does not affect the expression of NaSi-1 from the dexamethasone-inducible promoter. GH had a modest but significant effect on NaSi-1 expression (3.0% of that produced by dexamethasone); however, GH increased sodium-dependent sulfate transport by a mean value of 0.15 nmol/mg protein/5 min in the absence of dexamethasone compared with an increase of 1.78 nmol/mg protein/5 min in the presence of dexamethasone. Therefore, the effect of GH on sodium/sulfate uptake cannot be explained by considering only its effect on the dexamethasone-inducible promoter. The effect of IGF-1 on sodium/sulfate co-transport is similar to that of IGF-1 on sodium/phosphate co-transport. IGF-1 stimulates sodium-dependent phosphate transport in a dose-dependent manner in opossum kidney (OK) cells *via* a mechanism involving *de novo* protein synthesis (40).

PG and EST increased sodium-dependent sulfate uptake in MDCK/NaSi-1 cells, but only PG or the combination of PG and EST increased sulfate uptake in OK cells. These hormones stimulated sulfate uptake in a concentration-dependent fashion up to 10 n*M* in MDCK/NaSi-1 cells; further increases in the hormone concentrations reduced the sodium/sulfate co-transport activity. These observations are consistent with the findings of Beck *et al.* (41) that PG increased the sulfate uptake in a dose-dependent manner up to 10 n*M* in EST-primed endometrial epithelial cells, and at higher PG concentrations there was a decrease in the uptake of sulfate. An additive or synergistic effect of PG and EST on sodium/sulfate co-transport was not observed in MDCK/NaSi-I cells, which was consistent with the findings of Beck *et al.* (41) in guinea pig endometrial epithelial cells. The cells preincubated with PG, EST, or PG/EST exhibited NaSi-1 mRNA levels that were increased by 23%, 51%, and 45%, respectively, and NaSi-1 protein levels that were enhanced by 36%, 50%, and 49%, respectively. Preincubation of cells with PG or EST, in the absence of dexamethasone, had no effect on sodium/sulfate uptake, suggesting that these hormones do not affect the dexamethasone-inducible promoter. The present investigation provides the first evidence of gonadal hormonal regulation of sodium/sulfate co-transport in the kidney.

It has been reported that estrogen decreases the fluidity of rabbit ileal basolateral membrane (42) and of rat sinusoidal liver plasma membrane (43). In addition, progesterone reduces the motional order of the *Rana* oocyte plasma membrane (44) and of hamster spermatozoal plasma membrane (45). In this study, a significant decrease in membrane fluidity was observed in the MDCK/NaSi-1 cells preincubated with 0.1 n*M* GH, 100 n*M* IGF-1, 10 n*M* PG, and/or 10 n*M* EST compared with control (untreated) cells at both 25°C and 37°C. A decreased membrane fluidity of MDCK/NaSi-1 cells preincubated with cholesterol reduced sodium/sulfate co-transport activity (46). Therefore, the decrease in membrane fluidity of the cells treated with these hormones would be expected to produce a reduction of sodium-dependent sulfate transport. However, it appears that the increased NaSi-1 protein levels in the cells produced by these hormones has a greater influence on sodium/sulfate

co-transport and negates the opposing effect of decreased cell membrane fluidity.

In conclusion, GH, IGF-1, and PG/EST increased the activity of sodium/sulfate co-transport in MDCK/NaSi-1 and OK cells cultured *in vitro*. These observations suggest that GH and IGF-1 may play important roles in the regulation of renal sulfate reabsorption during growth and development. In addition, the stimulation of renal sulfate reabsorption during pregnancy could be mediated by PG and possibly EST. The increased steady-state levels of NaSi-1 mRNA and NaSi-1 protein produced by hormone treatment may represent the mechanism responsible for the enhanced sodium-dependent sulfate transport in MDCK/NaSi-1 cells preincubated with GH, IGF-1, PG, and/or EST.

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