Effect of experimentally induced hypothyroidism on sulfate renal transport in rats

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INORGANIC SULFATE IS a physiological anion that is utilized in the sulfation of many endogenous and exogenous compounds. Xenobiotics, such as steroids, anti-inflammatory drugs, and adrenergic blockers and stimulants, undergo biotransformation by sulfate conjugation (30). Sulfate conjugation is essential for the biological activity of many endogenous compounds, such as heparin, heparan sulfate, dermatan sulfate, gastrin, and cholecystokinin (31, 33). Inorganic sulfate is also necessary for the biosynthesis of numerous structural components of membranes and tissues, such as sulfated glycosaminoglycans or cerebroside sulfate (17).

Inorganic sulfate can be absorbed from the diet or formed from the oxidation of the sulfur-containing amino acids, cysteine and methionine. It is eliminated from the body mainly in unchanged form by urinary excretion (48). Homeostasis of inorganic sulfate occurs predominantly by renal mechanisms. Inorganic sulfate enters into the proximal tubule cell across the brush-border membrane (BBM) by sodium-dependent sulfate cotransport. This transport system is distinct from sodium-dependent amino acid, phosphate, or glucose cotransport (24, 47). The cDNA for the sodium-dependent sulfate transporter (NaSi-1), which contains 2,239 bp and encodes a protein of 595 amino acids, has been identified and cloned from rat kidney cortex (26).

Sulfate exits from the cell across the basolateral membrane (BLM) through sulfate/anion exchange transport for which hydroxyl ions, bicarbonate and oxalate can serve as counter ions (22, 36).

Thyroid hormone (triiodothyronine, T3) plays an important role in maturation of kidney growth and morphology (18) and can influence several transport processes in the kidney including that of sodium, phosphate, and adenosine (12, 27, 50). Serum sulfate concentrations are observed in hypothyroid patients (43). However, the effect of T3 on sulfate renal transport is controversial. Tenenhouse et al. (44) demonstrated that sulfate uptake by sodium-dependent sulfate cotransport in renal BLM was significantly higher in T3-treated mice. However, Beers and Dousa (3) reported no effect of T3 treatment on sodium-sulfate cotransport in BLM isolated from both rats and mice. T3 also did not affect sodium-dependent sulfate uptake in opossum kidney cells (45). Thyroid hormone treatment may also alter sulfate/anion exchange in the kidney, possibly in a different manner. Chou et al. (8) reported that thyroid hormone treatment results in a decreased Vmax for lysosomal sulfate/anion exchange in the rat liver.

The objectives of this study were 1) to examine serum concentrations, renal clearance, and the fractional reabsorption of sulfate in a rat model of 6-propyl-2-thiouracil (PTU)-induced hypothyroidism; 2) to determine the renal transport of sulfate (both sodium-sulfate cotransport in BLM and sulfate/anion exchange in BLM) in membrane vesicle preparations isolated from the kidneys of hypothyroid rats; and 3) to determine the mechanism(s) involved in the hypothyroid-induced alterations in renal sulfate transport (NaSi-1).

MATERIALS AND METHODS

Study design. Female Lewis rats weighing 200–220 g were used. Hypothyroid rats were produced by the administration of 0.05% PTU in the drinking water for 21 days (32). Control animals were given tap water during the treatment period. Food and water were provided to the rats ad libitum.

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Urine was collected for 12 h on the day before beginning the treatment (day 0) and on day 21. A blood sample was obtained at the midpoint of the urine collection period from the tail artery on days 0 and 21. Animals were weighed daily. Animals were killed by CO2 inhalation on day 21, and the kidneys were removed. Kidney cortex was trimmed and used for a membrane vesicle preparation or immediately frozen in liquid nitrogen for mRNA and crude membrane preparations.

Sulfate uptake rates from individual preparations were fitted to the Michaelis-Menten equation using the PCNONLIN nonlinear estimation program (Statistical Consultants, Lexington, KY) to obtain estimates of $K_m$ and $V_{max}$. Uptake values were determined in triplicate uptake studies from one vesicle preparation, and studies were repeated five to six times.

Tissue RNA preparation. Total RNA was prepared from rat kidney cortex by the guanidium isothiocyanate method (7). The tissue from animals in the same group was combined and ground under liquid nitrogen before the initial homogenization step. Total RNA was prepared in duplicate from one tissue pool from each study group and used for the RT-PCR. Final mRNA concentrations in the samples were determined by optical density at 260 nm.

RT-PCR. Primers, which were designed to produce a 700-bp DNA (native DNA), were prepared from the NaSi-1 cDNA (26). The 5' primer was constructed with a BamHI enzyme digestion site and a portion of the NaSi-1 cDNA corresponding to positions 492–513, CTGAGATCCACAGTGCTGAAGCAGAGC. The 3' primer was constructed with a PstI enzyme digestion site and a portion of the cDNA corresponding to positions 1172–1192, TGCTGTAGCCAACTAAGGGCAACAGGTAAG. A deletion standard cDNA (600 bp) was prepared by deleting a 100 bp of native cDNA located in the middle of the sequence. The cDNA transcribed in vitro from the deletion cDNA was added as an external standard to the RT-PCR mixture, and amplified with sample RNA in the same reaction tube to correct for amplification efficiency. Fifty nanograms of tissue RNA and 10 fg deletion standard cDNA were reverse transcribed in the same tubes using SuperScript (Promega) at 42°C for 45 min. After the reverse transcriptase reaction, additional reagents for PCR including UMP polymerase (Perkin-Elmer) were directly 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. Final extension was at 72°C for 7 min, and samples were kept at 4°C.

Southern hybridization. The RT-PCR products were size separated on 1.5% agarose gel and transferred to hybridization matrices (Duralon-UV, Stratagene). The RT-PCR products were loaded on the gel in duplicate. The hybridization probe was a 301-bp NaSi-1 cDNA fragment (492–792 bp). The random primer labeling reaction was prepared using a random primer labeling kit (Prime-It; Stratagene, La Jolla, CA). Matrices were prehybridized for a minimum of 4 h and hybridized overnight in hybridizing solution (5× SSC, 1% SDS, 5× Denhardt's, 50% formamide, and 100 µg/ml sheared salmon sperm DNA) at 42°C. Matrices were washed five times in 2× SSC plus 0.1% SDS at room temperature, twice in 0.1× SSC plus 0.1% SDS at room temperature followed by 0× SSC with 0.1% SDS at 65°C until the radioactivity was decreased to the background levels. Hybridization signals were visualized and analyzed using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The RT-PCR results were expressed as a ratio between amplified NaSi-1 mRNA and amplified deletion standard cRNA, added as an external standard, normalized by the amount of total RNA.

Crude membrane preparation for ELISA. Crude membrane fractions were prepared from kidney cortex to determine the protein expression levels in the tissue. Approximately 0.25 g of ground tissue powder was homogenized in the homogenizing buffer (250 mM sucrose, 10 mM triethanolamine-HCl, pH 7.6) and centrifuged at 1,250 g for 10 min at 4°C. The supernatant was further centrifuged at 100,000 g for 30 min at 4°C (46). The pellet containing crude membrane fractions was resuspended in 2.5% Triton X-100 in 1× PBS (sample...
buffer) to gently extract proteins. Protein concentrations were measured by the method of Lowry et al. (23).

Sandwich-type ELISA procedure. NaSi-1 polyclonal and monoclonal antibodies were raised against rabbits and mice, respectively, as previously described (40). The antigen used for antibody production consisted of a recombinant protein containing 119 amino acids, which corresponded to amino acids 159-277 of the NaSi-1 protein. Western analysis showed that both the NaSi-1 polyclonal and monoclonal antibodies detected a 69-kDa protein in BBM. The size and the location of our immunoblot suggested that our antibodies recognized the NaSi-1 transporter. The NaSi-1 protein was detected by sandwich-type ELISA (39). The assay plates (polyethylene flat-bottom microtiter plates; Maxisorp, Nunc) were coated with the NaSi-1 monodonal antibody (10 µg/ml), then incubated with 5% BSA/PBS overnight at 4°C to block nonspecific absorption. Wells were then incubated with samples (500 µg/well) at 4°C overnight. After incubation with NaSi-1 rabbit antiserum or preimmune serum (1:600 diluted in 0.3% BSA/PBS), wells were then incubated with horseradish peroxidase-conjugated mouse anti-rabbit IgG. Ten minutes after freshly prepared substrate solution (0.5 mg/ml o-phenylenediamine dihydrochloride, 0.045% H2O2) was added, the reaction was stopped with 2 M sulfuric acid, and the OD at 490 nm was measured using a Microkinetics Reader (Bio-Tek Instruments, Winooski, VT). The amounts of NaSi-1 in the tissue were calculated using a constructed standard curve using serial dilution of the NaSi-1 standard protein (6.58 to 164 fmol).

Evaluation of membrane motional order (fluidity). The motional order of BBM and BLM obtained from PTU-treated or control rat kidney cortex was determined by examining the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) as previously described (1, 41). To incorporate the probe, 2 µl of 2 mM DPH in tetrahydrofuran was added to the membrane vesicles and incubated at 37°C for 1 h. Fluorescence polarization measurements were done on a spectrofluorometer (model 8000; SLM Aminco, Urbana, IL) with film polarizers (FP110). Samples were excited at 355 nm, and the emission was monitored at 430 nm with 4-nm excitation and emission slits. The lipid order parameter (S) was calculated from the steady-state polarization value by the equation \( S^2 = \frac{(4r_0^3 - 0.1)r_o}{r} \), where \( r_0 \) is the maximal fluorescence anisotropy value in the absence of any rotational motion (taken as 0.40) and \( r \) is the steady-state anisotropy (35).

Data analysis. Renal sulfate and creatinine clearances were calculated as the urinary excretion rate divided by the midpoint serum concentration. The sulfate filtration rate was determined from the product of the serum sulfate concentration and glomerular filtration rate (GFR; estimated from creatinine clearance), since the serum protein binding of sulfate is negligible. The amount of sulfate reabsorbed was calculated as the amount of sulfate excreted in urine subtracted from the total amount filtered. The fraction of the filtered sulfate that was reabsorbed was calculated by 1 - (renal sulfate clearance/GFR).

Statistics analysis. All results are expressed as means ± SD. A paired t-test was used to compare the values before and after the treatment in each group, and an unpaired t-test was used to compare the values between the groups.

RESULTS

In vivo studies. Animals in both groups gained 25–50 g after 21 days of the treatment, and there were no differences in the weight gain in the two treatment groups. There were no significant differences in all parameters measured on day 0 between groups. Also there were no significant differences in the control group in all parameters (T3 values, creatinine clearance, and sulfate disposition) evaluated before and after the treatment. After 21 days of the treatment, animals that received PTU in their drinking water exhibited significantly lower serum T3 levels compared with the values in control animals (Fig. 1). The creatinine clearance was significantly lower in hypothyroid rats after 21 days of treatment compared with the values on day 0 (2.9 ± 0.6 vs. 4.2 ± 1.5 ml·min⁻¹·kg⁻¹; P < 0.01, n = 18). The mean values of serum sulfate concentration, urinary excretion rate, renal sulfate clearance, and renal reabsorption of sulfate on days 0 and 21 in each study group are shown in Table 1. In the hypothyroid rats, serum sulfate concentrations were significantly decreased on day 21 compared with the values for day 0 (P < 0.01, n = 26). There were no significant differences in urinary excretion of sulfate and renal sulfate clearance after PTU treatment. The fractional clearance of sulfate (sulfate clearance/creatinine clearance) was significantly increased, and the fraction of sulfate reabsorbed was significantly decreased in the hypothyroid group on day 21 compared with the values obtained on day 0 (P < 0.001, n = 15).

Sulfate transport studies. The marker enzyme enrichment ratios (vesicles/homogenate) were similar to values reported previously (10). Enrichment ratios for alkaline phosphatase were 13.1 ± 6.5 (n = 6) and 14.1 ± 8.0 (n = 6) for control and hypothyroid, respectively. Enrichment ratios for Na⁺-K⁺-ATPase were 11.4 ± 5.2 (n = 6) and 12.4 ± 3.2 (n = 6) for control and hypothyroid, respectively. The time course for sulfate uptake was examined in BBM and BLM vesicles. Sulfate uptake exhibited a characteristic overshoot.

![Fig. 1](http://ajprenal.physiology.org/)

Fig. 1. Serum triiodothyronine (T3) concentrations before and after 21 days of 6-propyl-2-thiouracil (PTU) treatment. Data are means ± SD of 16–17 animals in each group. ***P < 0.0001 compared with day 0.
Table 1. Effect of hypothyroidism on sulfate serum concentration, urinary excretion, renal clearance and renal reabsorption

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Hypothyroid</th>
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<tr>
<td>Serum concentrations, mM</td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>day 0</td>
<td>0.60 ± 0.14</td>
<td>0.65 ± 0.15</td>
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<tr>
<td>day 21</td>
<td>0.62 ± 0.12</td>
<td>0.53 ± 0.15*</td>
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<tr>
<td>Urinary excretion, µmol·kg⁻¹·h⁻¹</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
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<tr>
<td>day 0</td>
<td>740.1 ± 290.0</td>
<td>658.2 ± 237.3</td>
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<tr>
<td>day 21</td>
<td>762.0 ± 307.2</td>
<td>696.6 ± 328.0</td>
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<tr>
<td>Sulfate clearance, ml·min⁻¹·kg⁻¹</td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>day 0</td>
<td>0.90 ± 0.37</td>
<td>0.77 ± 0.45</td>
</tr>
<tr>
<td>day 21</td>
<td>0.79 ± 0.35</td>
<td>0.87 ± 0.40</td>
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<tr>
<td>Sulfate clearance/creatinine clearance</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 0</td>
<td>0.27 ± 0.05</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>day 21</td>
<td>0.22 ± 0.08</td>
<td>0.30 ± 0.02†</td>
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<tr>
<td>Fraction reabsorbed, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>day 0</td>
<td>75 ± 21</td>
<td>87 ± 5.0</td>
</tr>
<tr>
<td>day 21</td>
<td>78 ± 10</td>
<td>70 ± 16†</td>
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Values are means ± SD. *P < 0.01 and †P < 0.001 compared with day 0.

during the first minute of incubation in both BBM and BLM. Sulfate uptake in BBM by sodium-dependent sulfate transport was decreased at the 10-s time point in the hypothyroid group compared with that in the control group (0.43 ± 0.088 vs. 0.24 ± 0.087 nmol/mg protein; P < 0.01). There were no differences in the equilibrium uptake rates (evaluated at 60 min) in either membrane preparation, suggesting that there were no changes in sulfate binding or vesicle volume.

The BBM vesicles were incubated with or without sodium in the uptake medium to determine kinetic parameters for sulfate transport in BBM. Sulfate uptake into BBM vesicles increased linearly in the absence of sodium. The difference between the two represents the sulfate uptake by sodium-dependent sulfate cotransport. $K_m$ and $V_{max}$ values were estimated by fitting the data using the Michaelis-Menten equation. A representative fit of the sodium-dependent sulfate uptake process using nonlinear regression analysis is shown in Fig. 2. The $V_{max}$ value in the hypothyroid group was significantly lower compared with the control group (0.90 ± 0.31 and 0.49 ± 0.08 nmol·mg⁻¹·10 s⁻¹ for control and hypothyroid, respectively; P < 0.05, n = 5–6). The $K_m$ for sodium differences in BBM was not significantly different between groups (0.44 ± 0.10 and 0.47 ± 0.19 mM for control and hypothyroid, respectively; n = 5–6). The BLM vesicles were incubated in the absence and presence of the competitive inhibitor, thiocysteine. The difference between the two represents sulfate uptake by the bicarbonate-driven sulfate/anion exchange transporter in BLM. There were no significant differences in $K_m$ (0.48 ± 0.21 vs. 0.30 ± 0.10 mM for control vs. hypothyroid, respectively, n = 5–6) and $V_{max}$ (0.52 ± 0.17 vs. 0.37 ± 0.09 nmol·mg⁻¹·10 s⁻¹ for control vs. hypothyroid, respectively; n = 5–6) for the sulfate/anion exchange transport in BLM (Table 2).

Table 2. Summary of kinetic parameters for sulfate uptake in BBM and BLM vesicles

<table>
<thead>
<tr>
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<th>Control</th>
<th>Hypothyroid</th>
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<tr>
<td>BBM uptake $V_{max}$, nmol·mg⁻¹·10 s⁻¹</td>
<td>0.90 ± 0.31</td>
<td>0.49 ± 0.08*</td>
</tr>
<tr>
<td>$K_m$, mM</td>
<td>0.44 ± 0.10</td>
<td>0.47 ± 0.19</td>
</tr>
<tr>
<td>BLM uptake $V_{max}$, nmol·mg⁻¹·10 s⁻¹</td>
<td>0.52 ± 0.17</td>
<td>0.37 ± 0.09</td>
</tr>
<tr>
<td>$K_m$, mM</td>
<td>0.48 ± 0.21</td>
<td>0.30 ± 0.10</td>
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</table>

Values are means ± SD of 5–6 preparations. BBM, brush-border membrane; BLM, basolateral membrane. *P < 0.05 compared with control.
the purified NaSi-1 fusion protein. A linear relationship between the amounts of NaSi-1 standard protein vs. OD 490 was obtained ($r^2 = 0.99$). A crude membrane fraction was isolated from the rat kidney cortex obtained from animals after 21 days of the treatment. ELISA was performed in triplicate from duplicate crude membrane preparations from each study group. The NaSi-1 protein abundance was significantly lower in the kidney cortex obtained from the hypothyroid animals than in the control group (decrease of 35.7%, $P < 0.05$, n = 6; Fig. 4).

Membrane motional order (fluidity). The fluorescence polarization studies with DPH demonstrated that BLM and BBM differ from one another, in that the motional order of BBM is less than that of BLM. This is consistent with previous reports that examined the fluidity of these membranes (1, 5). However, the PTU treatment did not produce any observable changes in membrane fluidity or the lipid order parameter for either BBM or BLM (Fig. 5).

## DISCUSSION

Thyroid hormone significantly influences some sodium-dependent transporters in the kidney, while having no effect on others. Sodium-phosphate cotransport in BBM isolated from rat and mouse kidneys is increased following $T_3$ or $T_4$ treatment due to an increased $V_{\text{max}}$; no change in the $K_m$ for sodium-dependent phosphate transport occurs (13, 44, 49). This is consistent with the observation that hyperthyroid patients exhibit increased serum phosphate concentrations, whereas hypothyroid patients have lower serum phosphate levels (25). Sodium-dependent adenosine transport is decreased in BBM isolated from the kidney cortex of hypothyroid rats (27). However, unlike sodium-phosphate transport, this is due to a decreased affinity ($K_m$) for the transport, with no reported change in the $V_{\text{max}}$. $T_3$ treatment can increase the activity of the Na$^+/H^+$ antiporter in rat kidney BBM (19), but it does not alter sodium-proline, sodium/$\alpha$-glucose, or sodium-citrate cotransport processes (19, 49).

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**Fig. 3.** A: Southern hybridization signals of RT-PCR products from hypothyroid and control groups. Top bands (700 bp) are reverse transcribed and amplified sodium-dependent sulfate transporter (NaSi-1) RNA in the tissue. Bottom bands (600 bp) are reverse transcribed and amplified deletion cRNA added as an external standard. Signals are duplicate lanes from 1 preparation, and 2 RNA samples were prepared from 1 tissue pool. B: comparison of NaSi-1 mRNA levels. mRNA levels were compared as RT-PCR products which were expressed as the volume ratio of coamplified NaSi-1 DNA and deletion DNA. Values are means ± SD (n = 4). **$P < 0.01$.

**Fig. 4.** Comparison of NaSi-1 protein levels in crude membrane fractions isolated from the kidney cortex of hypothyroid and control rats. Data are means ± SD determined in triplicate from 1 sample. *$P < 0.05$ compared with control.

**Fig. 5.** Membrane motion order (fluidity) in kidney BBM and basolateral membrane (BLM) vesicles isolated from hypothyroid and control rats. Standard deviation for each point ranged from 0.003 to 0.008. There was no difference in membrane fluidity between groups.
Early evidence of the hormonal regulation of sulfate by thyroid status was provided by Tallgren (43). He reported that clinical hyperthyroidism is associated with elevated serum sulfate concentrations and that hypothyroidism is associated with decreased serum sulfate concentrations. Tenenhouse et al. (44) demonstrated that sodium-dependent sulfate uptake in renal BBM is significantly increased in mice treated with a pharmacological dose of T3 compared with vehicletreated controls due to an increased Vmax for sodium-sulfate cotransport. However, the effect of thyroid hormone on sodium-sulfate cotransport is controversial; Beers and Dousa (3) found no change in sodium-sulfate transport in renal BBM isolated from T3-treated rats or mice. Additionally, T3 does not stimulate sodium-dependent sulfate transport in opossum kidney cells (45).

The current investigation examined the effects of hypothyroidism on sulfate homeostasis and sulfate renal transport in the rat. Serum sulfate concentrations were significantly decreased in hypothyroid rats compared with those in control rats. This result is consistent with the lower serum sulfate concentrations reported for hypothyroid patients (43). GFR, estimated by creatinine clearance, was significantly decreased in hypothyroid rats compared with their pretreatment values, suggesting that amount of filtered sulfate was decreased in these animals. However, we observed no significant alterations in the urinary excretion or renal clearance of sulfate. Consequently, renal sulfate fractional reabsorption was significantly decreased in hypothyroid rats, supporting the findings of our in vitro study in membrane vesicles that showed a decreased Vmax for sodium-dependent sulfate uptake into BBM vesicles isolated from the hypothyroid rats. The lack of change in the renal clearance of sulfate in hypothyroid animals may be due to the changes in GFR observed in the present study, and which also occur in hypothyroid patients (18, 28). Decreased GFR results in elevated serum sulfate concentration, i.e., there is an inverse relationship between GFR and serum sulfate (2). One would expect decreased urinary excretion and renal clearance values in the presence of decreased GFR. However, it appears that the two opposing effects of decreased filtration and decreased reabsorption result in no apparent change in the renal clearance and urinary excretion of sulfate, although the renal clearance ratio (sulfate clearance/creatinine clearance) was significantly increased. It is also possible that the lack of change in the sulfate renal clearance in hypothyroid animals is partially due to reduced sulfate reabsorption in the papillary collecting duct. There is evidence indicating that sodium-sulfate cotransport-related mRNA and protein are expressed in collecting ducts (9).

We further investigated the mechanism of hypothyroidism-induced alterations on sodium-sulfate cotransport (NaSi-1). First, we investigated changes in Na+–K+–ATPase activity. Thyroid hormone regulates the activity of Na+–K+–ATPase in the kidneys possibly by direct upregulation of Na+–K+–ATPase in proximal tubule cells by a pretranslation mechanism (21). However, we found no significant difference in Na+–K+–ATPase activity between hypothyroid and control rats.

Second, we examined the membrane motional order (fluidity) in renal BBM and BLM, by examining the fluorescence polarization of DPH. Membrane fluidity affects the activity and kinetics of membrane-bound transport proteins and passive permeability properties (20). We have found that alterations in the membrane fluidity of Madin-Darby canine kidney (MDCK) cells due to preincubation with cholesterol or benzyl alcohol can alter sodium sulfate transport; the Vmax for sodium-sulfate cotransport increases with increased membrane fluidity (unpublished data). Thyroid hormones can influence the lipid composition of a number of cellular organelles in different tissues, resulting in changes in membrane fluidity. Thyroidectomy produces a decrease in the total cholesterol and phospholipid content of rat liver mitochondria (34). Hypothyroidism also alters the membrane fatty acid composition in rat brain mitochondria (42). However, the effect of hypothyroidism on membrane fluidity on kidney cortex BBM and BLM had not been previously examined. In this investigation, we found no significant differences in membrane fluidity in renal BBM and BLM isolated from the kidney of hypothyroid and control rats.

Third, we examined NaSi-1 mRNA and protein levels in kidney cortex of hypothyroid and control rats. NaSi-1 mRNA and protein levels were significantly lower in hypothyroid rats. It is possible that total tissue RNA and protein synthesis in the kidneys were lower in hypothyroid rats. Hayase et al. (16) reported that total tissue RNA and protein synthesis were lower in brain, kidney, and liver in hypothyroid rats. However, NaSi-1 mRNA and protein levels were significantly lower when normalized with total RNA and protein in tissue, suggesting that the NaSi-1 gene is regulated by thyroid hormone. These results indicate that the molecular mechanisms of the hypothyroidism-induced decrease of renal sulfate transport involves, at least in part, down-regulation of NaSi-1 protein. NaSi-1 mRNA and protein levels are also significantly decreased in vitamin D deficiency (14) and following ibuprofen treatment (37) and increased following a low-sulfate diet (38).

In summary, significantly decreased serum sulfate concentrations, the renal fractional reabsorption of sulfate, and BBM sodium-sulfate cotransport were observed in experimentally induced hypothyroid rats. NaSi-1 mRNA and protein synthesis levels were significantly lower in these rats, suggesting that thyroid hormone is involved in renal cellular regulation of NaSi-1 by a mechanism not yet elucidated in detail.

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