Renal expression of sodium transporters and aquaporin-2 in hypothyroid rats

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Submitted 11 October 2002; accepted in final form 28 January 2003

HYPOTHYROIDISM IS ASSOCIATED with significant abnormalities of renal function in humans and in experimental animals. The impairment of renal function typically manifests as a reduction in glomerular filtration rate (GFR), reduced diluting and concentrating ability, and exaggerated natriuresis in response to volume alterations caused by saline and water loading or other (for a review, see Refs. 7, 20, 25). The latter deficiency has been so marked that rats receiving antithyroid drugs together with a sodium-deficient diet were rapidly dying from negative sodium balance (16). The observed sodium loss was attributed to a diminished reabsorptive ability of the proximal (31) and the distal renal tubule (15, 21) as revealed by micropuncture studies. One of the mechanisms resulting in the reabsorptive defect was suggested to be the dependency of tubular sodium reabsorption on the activity of Na-K-ATPase, which is known to be reduced in hypothyroid rats (22).

However, it has been demonstrated that the reduced activity of Na-K-ATPase alone cannot account for the transport changes observed in hypothyroidism (10, 11). Although Na-K-ATPase actively maintains ionic gradients, it is the passive apical entry through various transport proteins that normally represents the rate-limiting step in tubular sodium reabsorption. Thus a potential mechanism accounting for the insufficient reabsorption of the hypothyroid kidney may be the decrease in the abundance or activity of the apical sodium transporters that normally represents the rate-limiting step in tubular sodium reabsorption. The influence of thyroid hormone on two major sodium transporters of the proximal tubule, the type 3 Na/H exchanger (NHE3) and the type 2 Na-phosphate cotransporter (NaPi2), has recently been investigated and it has been shown that both proteins are directly regulated by thyroid hormone (1, 9, 41).

On the other hand, no information is available on the expression of the major distal tubular sodium transporters, i.e., the bumetanide-sensitive type 2 Na-K-2Cl cotransporter (NKCC2) of the thick ascending limb of the loop of Henle (TAL), the thiazide-sensitive NaCl cotransporter (NCC) of the distal convoluted tubule (DCT), and the amiloride-sensitive epithelial Na channel (ENaC) of the late DCT, connecting tubule (CNT), and the cortical and medullary collecting tubule (CNT).

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duct (CCD, MCD), in altered thyroid status. We studied the hypothesis of whether renal expression of these proteins would be altered in rats pharmacologically rendered hypothyroid to elucidate their possible implication in impaired sodium handling. To this end, abundance of these proteins was analyzed by semiquantitative immunoblotting and immunohistochemistry. Further key sodium transporters such as the proximal tubular NHE3 and NaPi2 as well as the α1-subunit of Na-K-ATPase were also analyzed. To address water transport in the late distal tubule and collecting duct, we extended our observations to the expression of the water channel aquaporin-2 (AQP2), which mediates water permeability across the luminal membrane of these segments (27, 34) and may therefore be critical in another prominent feature of hypothyroidism, i.e., the impairment in water reabsorption (38).

MATERIALS AND METHODS

Animal model. Sixteen male Sprague-Dawley rats weighing 150 g (Schönwalde, Berlin, Germany) were divided randomly into two groups. Both groups were fed the same standard diet and had free access to drinking water. Hypothyroidism was induced by adding 0.05% methimazole to the water in one group. Body weights were measured weekly. After 7 wk of treatment, urine was collected for 20 h by placing the rats into individual metabolic cages. Thereafter, rats (n = 6) were in vivo perfusion-fixed for immunohistochemistry. For biochemical analysis of serum and kidneys, rats (n = 10) were killed by an overdose of pentobarbital sodium; blood was collected by puncture of the abdominal aorta, and both kidneys were quickly removed, individually sodium; blood was collected by puncture of the abdominal aorta, and both kidneys were quickly removed, individually

Antibodies. We used previously well-characterized rabbit polyclonal antibodies to the following proteins: NaPi2 [α-ENaC, commercially available polyclonal rabbit antibodies were used (Alpha Diagnostic International, San Antonio, TX). Mouse monoclonal antibody against NHE3 was purchased from Chemicon International (Temecula, CA), and monoclonal antibody against the α1-subunit of Na-K-ATPase was from Upstate Biotechnology (Lake Placid, NY). Polyclonal antibodies against NKCC2 were raised in guinea pigs against a rat NH2-terminal NKCC2-maltose-binding fragment protein (MBP) (DAKO) followed by rabbit anti-MBP fusion protein (MBP), which was produced using a DNA fragment encoding the 85 NH2-terminal amino acids of NKCC2. The fragment was cloned infrane into pMAL (New England BioLabs, Beverly, MA). For immunolabeling of NKCC2 and AQP2, detection by a 1-h incubation with Cy3-conjugated goat anti-rabbit IgG (DAKO) followed by rabbit anti-horse antibody was a gift from J. Biber, Department of Physiology, San Antonio, TX). Mouse monoclonal antibody against NHE3 and NaPi2 [anti-

Electrophoresis and immunoblotting of membrane proteins. After Laemmli’s sample buffer was added, the proteins were solubilized at 65°C for 10 min. SDS gel electrophoresis was performed on 6–10% polyacrylamide gels. After electrophoretic transfer of the proteins to polyvinylidene fluoride membranes, protein in loading and blotting was verified by membrane staining using 0.1% Ponceau red. Membranes were probed overnight at 4°C or at room temperature for 2 h and then exposed to horseradish peroxidase-conju- gated secondary antibodies (DAKO, Glostrup, Denmark) for 45 min at room temperature. Immunoreactive bands were detected on the basis of chemiluminescence, using an enhanced chemiluminescence kit (Amersham Pharmacia, Freiburg, Germany) before exposure to X-ray films (Hyperfilm, Amersham). For densitometric evaluation of the resulting bands, films were scanned and analyzed using BIO-PROFIL Bio-1D image software (Vilber Lourmat, Marue La Vallée, France).

Tissue preparation for immunohistochemistry. The animals were killed by in vivo perfusion fixation under pentobarbital sodium anesthesia. The kidneys were perfused retrograde through the abdominal aorta using PBS adjusted to 330 mosmol/kgH2O with sucrose, pH 7.4, for 20 s. Next, 3% paraformaldehyde in PBS was infused for 5 min, followed by the first solution for an additional minute. The kidneys were removed and cut into slices. Slices were processed for embedding in Epon and paraffin, or they were shock-frozen in liquid nitrogen-cooled isopentane for subsequent cryostat sectioning.

Immunohistochemistry. Immunohistochemical staining was performed on cryostat sections or on sections from Epon- and paraffin-embedded tissue. For labeling of NCC, 0.5-μm Epon sections were pretreated with 5-min steps of Na methanolate, methanol-toluol, and acetone before blocking with 5% skimmed milk in PBS. For detection of α1-, β-, γ-ENaC, and α1-Na-K-ATPase, 4-μm cryostat sections were cut and milk-blocked. After the sections were incubated with the respective primary antibody at 4°C overnight, bound antibody was detected by a 1-h incubation with Cy3-conjugated goat anti-rabbit or goat anti-mouse antibody (DIANova, Hamburg, Germany). For immunolabeling of NKCC2 and AQP2, de-waxing and rehydration of 4-μm paraffin sections were followed by 10 min in 0.5% H2O2 in methanol to block endogenous peroxidase. Improved antigen retrieval was achieved by heating the sections in 0.01 mM Na citrate using a microwave oven at maximal power for 15 min. After being blocked with 5% skimmed milk in PBS, sections were incubated overnight at 4°C with primary antibodies. Antibody detection was carried out using horseradish peroxidase-conjugated rabbit anti-guinea pig antibodies (DAKO) for NKCC2 or swine anti-rabbit IgG (DAKO) followed by horseradish peroxidase IgG (DAKO) for AQP2. Generation of signal was realized with 0.1% diaminobenzidine and 0.02% H2O2 in PBS for standardized duration. Sections were counterstained with hematoxylin and analyzed using a Leica DMRB microscope.
Blood and urine analysis. Thyroid hormones were measured by a chemiluminescent immunoassay (Centaur analyzer, Bayer, Germany) in plasma samples collected as described above. Electrolytes in serum and urine were determined by indirect ion-selective electrode analysis (Modular Analytics, Roche Diagnostics), and urinary osmolality was measured by an osmometer (Gonotec, Berlin, Germany). Blood Urea Nitrogen (BUN) and creatinine concentrations were determined enzymatically and by the kinetic Jaffé method (Modular Analytics, Roche Diagnostics). Creatinine clearance and fractional sodium excretion were calculated using standard equations. Measurement of urinary vasopressin in 20-h urine collections was kindly performed by S. Diederich, Free University Berlin, using a radioimmunoassay as previously described (12).

Presentation of data and statistical analysis. Quantitative data are presented as means ± SE. For statistical comparison, the unpaired t-test (when variances were the same) or the Mann-Whitney rank sum test (when variances differed significantly between groups) was employed. P values of less than 0.05 were considered statistically significant.

RESULTS

Clinical parameters and functional data. As shown in Table 1, treatment with methimazole resulted in dramatically decreased serum levels of free T3 and thyroxine (T4) and was associated with a significant lack of weight gain (mean body wt 179 ± 6 vs. control 358 ± 8 g). In addition to the lower body weight, kidneys showed a disproportionately reduced weight, resulting in a lowered kidney-to-body weight ratio (0.0046 ± 0.02 vs. control 0.055 ± 0.02). Analysis of renal function revealed a significantly increased 20-h urinary output (15.7 ± 0.9 vs. control 9.4 ± 1 ml), which was paralleled by a marked reduction in urinary osmolality (534 ± 43 vs. control 1,805 ± 308 mosmol/kg H2O). The creatinine clearance was significantly reduced (0.7 ± 0.09 vs. control 1.64 ± 0.5 ml/min−1·g kidney wt−1), whereas the fractional Na excretion (FE Na) was markedly increased (0.69 ± 0.3 vs. control 0.2 ± 0.12%). There were no significant changes in serum Na (143.4 ± 0.7 vs. control 142.2 ± 2.1 mmol/l), but there was a significant elevation in serum levels of creatinine and urea nitrogen (0.61 ± 0.05 vs. control 0.32 ± 0.05 mg/dl and 95.2 ± 7.9 vs. control 46.8 ± 8.2 mg/dl). A possible role of an altered vasopressin status was studied by measuring the hormone in 20-h urine collections, since urinary vasopressin concentrations are known to indicate changes in plasma vasopressin concentrations (12). Urinary concentrations of vasopressin were not different between hypothyroid and controls (70.1 ± 23 vs. 72.0 ± 12 pg/ml). Related to the highly divergent urinary volumes between groups, however, a significantly higher vasopressin excretion was found in the hypothyroid rats (1,084 ± 203 vs. 678 ± 119 pg/20 h).

Histology and abundance/distribution of tubular transporters and channels. General histology revealed a marked reduction in size of the glomeruli, and tubules were also significantly smaller in profile in hypothyroid animals than in controls. Apart from these changes, there were no further obvious alterations in renal structure.

NHE3 and NaPi2. Antibodies against NHE3 and NaPi2, applied in immunoblots from whole kidney membrane preparations, recognized bands at ~85 and ~80–85 kDa, respectively, corresponding to published data (8, 9). Densitometric analysis of blots showed dramatic decreases in the expression of NHE3 and NaPi2 in the hypothyroid group compared with controls (12 ± 1.4 vs. 100 ± 7.3 and 14.4 ± 4.2 vs. 100 ± 8.4%, respectively; Fig. 1).

NKCC2. Antibody to NKCC2 labeled a broad band of ~160–165 kDa in the blots, which agreed with the expected size of 165 kDa (8). Densitometric analysis showed a marked increase in NKCC2 expression in the hypothyroid animals (196 ± 18.7 vs. 100 ± 12%; Fig. 1). Immunohistochemical staining showed a marked increase in NKCC2 labeling in medullary TAL of hypothyroid kidneys (Fig. 2, A and B) and unchanged signal intensity in the cortical TAL (not shown).

NCC. Antibody to NCC recognized broad bands at ~160–170 kDa in immunoblots, as previously reported (4). Densitometric evaluation revealed no difference in band density (108 ± 1.1 vs. control 100 ± 6%; Fig. 1). Immunohistochemical analysis of NCC distribution did not reveal any changes between groups (Fig. 3, A and B); signal in both groups was restricted to the apical membrane of DCT cells, confirming previously published data (4, 39).

α-, β-, and γ-ENaC. Antibodies directed to each of the ENaC subunits recognized bands in the 80- to 90-kDa range, as previously published (18). Densitometry of the blots did not show differences between groups (α-ENaC: 111.6 ± 6 vs. control 100 ± 7.4%; β-ENaC: 102.3 ± 9.7 vs. control 100 ± 12.8%; γ-ENaC: 103 ± 7.1 vs. control 100 ± 13.3%; Fig. 1). Figure 3 shows immunofluorescence labeling of α-ENaC in controls and methimazole-treated rats (Fig. 3, C-F). Strong labeling of α-ENaC was found in the apical

| Table 1. Clinical parameters and functional data on renal function |
|-------------------|-------------------|-------------------|
|                   | Methimazole       | Control           |
| Free T3, ng/l     | 0.8 ± 0.05 in 3,  |
|                   | and < in 2 rats   | 2.98 ± 0.08       |
| Free T4, ng/dl    | 1.81 ± 0.08       |                   |
| Body wt, g        | 179 ± 6†          | 358 ± 8           |
| Kidney wt, g      | 0.41 ± 0.02†      | 1.02 ± 0.02       |
| Kidney wt/body wt | 0.0046 ± 0.0002†  | 0.055 ± 0.0003    |
| PNa, mmol/l       | 143.4 ± 0.7       | 142.2 ± 2.1       |
| PCr, mg/dl        | 0.61 ± 0.05†      | 0.32 ± 0.05       |
| PUre, mg/dl       | 95.2 ± 7.9*       | 46.8 ± 8.2        |
| Urate, mg/20 h    | 15.7 ± 0.9†       | 9.4 ± 0.4         |
| CCr, ml/min       | 0.58 ± 0.09†      | 3.33 ± 1.2        |
| Ccr/g kg kidney   | 0.7 ± 0.09†       | 1.64 ± 0.5        |
| FE Na, %          | 0.69 ± 0.3*       | 0.2 ± 0.12        |
| Uosmol, mosmol/kgH2O | 534 ± 43†    | 1,805 ± 203       |
| Urinary vasopressin, pg/ml | 70.1 ± 23 | 72.0 ± 12 |
| Vasopressin excretion, pg/20 h | 1,084 ± 203 | 678 ± 119 |

Values are means ± SE; T3, triiodothyronine; T4, thyroxine; Ccr, creatinine clearance; FE Na, fractional Na excretion; Uosmol, urine osmolality. *P < 0.05; †P < 0.005. ‡Below detection limit; n = 5/group.
membrane of principal cells in the CCD (Fig. 3, C and D), whereas staining in the MCD was distributed more evenly throughout the cytoplasm (Fig. 3, E and F), which agrees with previous studies in healthy rats (18, 39). No differences in intensity or cellular distribution of α-ENaC were detected between groups. Anti-β- and γ-ENaC-immunoreactive signals were weaker, but the distribution corresponded to published data (18, 39). Differences between groups, however, were not established either (results not shown).

**AQP2.** Polyclonal antibody against AQP2 recognized a narrow ~27-kDa band and a broader 35- to 40-kDa band in the blots, which agreed with published data (28). Densitometric analysis of the blots revealed a sharp increase in AQP2 in the hypothyroid group (250.3 ± 27.8 vs. 100 ± 15.7%; Fig. 1). Immunohistochemistry as well showed enhanced AQP2 signals in CCD and MCD principal cells of the hypothyroid group (Fig. 2, C and D). The association of AQP2 immunostaining with the apical plasma membrane apparently was more pronounced in the hypothyroid kidneys, whereas in controls, the signal showed a more cytosolic distribution. In both groups, weaker AQP2 staining was also recorded in DCT and CNT. However, no changes in signal abundance were observed between groups in these segments.

**α1-Na-K-ATPase.** Antibody directed against α1-Na-K-ATPase recognized an expected band at ~96 kDa (8). Densitometric analysis of the respective immunoblots showed no significant differences between hypothyroid and control animals (98 ± 6.1 vs. 100 ± 2.8%; Fig. 1). In the histochemical analysis, no differences were

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Fig. 1. Abundance of type 3 Na/H exchanger (NHE3), type 2 Na-Pi cotransporter (NaPi2), bumetanide-sensitive type 2 Na-K-2Cl co-transporter (NKCC2), thiazide-sensitive Na-Cl cotransporter (NCC), α-, β-, and γ- subunits of the epithelial Na channel (ENaC), aquaporin-2 (AQP2), and α1-subunit of Na-K-ATPase by semiquantitative immunoblotting. Each lane was loaded with an equal amount of total protein extracted from control or methimazole-treated rat kidneys. **+P < 0.0001, + P < 0.005.

Fig. 2. Abundance of NKCC2 (A, B) and AQP2 (C, D) by immunohistochemical analysis. Immunoperoxidase signal for NKCC2 is markedly stronger in the apical part of the medullary thick ascending limb epithelium (*) in hypothyroid rats (B) compared with controls (A). Immunoperoxidase signal for AQP2 is markedly stronger in the apical part of the collecting duct epithelium in hypothyroid rats (CD in D) compared with controls (CD in C) and is more concentrated at the luminal cell border; paraffin sections were counterstained with hematoxylin. Note the smaller size of tubules in the treated group. Magnification ×1,000.
found between groups regarding signal intensity and cellular or zonal distribution of α1-Na-K-ATPase (results not shown).

Calculation of total kidney protein abundance and normalization by creatinine clearance. As equal amounts of total protein were loaded in each lane for immunoblots, but less total protein was harvested from kidneys of methimazole-treated rats, lanes of treated rats contained greater total kidney fractions of any given protein. In addition to the raw densitometric values, we therefore calculated the absolute protein abundance for each transporter by forming the product of protein content of the kidney homogenates and the densitometric intensity levels. The results showed a dramatic reduction in absolute abundance of NHE3 and NaPi2, decreased absolute abundance of NCC, α-, β-, γ-ENaC, and α1-Na-K-ATPase, and unchanged absolute abundance of NKCC2 and AQP2, respectively (Table 2). As these values largely reflect changes in kidney size, it appears essential to relate them to a more functional parameter linked to the tubular transport process. When protein abundance was thus normalized by creatinine clearance, NHE3 and NaPi2 were decreased, whereas the remaining transporters were increased (Table 2). The increase in abundance was particularly pronounced for NKCC2 and AQP2, which was confirmed on the cellular level by immunohistochemistry (Fig. 2).

Methimazole-treated, T3-substituted rats. In the T3-substituted group, serum-free T3 concentrations were significantly higher than in the group solely receiving methimazole (1.87 ± 0.015 vs. 0.8 ± 0.05 ng/l) but lower than in the euthyroid control group (2.98 ± 0.08 ng/l). T3 substitution normalized body weight gain (280 ± 5.7 g after 4 wk vs. control 276 ± 4 g), kidney-to-body weight ratio (0.0059 ± 0.0002), creatinine clearance (1.4 ± 0.1 ml·min⁻¹·g kidney wt⁻¹), and FENa (0.23 ± 0.02%). Densitometric analysis of immunoblots revealed normalization of abundance of NHE3 (81.5 ± 6.2 vs. control 100 ± 4.1%) and NaPi2 (139.5 ± 4 vs. control 100 ± 3.1%) and largely normalized NaPi2 (56 ± 2 vs. control 100 ± 8%) and AQP2 (158.9 ± 6.2 vs. control 100 ± 5.1%).

DISCUSSION

In this study, we showed that rats with methimazole-induced hypothyroidism showed a dramatic deficit in body and kidney weight gain, reduced creatinine clearance, and a marked increase in urinary output
and NaPi2 in hypothyroid kidneys is in agreement with previous data (1, 9). Decreased NHE3 and NaPi2 expression has furthermore been found in rats with chronic renal failure (29), and since we observed increased BUN and serum creatinine levels, this may have influenced the transporters accordingly. Reduced GFR and luminal flow, which are both likely to attenuate luminal Na/H exchange, may have caused a reduced NHE3 expression as well (36). However, lowered proximal tubular transport has also been observed in hypothyroidism when GFR was still normal (17), which agrees with T3 acting as a direct stimulator of NHE3 and NaPi2 transcription rate (9, 41).

Despite the low GFR, the distal tubular NKCC2 was unchanged in hypothyroidism and increased when normalized for GFR and kidney weight or analyzed by histochemistry, respectively. Impaired proximal tubular reabsorption with a resulting increase in distal sodium delivery may, in fact, be causative for this difference (14). Parallel stimulation of NKCC2 and AQP2 is probably not related to T3 deficiency but rather to the enhanced secretion of vasopressin. This hormone may determine the expression of various sodium transporters including NKCC2 in medullary TAL besides regulating water channel abundance in the collecting duct (13). Effects of vasopressin on salt reabsorption in medullary TAL have been established (for review, see Ref. 37). Effects of hypothyroidism on vasopressin release have been investigated in patients and animal models with inconsistent results (23, 24, 26, 40). However, only plasma vasopressin levels were determined, which critically may be subject to short-term variations induced by stress and experimental conditions (23). To avoid this, we therefore measured vasopressin excretion since the hormone is relatively stable in the urine (2), and we found its excretion substantially increased.

Vasopressin may as well raise the expression of other distal tubular proteins such as the ENaC subunits and NCC (13, 33). In fact, these appeared to be increased when normalized for GFR, but since they were not changed in relation to kidney mass and histochemical signal intensity, we consider their definitive stimulation in the hypothyroid organism unlikely. Alternatively, a modified vasopressin responsiveness may be considered in hypothyroidism since a diminished V2 receptor-mediated release of cAMP has been reported in kidneys under this condition (19), and related findings were reported in hepatocytes (30). The efficiency of vasopressin may as well be reduced by a diminished concentration of medullary solutes in hypothyroidism, hereby aggravating urinary concentration deficit and polyuria (38).

Another potential cause for hypothyroid sodium losses may be a reduced activity of Na-K-ATPase and the resulting decline of ion gradient driving forces as and may in particular trigger adaptive steps related to impaired proximal tubular reabsorption. These considerations must be regarded with some reservation, however, as the creatinine clearance as an indicator of GFR is a rather rough marker in rats.

The demonstrated parallel reduction of NHE3 and NaPi2 in hypothyroid kidneys is in agreement with previous data (1, 9). Decreased NHE3 and NaPi2 expression has furthermore been found in rats with chronic renal failure (29), and since we observed increased BUN and serum creatinine levels, this may have influenced the transporters accordingly. Reduced GFR and luminal flow, which are both likely to attenuate luminal Na/H exchange, may have caused a reduced NHE3 expression as well (36). However, lowered proximal tubular transport has also been observed in hypothyroidism when GFR was still normal (17), which agrees with T3 acting as a direct stimulator of NHE3 and NaPi2 transcription rate (9, 41).

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reported in proximal tubules and collecting ducts (3). Decreased Na-K-ATPase activity has been attributed to a selective decrement of β-Na-K-ATPase abundance (22). In accordance with this, we found the abundance of the α1-subunit of Na-K-ATPase only lowered to the same extent as kidney size and unchanged in immunohistochemical staining. In addition to its direct stimulatory effect, T3 also enhances Na-K-ATPase activity by increasing the sensitivity of target cells to mineralocorticoids (3). Besides Na-K-ATPase, NCC and ENaC are also aldosterone-dependent products, and a permissive role of T3 on the action of mineralocorticoids must thus be considered. Because the products were not altered in our hands, however, one may only speculate that mineralocorticoid-dependent adaptation under additional challenges may be impaired by this deficit as observed elsewhere (35).

Finally, structural changes of the hypothroid kidney may contribute to the presumed deficit in late distal sodium reabsorption. We could confirm data showing that renal growth is proportionately more affected than total body growth (5, 6). Measuring the length of microdissected tubules from hypothroid rat kidneys had previously revealed that renal growth reduction mainly resulted from a decrement in length of the proximal and distal renal tubule, whereas glomerular growth was retarded only to the extent of total body growth (6). Assuming a distal filtrate delivery as high in hypothroid rats as in controls (32), it therefore seems likely that distal sodium handling may be related to disproportionately shortened distal convolutions.

Despite the kidney’s need for thyroid hormone to maintain sodium balance (42), we found no indication for a disturbed balance in this study consistent with previous studies (reviewed in Ref. 25). Additional manipulations such as a sodium-deficient diet, saline or water loading, or others, however, cause excessive natriuresis in the hypothroid organism (7, 16, 21, 42).

In summary, we demonstrate that hypothyroidism in rats is associated with an altered abundance of renal sodium entry pathways along with a decrement in creatinine clearance, increased FENA, increased vasopressin excretion, and polyuria. A proximal decrease of NHE3 and NaPi2 is likely to be related to the low T3 levels as well as the reduced GFR. Distal straight tubule and collecting duct increases in NKCC2 and AQP2 probably reflect a vasopressin-induced adaptation of the tubule to preserve water and sodium. The observed changes were largely corrected by substitution of exogenous T3.

We express our gratitude to P. Exner and S. Diederich for the generous help measuring urinary vasopressin and J. Biber for providing the NaPi2 antibody.

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