Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition

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Submitted 30 January 2004; accepted in final form 4 March 2004

The kidneys play a crucial role in the elimination of organic anions that include a variety of endogenous substances and xenobiotics (2, 10, 29, 36, 48, 53). Glomerular filtration is of minor importance in this process. The major contribution to elimination comes from proximal tubular secretion, which is mediated by specific transporters located in basolateral (BLM) and apical membranes of the epithelial cells. Whether transcellular transport via microtubule-dependent transcytosis of intracellular vesicles is also involved in secretion is unclear (Ref. 48 and references therein).

Previous studies of probenecid-sensitive p-aminohippurate (PAH) secretion have indicated a multispecific transporter, represented by a tertiary active PAH/α-ketoglutarate exchange localized in the proximal tubule cell BLM, as the pivotal mechanism for organic anion secretion in the nephron (29, 39, 45, 46). In the rabbit, tubular secretion of PAH occurs along the entire proximal tubule, with the highest transport activity in the S2 segment (40, 41, 51). In the mammalian kidney, two multispecific organic anion transporters, OAT1 (Slc22a6) and OAT3 (Slc22a8), which belong to a larger OAT family, have been cloned, immunocytochemically and functionally characterized, and found to transport PAH in addition to a variety of other substrates (36, 42, 48). A third member of this family, OAT2, was also demonstrated in the kidney (21, 37), but its PAH-transporting capability is negligible compared with OAT1 and OAT3, and it is thus assumed to be less important for this process. Although recent data have indicated that OAT1, with its 5–10 times higher affinity for PAH than that of OAT3, may be a major player in the secretion of PAH and some other organic anions in the mammalian kidney (12, 36, 42), the relative importance of both transporters in secretion may be influenced by their expression levels along the nephron.

OAT1 was cloned from rat (38, 43), human (13, 16, 24, 30, 31), and flounder kidneys (50). By Northern blotting, OAT1 mRNA was detected in rat kidney cortex more than in the outer medulla, whereas by in situ hybridization the message was more abundant in deeper parts of the cortex and in medullary rays (38). By immunocytochemistry in the rat kidney, the protein was localized to the BLM of predominantly the S2 proximal tubule segments and less in the S1 and S3 segments (21, 27, 44), and a similar localization was found for human OAT1 (12, 16, 26). OAT3 was originally cloned from the rat brain (22) and was also demonstrated in the rat and human kidney at the level of both mRNA and protein (12, 21, 22, 26). In the human kidney, OAT3 was immunostained in the BLM of the cortical proximal convoluted tubule, where it colocalized with OAT1 (12, 26), whereas in the rat kidney, the transporter was immunolocalized to the BLM along the entire proximal tubule, thick ascending limb of Henle (TALH), connecting segment, and collecting duct (21).

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Previous data indicate that the rate of renal organic anion transport is regulated by sex steroids (3, 5, 17, 19, 32). In rats in vivo, the rate of PAH transport in male kidneys was greater than that in female kidneys. PAH uptake studies with kidney cortex slices in vitro showed that 1) uptake in male rats was greater than that in female rats; 2) castration of male rats significantly reduced uptake, whereas ovariectomy of female rats had no major effect; 3) treatment of gonadectomized rats with testosterone, but not with estrogen, stimulated uptake; and 4) gender differences in uptake did not exist in very young rats and appeared after maturation.

Cerutti et al. (11) recently demonstrated gender differences in OAT1 protein abundance in isolated rat renal cortical BLM by immunoblotting. However, at the mRNA level, the data on gender differences in renal cortical OAT1 in adult rats are inconsistent and vary from negative (20, 47) to marginal (males > females) (7) or clearly positive (males > females) and androgen dependent (8). The OAT3-related mRNA, however, exhibits strong gender differences (male > female) in the rat liver, but in the kidney these differences are either absent (20) or marginal in favor of males (7). The renal expression of mRNAs for both transporters was lower in prepubertal than adult rats (7). These contradictory data prompted us to investigate in more detail the distribution and possible gender differences at the protein level of OAT1 and OAT3, as well as the sex hormones responsible for these differences along the rat nephron.

**MATERIALS AND METHODS**

**Animals and Treatment**

Male and female Wistar rats aged 10–12 wk (adult) or 25 days (prepubertal) from the breeding colony at the Institute for Medical Research and Occupational Health in Zagreb were used. Animals were bred and maintained according to the Guide for Care and Use of Laboratory Animals (Washington, DC: National Academy Press, 1996). Before and during experiments, animals had free access to standard pelleted food and tap water. The studies were approved by the Institutional Ethics Committee.

Males were castrated by the scrotal route, whereas females were ovariectomized by the dorsal (lumbar) approach. The sham-operated animals underwent the same procedure, except that the respective organs were not removed. The operations were performed under proper anesthesia (Narketan, 80 mg/kg body mass/Xylapan, 12 mg/kg body mass ip). Eight days after surgery, the animals began treatment (sc) with either testosterone enanthate, estradiol dipropionate, or progesterone (2.5 mg (sc) with either testosterone enanthate, estradiol dipropionate, or progesterone were from RotexMedica (Trittau, Germany), Galenika (Zemun, Serbia), and Prolek (Belgrade, Serbia), respectively. The molecular mass standards, used in immunoblotting, were either from Gibco BRL/Invitrogen (Carlsbad, CA) or from Bio-Rad (Hercules, CA). Various other chemicals in the study were the highest purity available and were purchased from either Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

**Tissue Fixation and Immunocytochemistry**

In anesthetized rats the circulatory system was perfused via the left ventricle of the heart, first with aerated (95% O2-5% CO2) and temperature-equilibrated (37°C) PBS (in mM: 140 NaCl, 4 KCl, 2 K2HPO4, pH 7.4) for 2–3 min, and then with ~180 ml PLP fixative (2% paraformaldehyde, 75 mM lysine, 10 mM sodium periodate) for 4–5 min (25). Kidneys were removed, sliced, and kept overnight in the same fixative at 4°C, followed by extensive washing in PBS and storage in PBS containing 0.02% NaN3 at 4°C until use.

To cut 4-µm frozen sections, tissue slices were infiltrated with 30% sucrose (in PBS) overnight, embedded in OCT medium (Tissue-Tek, Sakura, Japan), frozen at −25°C, and sectioned in a Leica CM 1850 cryostat (Leica Instruments, Nussloch, Germany). Sections were collected on Superfrost/Plus Microscope slides (Fischer Scientific), dried at room temperature for 2–3 h, and kept refrigerated until further use.

To perform double staining for OAT1 and megalin, the sections were rehydrated in PBS for 10 min and treated for 5 min with 1% SDS (in PBS) to expose cryptic antigenic sites (6). SDS was removed by an extensive washing with PBS. Nonspecific binding of antibodies was prevented by incubating the sections with 1% bovine serum albumin (in PBS) for 15 min, followed by one of the polyclonal anti-OAT1 antibodies (diluted with PBS: Ab-1, 100 µg/ml; Ab-2, 1:400) at 4°C overnight (for 12–14 h). Two washings followed with high-salt PBS (PBS containing 2.7% NaCl) and two washings with regular PBS (5 min each), incubation with the monoclonal anti-megalin antibody (1:100) at room temperature for 3 h, washing with high-salt and regular PBS (2 × 5 min each), incubation with GAMF (8 µg/ml in PBS) at room temperature for 60 min, washing with high-salt and regular PBS (2 × 5 min each), and mounting in a fluorescence fading retardant (Vectashield, Vector Laboratories, Burlingame, CA).

The retrieval technique with SDS for showing OAT1 proved to give poor and inconsistent data for OAT3, and a harsher approach with several steps that included treatment with organic solutions, detergents, acidic buffers, and heating in a microwave oven, etc., with steps usually employed in processing the paraffin sections, had to be used to expose OAT3. As found by extensive preliminary testing, a significant deviation from these steps yielded much weaker staining. Therefore, the dried tissue sections were incubated (steps) in xylol (30 min), isopropanol (5 min), 98% ethanol (5 min), 75% ethanol (5 min), 60% ethanol (5 min), water (5 min), and PBS (15 min), followed by four cycles (5 min each at 800 W) of heating in a microwave oven in 10 ml citrate buffer, pH 3, and cooling down to room temperature in the same buffer for 20 min. This followed (steps) washing in PBS (3 × 5 min), incubation in 0.5% Triton X-100 (in PBS; 15 min), 2% Triton X-100 (in PBS; 30 min), 1% bovine serum albumin (in PBS; 30 min), anti-OAT3 antibody (1:100 in PBS; at 4°C overnight), washing with 0.5% Triton X-100 (in PBS, 2 × 10 min), incubation in GARCY3 (1.6 µg/ml in PBS) at room temperature for 2 h, washing

**Antibodies and Other Materials**

Polyclonal (anti-peptide) rabbit anti-rat OAT1 antibodies used in these studies were from two sources, i.e., the commercial antibody (Ab-1; affinity-purified immune serum) and the respective peptide (OAT1-P; Alpha Diagnostic, San Antonio, TX), whereas the Ab-2 (crude immune serum) and the respective peptide were noncommercial (27, 44). The affinity-purified polyclonal (anti-peptide) rabbit anti-rat OAT3 antibody and its peptide were also noncommercial and have been described previously (21). The use of monoclonal anti-megalin holoprotein antibody (1H2) and of the affinity-purified polyclonal chicken-raised anti-V-ATPase 31-kDa subunit antibody has been described (1, 34). Secondary antibodies, which included the CY3-labeled (GARCY3) or alkaline phosphatase-labeled (GARAP) goat anti-rabbit IgG and fluorescein-labeled goat anti-mouse (GAMF) or donkey anti-chicken IgG (DACF), were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA) and Kirkegaard and Perry (Gartersburg, MD).

Narketan and Xylapan were purchased from Chassot (Bern, Switzerland). Oil solutions of testosterone enanthate, estradiol dipropionate, and progesterone were from RotexMedica (Trittau, Germany), Galenika (Zemun, Serbia), and Prolek (Belgrade, Serbia), respectively. The molecular mass standards, used in immunoblotting, were either from Gibco BRL/Invitrogen (Carlsbad, CA) or from Bio-Rad (Hercules, CA). Various other chemicals in the study were the highest purity available and were purchased from either Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).
with 0.5% Triton X-100 (in PBS, 2 x 10 min) and PBS (1 x 10 min), and mounting in Vectashield.

To double stain OAT3 and V-ATPase, the sections were first processed and stained for OAT3 as described above and then incubated with the anti-V-ATPase 31-kDa subunit antibody (1:20) at room temperature for 3 h, washed, and incubated with DCF (8 μg/ml) at room temperature for 60 min, washed, and covered with Vectashield.

To test the staining specificity, the anti-OAT1 (Ab-1) and anti-OAT3 antibodies were blocked with the corresponding synthetic peptides (final concentration of the peptide: 0.25 mg/ml and 0.5 mg/ml, respectively) for 4 h at room temperature and then used in an immunofluorescence assay as described above.

The staining was examined and photographed with an Opton III RS fluorescence microscope (Opton Feintechnik, Oberkochen, Germany) using a Spot RT Slider camera and software (Diagnostic Instruments, Sterling Heights, MI). The photos were imported into Adobe Photoshop 6.0 and processed and labeled as required.

**SDS-PAGE and Western Blotting**

The rats were killed by decapitation. The kidneys were removed, and the cortex, outer stripe, inner stripe, and inner medulla (papilla) were dissected manually. Due to the small amount of the tissue, in some cases the inner stripe and inner medulla were pooled and processed as a single sample. The tissue collected from one rat was homogenized in 1-5 ml chilled buffer (in mM: 300 mannitol, 5 EGTA, 12 Tris-HCl, pH 7.4, 1 PMSF, 0.1 benzamidine, and 0.1 μg/ml antipain) with a Powergen 125 homogenizer (Fisher Scientific) at the maximal setting (1-min homogenization-1-min pause-1-min homogenization). The total cell membranes were isolated from these homogenates by first removing cell debris by centrifugation in a refrigerated high-speed centrifuge (Sorvall RC-5C, rotor SS34, Sorvall Instruments, Newtown, CT) at 5,000 g for 15 min. The pellets were discarded, and the supernatants were then centrifuged at 150,000 g for 1 h (ultracentrifuge Sorvall OTD-Combi, rotor T-875). The final pellets (total cell membranes) were resuspended in homogenizing buffer. BLM were isolated from the renal cortical homogenate by the Percoll density gradient centrifugation method of Scalera et al. (35). After dispersion of the membranes in an appropriate volume of homogenizing buffer and measurement of proteins by a dye-binding assay (4), all membrane preparations were stored at -70°C until further use in immunoblotting experiments.

Before electrophoresis, the membrane samples were thawed, mixed with sample buffer [final: 1% SDS, 12% vol/vol glycerol, 30 mM Tris-HCl, pH 6.8, with or without 5% β-mercaptoethanol (β-ME)], and denatured at either 37°C for 30 min or 65°C for 15 min, or 95°C for 5 min. Proteins (40–50 μg/lane and 50–100 μg/lane for BLM and total cell membrane preparations, respectively) were separated through 10% SDS-PAGE minigels using the Vertical Gel Electrophoresis System and then electrophoretically wet-transferred using a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad Laboratories) to an Immobilon membrane (Millipore, Bedford, MA). After transfer, the Immobilon membrane was briefly stained with Coomassie brilliant blue to check the efficiency of the transfer, destained, blocked in blotting buffer (5% nonfat dry milk, 0.15 M NaCl, 1% Triton X-100, 20 mM Tris-HCl, pH 7.4), and incubated at 4°C overnight (12–14 h) in the same buffer that contained one of the anti-OAT1 antibodies (Ab-1: 2.5 μg/ml; Ab-2: 1:1,000) or anti-OAT3 antibody (1:1,000), washed with four changes (15 min each) of blotting buffer, incubated for 60 min in the same buffer that contained 0.1 μg/ml GARAP, washed again, and stained for alkaline phosphatase activity using the 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium method as an indicator.

To block the specific labeling of OAT3, the anti-OAT3 antibody was preincubated with the corresponding synthetic peptide (final concentration of the peptide: 0.5 mg/ml) for 4 h at room temperature and then used in an immunoblotting assay as described above.

Fig. 1. Immunoblotting of organic anion transporter OAT1 in rat renal cortical basolateral membranes (A) and peptide protection of OAT1-related immunostaining in cryosections of the kidney cortex (B and C). Both commercial (Ab-1) and noncommercial (Ab-2) antibodies labeled a specific protein band of 70–75 kDa and an additional band (top). The immunoblots were performed with 50 μg (Ab-1) or 40 μg (Ab-2) protein/lane. By immunostaining, OAT1 (red fluorescence) was present in the basolateral membrane of the proximal tubule S2 segment, whereas glomeruli (G) and the S1 segments remained unstained (B). The OAT1-related staining was abolished after preincubation of the anti-OAT1 antibody (Ab-1) with the immunizing peptide (C). Green fluorescence represents megalin staining. β-ME, β-mercaptoethanol. Bar = 20 μm.
The labeled protein bands were evaluated by densitometry. The density of each band was scanned (Ultrascan Laser Densitometer, Bromma, Sweden), and the integrated scan surface was used in further calculations. In preliminary experiments we evaluated band densities with different amounts of protein and found that these parameters correlated well with up to 50 μg protein/lane for OAT1 and 100 μg protein/lane for OAT3 (data not shown). The integrated surface of each scan was expressed in arbitrary units, relative to the medium band density (= 100 arbitrary units) in the corresponding control samples.

Presentation of Data

The immunocytochemical and numeric data represent findings in four animals in each experimental group. The numeric data, expressed as means ± SE, were statistically evaluated by using Student’s t-test and ANOVA, Duncan’s test, at the 5% level of significance.

RESULTS

OAT1

Characterization of anti-OAT1 antibodies. The commercial (Ab-1) and noncommercial (Ab-2) anti-OAT1 antibodies were first tested for efficiency and specificity of the staining by immunoblotting and immunocytochemistry. BLM were isolated from the kidney cortex of male rats, dissolved in sample buffer without or with 5% β-ME, heated to 37, 65, or 95°C for 30, 15, or 5 min, respectively, and blotted with the antibodies. As shown in Fig. 1A, the membrane samples blotted with Ab-1 exhibited the best labeling of the OAT1-related protein band(s) after denaturation at 37°C for 30 min in the presence of β-ME, indicating that the binding epitope was temperature sensitive. In addition to the protein band of 70–75 kDa, the antibody also labeled a band on top of the gel, reflecting the presence of partially nondenatured and/or polymeric forms of the protein that did not enter the gel. Ab-2 labeled the same protein bands, however, with much greater intensity. After various experimental manipulations and hormonal treatments, the density of the protein band on top of the gel was always weaker than that of the 70- to 75-kDa band (data not shown). The 70- to 75-kDa band, labeled with either antibody, was not compact but rather consisted of a strong middle band and two weaker side bands.

Fig. 2. Immunostaining of OAT1 along the proximal tubule of adult male (A, C, E) and female (B, D, F) rats. OAT1 staining (red fluorescence) was brightly basolateral in the proximal tubule S2 segments in the superficial (A, arrow) and deep cortex (C). Some proximal tubule profiles, which may be the S1 segments (cf. Fig. 1B), were unstained (A and C, asterisk). In females, OAT1 in S2 segments of the superficial (B) and deep cortical nephrons (D) was stained with less intensity. In the outer stripe, the S3 segment in males exhibited a weak basolateral and, in some cells, granular intracellular staining, which was more abundant in the proximal parts of the S3 segment (E), and diminished toward its end (not shown). In females, the basolateral staining in the S3 segment was also weak (F) and present along the entire segment (not shown). The green fluorescence (A, arrowhead) represents the staining of megalin. Bar = 20 μm.
proximal tubule segments was strongly stained for megalin. Previous observations (1, 34), the brush border in all cortical and proximal tubule S1 segments (Fig. 1B and megalin, OAT1 was not significantly present in glomeruli and proximal tubule S1 segments (Fig. 1B) or in distal tubules and collecting ducts (not shown), whereas the BLM of the S2 segments was strongly stained (Fig. 1B). In accordance with previous observations (1, 34), the brush border in all cortical proximal tubule segments was strongly stained for megalin.

After preincubation of the anti-OAT1 antibody with the immunizing peptide, the specific basolateral OAT1-related staining was abolished, whereas the staining of megalin in brush-border membrane remained unchanged (Fig. 1C).

The data obtained with both commercial and noncommercial antibodies were entirely comparable (not shown). Taking into account optimal experimental conditions from the preceding experiments, in further studies we used both antibodies for immunocytochemical and Ab-2 for immunoblotting.

Gender differences in expression of OAT1. To study gender differences in OAT1 expression, tissue cryosections from the kidney cortex and outer stripe of the adult male and female rats were double stained for OAT1 and megalin (Fig. 2). In the male kidney, OAT1 was brightly stained in the BLM of the S2 segments in the superficial cortex (Fig. 2A) and even more strongly in the S2 segments around the juxtamedullary glomeruli (Fig. 2C). In the outer stripe, the antibody weakly stained the BLM of many cells, and some cells in more proximal parts of the S3 had weak intracellular granular staining (Fig. 2E). The cells in this zone were stained with heterogeneous intensity, giving to this part of the S3 segment a mosaic appearance. Terminal parts of the S3 segments, as well as the loop of Henle, distal tubules, collecting ducts, and various structures around the tubules, were negative (not shown). In the female kidney, the pattern of OAT1 staining in proximal tubule segments in the superficial (Fig. 2B) and deep cortex (Fig. 2D) was similar to that in males, but the staining intensity was considerably weaker.

Fig. 3. Immunostaining of OAT1 in the kidney cortex of prepubertal male (A) and female (B) rats and immunoblotting of OAT1 protein in renal cortical basolateral (BLM) and total cell membranes (TCM) from adult and prepubertal (young) male and female rats (C). OAT1 staining (red fluorescence) in proximal tubules of male (A) and female (B) kidneys was weak and similar in intensity. Green fluorescence represents the staining of megalin. In (C), the OAT1-related protein band in BLM and TCM from the cortex was stronger in adult males than in females, whereas in prepubertal rats, the band was weak and similar in TCM from both sexes. Each lane represents a membrane preparation from a separate animal. Bar = 20 μm.

which could represent the protein in different states of glycosylation.

By immunofluorescence cytochemistry, in cryosections of the male kidney cortex double stained for OAT1 (with Ab-1) and megalin, OAT1 was not significantly present in glomeruli and proximal tubule S1 segments (Fig. 1B) or in distal tubules and collecting ducts (not shown), whereas the BLM of the S2 segments was strongly stained (Fig. 1B). In accordance with previous observations (1, 34), the brush border in all cortical proximal tubule segments was strongly stained for megalin.

Table 1. Density of the 70- to 75-kDa OAT1-related protein band in basolateral and total cell membranes isolated from kidney cortex homogenates from variously treated male and female rats

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<td>Intact rats</td>
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<tr>
<td>BLM (adult)</td>
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<td>TCM (adult)</td>
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<td>26±5b</td>
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<td>TCM (prepubertal)</td>
<td>100±27</td>
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<td>Effect of castration in male rats (TCM)</td>
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<td>Sham-operated</td>
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<td>Castrated</td>
<td>54±3b</td>
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<td>Effect of testosterone and estradiol treatment in castrated male rats (TCM)</td>
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<tr>
<td>Sham-operated+oil</td>
<td>100±5</td>
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<tr>
<td>Castrated+oil</td>
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<tr>
<td>Castrated+testosterone</td>
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<td>Castrated+estradiol</td>
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<td>Effect of progesterone treatment in castrated male rats (TCM)</td>
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<td>Castrated+progesterone</td>
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<td>Effect of ovariectomy in female rats (TCM)</td>
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Values are means ± SE from the data in membrane preparations from 4 animals in each group. OAT, organic anion transporter; BLM, basolateral membranes; TCM, total cell membranes. Vs. males: †P < 0.01 and *P < 0.02 vs. respective sham-operated; ‡P < 0.001 and ≠ not significant (NS); vs. sham-operated+oil: ⁠*P < 0.01, NS, and ⁠≠NS, †P < 0.01 vs. respective castrated+oil; ⁠‡P < 0.05, ⁠*P < 0.001, and ⁠≠P < 0.05 vs. ovariectomized+oil: ⁠kP < 0.001. Other relationships, NS.
weaker. In the outer stripe, the cells along the entire S3 segment were weakly stained basolaterally and (less) intracellularly (Fig. 2F) with an intensity similar to, or slightly stronger than, that in males.

Gender differences in the expression of renal OAT1, as found above in adult animals by immunocytochemistry, were not observed in prepubertal (25 days old) rats; the basolateral staining of OAT1 in cortical proximal tubules of the prepubertal males and females was weak, and similar in both sexes (Fig. 3, A and B, respectively).

The gender- and age-dependent abundance of OAT1 in the cortical tubules, as observed by immunocytochemistry, was confirmed in immunoblotting studies by comparing the density of the 70- to 75-kDa protein band (Fig. 3C and Table 1). In both BLM and total cell membranes, isolated from the renal cortex, male rats exhibited four- to fivefold stronger densities of the OAT1-related protein band than did females, whereas the respective band in total cell membranes in prepubertal rats was much weaker and exhibited no sex differences.

**Effect of gonadectomy and replacement therapy with sex hormones.** To define hormone(s) responsible for gender-dependent expression of renal OAT1, adult rats were gonadectomized and treated with either vehicle (oil) or various sex hormones. As shown in Fig. 4, compared with intact rats (Fig. 4A), sham operation + oil treatment caused no change in the staining intensity and distribution of OAT1 in cortical tubules (Fig. 4B). However, 16 days after castration (oil-treated rats), the intensity of OAT1 staining in the cortical convoluted tubules was downregulated (Fig. 4C). In testosterone-treated castrated rats, the respective staining was restored to levels found in sham-operated controls (Fig. 4D), whereas in estradiol-treated castrated animals, the staining intensity was even weaker than that in castrated rats (Fig. 4E). Progesterone treatment of castrated rats caused an enhancement of staining (Fig. 4F). These immunocytochemical findings were supported by immunoblotting data in total cell membranes from the kidney cortex (Fig. 5 and Table 1). Versus sham-operated males, castration decreased the density of the 70- to 75-kDa band in these membranes by 46%, and this drop was not significantly affected by oil treatment of castrated rats (41%). However, the treatment of castrated animals with testosterone increased the abundance of protein (by 103% vs. castrated + oil-treated rats and 20% vs. sham-operated + oil-treated rats), whereas estradiol treatment strongly downregul-
E
demized females downregulated OAT1 abundance (in females slightly increased (D)), whereas estrogen treatment in ovariectomized rats. Furthermore, after ovariectomy in females, the protein abundance in total cell membrane vs. ovariec
tomized oil-treated females (Fig. 5 and Table 1), whereas estradiol treatment of castrated males with testoste
tone (TEST) and estradiol (ESTR) led to upregulation and further downregulation of protein abundance, respectively (B). Progesterone (PROG) treatment of castrated males also upregulated the abundance of OAT1 (C). Ovariectomy in females slightly increased (D), whereas estrogen treatment in ovariec
tomized females downregulated OAT1 abundance (E): E1 and E2 denote 2 independent experiments. Each lane represents a membrane preparation from a separate animal.

lated the protein abundance (by 93% vs. sham-operated+oil
treated rats and 88% vs. castrated+oil-treated rats). In a sep
aprate experiment, progesterone treatment increased the OAT1 abundance in total cell membrane by 30% vs. castrated oil
treated rats. Furthermore, after ovariectomy in females, the staining intensity of OAT1 in the renal cortical tissue slightly increased (data not shown), and the protein abundance in the cortical total cell membranes increased ~30% (Fig. 5 and Table 1), whereas estradiol treatment of ovariec
tomized females further decreased the staining (data not shown) and downregulated (by 74%) the abundance of protein in total cell membrane vs. ovariec
tomized oil-treated females (Fig. 5 and Table 1).

**OAT3**

Characterization of anti-OAT3 antibody. By immunostaining of the male cortex, OAT3 was localized to the BLM of the S1 and S2 proximal tubule segments, distal tubules, and collecting ducts (Fig. 6A). The staining in all these localizations was abolished after preincubation of the antibody with the respective peptide (Fig. 6B). The immunoblotting was per
duced using the same conditions as for OAT1; in preliminary experiments the pattern of labeling of different OAT3-related bands was similar using the denaturing conditions without or with β-ME and at different temperatures, but the respective protein bands were strongest after denaturation at 37°C for 30 min in the presence of β-ME (data not shown). In immunoblots of the renal cortical BLM (Fig. 6C), the anti-OAT3 antibody labeled two distinct bands: the bottom band at 55–70 kDa was more complex and consisted of several smaller bands of different mobility, the broadest one being at ~66 kDa; the top band at ~116 kDa was more compact. These bands were clearly different from the 70- to 75-kDa band related to OAT1, indicating no cross-reactivity of the antibodies, and were completely blocked after preincubation of the antibody with the respective peptide (OAT3+PEP). Both bands were also detected in the total cell membrane preparations from the male kidney cortex (Fig. 6D; –PEPTIDE), except that the density of the ~116-kDa band was variable in different membrane preparations. Therefore, in all further studies we used the more persistent 55- to 70-kDa band complex as a parameter for comparison of gender differences and different hormonal states and treatments. In total cell membranes from the inner stripe and inner medulla (Fig. 6D), we observed 1) a top band being much stronger than the bottom band and 2) a greater heterogeneity of the bottom band with a predominant mobility at 55–60 kDa. In the membranes from the outer stripe, only a weak top band was found. These bands were completely blocked by preincubating the antibody with the respective peptide (Fig. 6D; +PEPTIDE).

Distribution and gender differences of OAT3 expression along the nephron. The preceding immunoblotting data indicated the presence of OAT3 not only in the kidney cortex but also in other parts of the nephron. As shown by immunofluorescence cytochemistry in the male kidney (Fig. 7, A–D), the strongest staining of OAT3 was observed basolaterally in various cortical tubules (Fig. 7A; see also Fig. 6A). In the outer stripe (Fig. 7B), the proximal tubule S3 segments were negative, but the thick ascending limbs and collecting ducts were positive. In the inner stripe (Fig. 7C), the BLM of the TALH and collecting ducts (not shown) was brightly and weakly stained, respectively, whereas in the inner medulla (Fig. 7D), only the BLM in collecting ducts was specifically stained. The observed pattern of the staining along the nephron in the male kidney was present also in the female kidney (Fig. 7, E–H). However, the overall staining intensity in the cortical proximal and distal tubules in females was significantly weaker (Fig. 7E), indicating the presence of gender differences, whereas in other tissue zones (Fig. 7, F–H), the intensity of the staining in different animals was very heterogeneous, and the absence of gender differences in the expression of OAT3 in these regions could not be established with certainty.

The presence of OAT3 in the collecting ducts, which consist of principal and intercalated cells, led us to perform the double staining of collecting ducts in the kidney cortex and inner medulla for OAT3 and V-ATPase to reveal which of the cell type(s) is OAT3 positive. As shown previously (33), the staining of V-ATPase can discriminate the intercalated (V-ATPase positive) from principal (V-ATPase negative) cells, and various phenotypes of intercalated cells, with the apical, basolateral, and bipolar/diffuse localization of V-ATPase. As found in the cortical collecting ducts (Fig. 8A), intercalated

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Fig. 5. Immunoblots of OAT1 in TCM from the kidney cortex of variously treated male (M) and female (F) rats. Castration in adult males led to a decrease in OAT1 abundance (A), whereas treatment of castrated males with testosterone (TEST) and estradiol (ESTR) led to upregulation and further downregulation of protein abundance, respectively (B). Progesterone (PROG) treatment of castrated males also upregulated the abundance of OAT1 (C). Ovariectomy in females slightly increased (D), whereas estrogen treatment in ovariec
tomized females downregulated OAT1 abundance (E): E1 and E2 denote 2 independent experiments. Each lane represents a membrane preparation from a separate animal.
cells with the apical, basolateral, or bipolar/diffuse localization of V-ATPase were negative for OAT3, whereas the BLM of the adjacent, V-ATPase-negative principal cells was positive for OAT3. Similarly, in the inner medulla, the A-type intercalated cells, with apically located V-ATPase, were negative for OAT3, whereas the principal cells were stained at the BLM (Fig. 8B). In a detailed study in tissue sections from four male rats, the staining intensity in the cortical and medullary collecting ducts varied significantly, so that gender differences in the expression of OAT3 could not be confirmed in these nephron segments (not shown).

The expression of OAT3 in kidneys from prepubertal rats has been tested. In these animals, only the proximal tubules in the cortex were stained for OAT3, with an intensity that was similar in males and females and weaker than in adult animals (Fig. 9, A and B). No significant staining was observed in other tissue zones in either sex in prepubertal rats (data not shown).

The confirmation of gender differences in the expression of renal cortical OAT3 in adult rats was achieved by immunoblotting renal cortical BLM (Fig. 9C) and total cell membranes (Fig. 9, D and E) and by densitometric evaluation of these data (Table 2). In immunoblots in these and other studies, the top (116 kDa) and bottom (55–70 kDa) protein bands largely followed a similar pattern. However, the 116-kDa band was more variable and sometimes absent in preparations of total cell membranes, whereas the 55–70-kDa complex was more persistent and thus was used for densitometric evaluation. Therefore, the immunoblots (Fig. 9, C–E) and the respective densitometric data in Table 2 indicate that the abundance of OAT3 in the renal cortical BLM and total cell membranes in adult females was 35–40% lower than that in males. In prepubertal rats, OAT3 abundance was much lower than that in adults: compared with intact adult males, the density of the 55- to 70-kDa protein complex in the cortical total cell membranes from prepubertal male and female kidneys was only 15 ± 5.4 (n = 4) and 11 ± 1.5 (n = 4 vs. prepubertal males; not significant) relative units, respectively (blots not shown), thus showing no significant sex differences (Table 2). However, in the total cell membranes from pooled inner stripe + inner medulla tissue, the abundance of both protein bands, at ~116 kDa and the 55- to 60-kDa complex, was strong, but gender differences in the density of either the top (not shown) or the bottom band complex (Table 2) were not observed. Further experiments were aimed to resolve which of the sex hormones caused the observed gender differences in the expression of cortical OAT3 in adult rats.

Expression of renal OAT3 after gonadectomy and replacement therapy with sex hormones. As shown in Fig. 10, compared with intact rats (Fig. 10A), sham operation + oil treatment had no effect on the overall staining intensity and distribution of cortical OAT3 in adult rats.
of OAT3 in cortical tubules (Fig. 10B). However, 16 days after castration (oil-treated rats), the intensity of OAT3 staining in the convoluted and distal tubules was visibly downregulated (Fig. 10C). Furthermore, in castrated animals the respective staining was 1) partially restored by testosterone treatment (Fig. 10D), 2) further diminished by estradiol treatment (Fig. 10E), and 3) not affected by progesterone treatment (Fig. 10F).

These hormone-induced changes in OAT3 expression were clear for proximal and distal tubules, whereas in the collecting ducts, hormonal dependence could not be clearly confirmed due to a significant staining variability in various animals (data not shown). Furthermore, in female rats compared with sham-operated animals, ovariectomy had no affect, whereas the treatment of ovariectomized animals with estrogen diminished the cortical expression of OAT3 (data not shown).

The immunoblotting data for OAT3 in total cell membranes from the kidney cortex of gonadectomized and sex hormone-treated rats are summarized in Fig. 11 and Table 2. Versus sham-operated males, castration decreased the density of the 55- to 70-kDa band complex in these membranes by 60%, and this drop was not significantly changed by oil treatment of castrated rats (59%). However, the treatment of castrated animals with testosterone partially restored the abundance of protein (by 88% vs. castrated+oil-treated rats), whereas estra-
diol treatment strongly downregulated the protein abundance (by 86% vs. sham-operated-oil-treated rats and 66% vs. castrated-oil-treated males), as well as ovariectomy in females (vs. sham-operated females), had no effect, whereas estradiol treatment in ovariectomized females (vs. oil-treated ovariectomized females) further downregulated (by 60%) the expression of OAT3 protein in the renal cortical total cell membranes.

In addition to the studies of the classic PAH-transporting proteins OAT1 and OAT3, we have also tried to test whether gender differences at the protein level exist for OAT2, which transports PAH only weakly (36, 37). Previously, it was shown that the expression of OAT2-related mRNA in the rat kidney is strongly gender dependent (female >> male), and it increased after castration in males (7, 20), indicating female sex hormones as the ruling factor. In the rat kidney, with a rabbit-raised polyclonal antibody against the COOH-terminal peptide of the protein, OAT2 immunoreactivity was localized to the apical domain of the medullary thick ascending limb of Henle’s loop and the cortical and medullary collecting duct (21). However, by using the anti-OAT2 antibody from the same source (21) and a variety of unmasking techniques, in our study the antibody stained the apical domain of the medullary collecting ducts and cortical proximal convoluted tubules, whereas the TALH remained unstained (data not shown). The staining intensity varied in tissues from different rats, without obvious gender differences. By immunoblotting of the total cell membrane preparations from the kidney cortex and inner medulla (4 animals of each sex), many protein bands were labeled in the range of 20–150 kDa, but none exhibited gender differences. Moreover, neither the immunocytochemical staining nor the labeling of any band in immunoblots was blocked after the antibody had been preincubated with the immunizing...
Table 2. Density of the 55- to 70-kDa OAT3-related protein band complex in BLM and TCM isolated from kidney cortex homogenates from variously treated male and female rats

<table>
<thead>
<tr>
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<th>Male</th>
<th>Female</th>
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<tr>
<td>Intact rats (cortex)</td>
<td></td>
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<tr>
<td>BLM (adults)</td>
<td>100 ± 9 (7)</td>
<td>66 ± 12* (7)</td>
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<tr>
<td>TCM (adults)</td>
<td>100 ± 7 (4)</td>
<td>61 ± 6* (4)</td>
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<tr>
<td>TCM (prepubertal)</td>
<td>100 ± 10 (4)</td>
<td>80 ± 12 (4)</td>
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<tr>
<td>Intact rats (inner stripe + inner medulla, TCM)</td>
<td>100 ± 20 (4)</td>
<td>118 ± 12 (4)</td>
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<tr>
<td>Effect of castration in male rats (TCM)</td>
<td></td>
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<tr>
<td>Sham-operated</td>
<td>100 ± 9 (4)</td>
<td>40 ± 7* (4)</td>
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<tr>
<td>Castrated</td>
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<td>Effect of testosterone and estradiol treatment in castrated male rats (TCM)</td>
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<tr>
<td>Sham-operated + oil</td>
<td>100 ± 4 (4)</td>
<td>41 ± 7* (4)</td>
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<tr>
<td>Castrated + oil</td>
<td>77 ± 8* (4)</td>
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<tr>
<td>Castrated + testosterone</td>
<td>14 ± 2* (4)</td>
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<tr>
<td>Castrated + estradiol</td>
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<tr>
<td>Effect of progesterone treatment in castrated male rats (TCM)</td>
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<tr>
<td>Castrated + oil</td>
<td>100 ± 7 (4)</td>
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<td>Castrated + progesterone</td>
<td>109 ± 27 (4)</td>
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<td>Effect of ovariectomy in female rats (TCM)</td>
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<tr>
<td>Sham-operated</td>
<td>100 ± 6 (7)</td>
<td>96 ± 11 (7)</td>
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<td>Ovariectomized</td>
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<td>Effect of estradiol treatment in ovariectomized female rats (TCM)</td>
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<tr>
<td>Ovariectomized + oil</td>
<td>100 ± 7 (4)</td>
<td>40 ± 6* (4)</td>
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<td>Ovariectomized + estradiol</td>
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Shown are means ± SE (relative units) from the data in membrane preparations from (n) animals in each group. vs. males: *P < 0.05 and **P < 0.001 vs. respective sham-operated; †P < 0.05 vs. sham-operated + oil, ‡P < 0.001, §P < 0.05 vs. respective castrated + oil, ¶P < 0.02, ‡P < 0.01 vs. ovariectomized + oil, ‧P < 0.001. Other relationships, NS.

peptide. The reason for these discrepancies between our and previously published data, performed with the same antibody, cannot be explained at present.

**DISCUSSION**

To understand in more detail the molecular basis of gender differences in renal secretion of PAH and other organic anions in rats, we used specific antibodies to study the expression of two major PAH-transporting proteins, OAT1 and OAT3, in tissue cryosections and isolated membranes from the kidney cortex in intact, gonadectomized, and sex hormone-treated gonadectomized male and female rats. The principal findings by immunochemical methods in this work show that 1) OAT1 was labeled as a 70- to 75-kDa protein, whereas the anti-OAT3 antibody labeled two bands, a broad one at 55–70 kDa and a more compact one at ~116 kDa; 2) OAT1 was largely stained in the BLM of the proximal tubule S2 segment, but the S3 segments also exhibited a limited staining both in the BLM and (less) intracellularly, whereas OAT3 was localized also basolaterally in the S1 and S2 proximal tubule segments (S3 was negative), TALH, distal tubule, and principal cells of the collecting duct; 3) gender differences in the expression of OAT1 in proximal convoluted tubules of adult rats were confirmed (males > females), whereas gender differences for OAT3 were clearly observed only in the cortical (proximal and distal) tubules; 4) in prepubertal rats, the expression of OAT1 and OAT3 in the cortical tubules was low and exhibited no gender differences; 5) castration in adult males caused a strong downregulation of both transporters in the cortical tubules, whereas ovariectomy in adult females led to the slight enhancement and no change in OAT1 and OAT3 expression, respectively, in cortical tubules; 6) in gonadectomized animals, the expression of both OAT1 and OAT3 in cortical tubules was strongly elevated by testosterone, whereas estradiol treatment caused an additional decrease; and 7) the expression of OAT1, but not of OAT3, in cortical tubules was upregulated by progesterone treatment in gonadectomized rats.

In immunoblots of the renal cortical BLM, the anti-OAT1 antibodies labeled a protein band of 70–75 kDa, the intensity of which was inversely related to the denaturing temperature, thus indicating that the antibody-binding epitope was temperature sensitive. In previous studies, OAT1 in the rat kidney was identified as either a 57 (11, 44)- or 77-kDa protein (27), whereas the human homolog was labeled as an 80- to 90-kDa protein band (13). At present, we have no explanation for the obvious discrepancies in the electrophoretic mobility of the OAT1 protein in previous and our studies. Rat OAT1 is a protein of 551 amino acid residues, with four potential N-glycosylation sites and a predicted molecular mass of the nonglycosylated form of ~60 kDa (36, 43). Accordingly, human OAT1 decreased from 80–90 to 60 kDa after deglycosylation (13). Therefore, the size of the OAT1 protein band of the 70–75-kDa protein, as found in this study, is probably related to the glycosylated form of the protein. This possibility is also indicated by the pattern of the protein band, which was a complex of one strong middle band and two weaker side bands, which may represent different glycosylation states of the protein. In addition, in our blots the antibody also labeled a band on the gel top, which may reflect the presence of either a partially nondenatured protein and/or a hitherto unrecognized polymeric forms of the protein. On the other side, OAT3 was previously labeled as a single band of 130 and 80 kDa in a crude plasma membrane fraction from rat (21) and human (26) kidneys, respectively, whereas in this study the anti-OAT3 antibody labeled two major protein bands, one being more complex with multiple bands at 55–70 kDa, which was strongest in the membranes from the kidney cortex, where it peaked at ~66 kDa, and another more compact band at ~116 kDa, which was strongest in the membranes from the inner stripe and inner medulla. Both band complexes were strongly labeled in the BLM isolated from the kidney cortex, and both were completely blocked by the immunizing peptide in the membranes from various tissue zones, indicating specificity. As rat OAT3 is a polypeptide of 536 amino acid residues, with four putative N-glycosylation sites (22), our findings indicate that, under our experimental conditions, the complex band at 55–70 kDa may reflect monomeric proteins in various states of glycosylation, whereas the 116-kDa band may represent a dimeric form of the protein. The distribution of both protein complexes in the cell membranes from various tissue zones, as well as a generally similar pattern of their labeling in the cortical membranes from the intact, gonadectomized, and hormone-treated animals, indicates that both monomeric and dimeric forms exist in the same membrane preparations, except that the heterogeneously glycosylated monomeric form is more prevalent and consistent in the cortical membranes, where the dimeric form is present in an inconsistent abundance, whereas in membranes from the inner stripe and inner medulla the dimeric form prevails. A similar pattern of expression of both forms was also observed after heating of the membranes at
different temperatures, except that the density of the final protein bands was strongest at 37 °C (data not shown), which speaks against the temperature-dependent digestion of the membrane proteins as an explanation. The basis for the zone-related expression of monomers and putative dimers remains unexplained and was not further studied.

In accordance with recently published immunolocalization data in rat (21, 27, 44) and human kidney (26), our studies proved the presence of OAT1 in the BLM in cells along the proximal tubule, with predominant localization in the S2 segments. The S3 segments also exhibited a limited staining of OAT1 in both BLM and (less) intracellularly. This axial distribution of OAT1 along the rat renal proximal tubule fits previous functional data obtained with rabbit proximal tubules, showing highest PAH secretion in S2 segments (40, 41, 51), and suggests that OAT1 may have a similar distribution in the rabbit. The intracellular granular staining in some cells indicates a possible localization of the protein in a vesicle population that may be involved in the recycling of this transporter by means of endo- and exocytosis. Furthermore, previous studies identified OAT3 in the BLM of cortical proximal convoluted tubules in the human kidney and along the entire proximal tubule, TALH, connecting segment, and collecting duct in the rat kidney (21). The same distribution of OAT3 along the rat nephron was confirmed in our study. In addition, we showed that the basolateral localization along the collecting duct is restricted to the principal cells; the V-ATPase-positive A and B type intercalated cells were negative for OAT3. These data raise the question of a largely neglected function(s) and substrate specificity of OAT3 in the nephron segments that are distal from the proximal tubules; so far, the OAT-related transport of PAH and other organic anions has been characterized only in the proximal tubule segments.

Gender differences in the renal secretion of PAH and various other organic anions have been known for a long time (3, 5, 17, 19, 32). These differences in transport may reflect the differences in the respective transporter(s). Indeed, Cerrutti et al. (11) recently demonstrated by immunoblotting of isolated renal cortical BLM a 60% stronger OAT1-related protein band in male than in female kidney. We confirmed this finding for OAT1, however, with a much larger difference: in our hands, the density of the 70- to 75-kDa protein band in isolated renal cortical BLM from adult male rats was five times stronger than that in females. A similar difference in protein band density

Fig. 10. Immunostaining of OAT3 in the cortex of intact (A), sham-operated + oil-treated (B), castrated + oil-treated (C), castrated + testosterone-treated (D), castrated + estradiol-treated (E), and castrated + progesterone-treated (F) male rats. Castration and estradiol treatment downregulated, whereas treatment with testosterone upregulated, the expression of OAT3 in the cortical tubules. Sham operation (B) and progesterone treatment in castrated rats (F) had no effect. Bar = 20 μm.
were supported by immunocytochemistry; much higher stain-
for studies of protein expression. The immunoblotting data
was retained in total cell membranes isolated from the renal
cortex, allowing for a simpler membrane preparation procedure
for studies of protein expression. The immunoblotting data
were supported by immunocytochemistry; much higher stain-
ing intensity of OAT1 in the tubules of superficial and (even
higher in) deep cortex in male than in female rats confirmed
gender differences in OAT1 expression. Similar gender differ-
ences were observed for OAT3, however, only in the cortex.
Compared with the respective data in females, the density of
the OAT3-related 55- to 70-kDa protein band complex was
stronger in the male cortical tubules, whereas the immunostain-
ing in other segments along the nephron and the abundance of
OAT3 protein in isolated membranes from other tissue zones
was rather heterogeneous, showing no clear-cut gender differ-
ences.

The gender differences in OAT1 and OAT3 existed in adult
animals but not in prepubertal rats, which, in both sexes,
exhibited a low abundance of these proteins in the cell mem-
branes and a low intensity of the staining in the cortical
tubules, thus explaining previously observed gender-indepen-
dent and low PAH accumulation in renal cortical slices in
young rats with immature kidney function (5, 27). The low
abundance of renal OAT1 in prepubertal rats may be related to
the lower expression level of the specific mRNA in these
animals; Buist et al. (7) observed a gradual increase in mRNA
eexpression from birth through day 35 thereafter. However, the
findings by Nakajima et al. (27) were just the opposite and
indicated a much higher expression of mRNA and OAT1
protein in very young than in adult rats. In addition, in one
study mRNA for renal OAT3 already reached mature values
10–15 days after birth (7). These and other studies indicate that
gender differences in cortical OAT1 and OAT3 proteins may
not be a mere reflection of the expression of mRNA; clear
gender differences in mRNA for OAT1 in the adult rat kidney
were observed in one study (8), but not in a few others (7, 20,
47), and not for OAT3 (7, 20). However, our data with
prepubertal (25 day) rats revealed abundances of the protein
well below the adult values. This lack of correlation between
OAT1 and OAT3 proteins and their mRNAs may be apparent,
e.g., because different animal strains have been used in these
studies. The possibility that mRNA-independent mechanisms
affecting synthesis and/or degradation of OAT proteins may, in
part, contribute to age and gender differences cannot be disre-
garded and requires additional experiments.

The absence of gender differences in the abundance of
cortical OAT1 and OAT3 in prepubertal rats and their presence
in adult rats indicated that the expression of these transporters
depends on maturation and thus may be regulated by sex
hormones. This possibility was supported by the findings of
diminished PAH uptake in kidney cortical slices from castrated
male rats and its stimulation after treatment of gonadectomized
animals with testosterone, but not with estrogens (3, 5, 17, 19,
32). Our immunoblotting and immunocytochemical data cor-
raborate previous PAH uptake studies in tissue slices in vitro.
According to our data, both androgens and estrogens are
responsible for the observed gender differences in OAT ex-
pression, however, by acting in opposite directions: after cas-
tration of adult males, cortical OAT1 and OAT3 expression
was downregulated and was reversed by testosterone treat-
ment, whereas treatment of castrated rats with estradiol caused
additional downregulation. Furthermore, after ovariectomy of
adult females, OAT1, but not OAT3, expression was slightly
enhanced, whereas estrogen treatment in these rats further
downregulated both proteins. In addition, progesterone treat-
ment led to enhanced protein expression of OAT1 in castrated
males. At present, it is unclear whether these effects of sex
steroids are at the genomic and/or nongenomic levels, mediat-
ed by specific steroid receptors. At the message or protein
level, steroid receptors for androgens, estrogen, and progester-
one have been reported in animal and human kidneys (9, 15,
23, 28 and references therein; 49, 52). In one report, estrogen

Fig. 11. Immunoblots of OAT3 in TCM from the kidney cortex of variously
treated male (M) and female (F) rats. Castration in adult males led to a decrease
in OAT3-related bands (A, B). The treatment of castrated males with testos-
terone or estradiol led to an upregulation or a further downregulation of protein
abundance, respectively (B), whereas progesterone treatment of castrated
males had no significant effect (C). Ovariectomy in females did nothing (D),
whereas the estrogen treatment in ovariectomized females strongly downregu-
lated OAT3 abundance (E). E1 and E2 denote 2 independent experiments. Each
lane contains 80 (males) or 100 µg (females) protein and represents a
membrane preparation from a separate animal. The positions of molecular
mass markers of 66 and 116 kDa are indicated by an arrowhead and an arrow,
respectively.
receptors have been localized to the proximal tubule of the Rhesus monkey (14), but a detailed immunolocalization of various sex steroid receptors in rat and human kidneys, which would allow correlation with our OAT data in male and female rat kidneys, has to our knowledge not been reported. Our findings nevertheless indicate the possibility that various physiological states, associated with variable levels of endogenous sex hormones, such as sexual cycle, pregnancy and menopause in women, and aging in both sexes, may influence renal organic anion secretion via hormone-dependent expression of the two major transporters, OAT1 and OAT3, that are located in the BLM of proximal (both transporters) and distal (OAT3) tubules. Further examples of sex hormone-dependent transporter mRNA expression are rat renal OAT2 (females > males; 7), organic cation transporter OCT2 (males > females; 47), and Oatp1 (males > females; 18). Thereby, gender differences may have profound effects, at least in the rat, on renal drug clearance mediated by OATs, OCTs, and Oatp1.

In conclusion, our data indicate that gender differences in OAT1 and OAT3 in rat renal cortical tubules appear after puberty and are determined by both a stimulatory effect of androgens (and progesterone in case of OAT1) and an inhibitory effect of estrogens. The presence of OAT3 in the distal parts of the nephron, including the TALH and collecting duct, indicates that the distal parts of the nephron may also contribute to the organic anion secretion, however, with an unknown efficiency and substrate specificity. The possible impact of these gender differences and transporter localizations on renal drug excretion and pharmacokinetics of anionic drugs may be of clinical importance and remains to be determined.

ACKNOWLEDGMENTS

The authors thank Eva Herfak for technical assistance.

GRANTS

This work was supported by Croatian Ministry of Science and Technology Grant 022111 (I. Sabolić) and by a collaborative Croatian-German grant (I. Sabolić and G. Burckhardt).

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