Influence of testosterone on regulation of ODC, antizyme, and N\(^1\)-SSAT gene expression in mouse kidney

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Influence of testosterone on regulation of ODC, antizyme, and N\(^1\)-SSAT gene expression in mouse kidney. Am J Physiol Renal Physiol 285: F498–F506, 2003. First published April 22, 2003; 10.1152/ajprenal.00407.2002.—Polyamines are involved in the control of the cell cycle and cell growth. In murine kidney, testosterone enhances gene expression of ornithine decarboxylase (ODC), the first enzyme in polyamine biosynthesis. In this study, we document the time course effect of testosterone on regulation of ODC, antizyme, and N\(^1\)-SSAT gene expression in mouse kidney. Am J Physiol Renal Physiol 285: F498–F506, 2003. First published April 22, 2003; 10.1152/ajprenal.00407.2002.—Polyamines are involved in the control of the cell cycle and cell growth. In murine kidney, testosterone enhances gene expression of ornithine decarboxylase (ODC), the first enzyme in polyamine biosynthesis. In this study, we document the time course effect of testosterone on 1) gene expression of ODC, antizyme 1 (AZ1), and spermidine/spermine-N\(^1\)-acyethyltransferase (N\(^1\)-SSAT); 2) ODC activity in proximal convoluted tubules (PCT) and cortical proximal straight tubules (CPST); and 3) renal polyamine levels. Female mice were treated with testosterone for a period of 1, 2, 3, and 5 consecutive days. ODC gene expression was extremely low in kidneys of untreated female mice compared with that of males. Consequently, the renal putrescine level was sevenfold lower in females than in males, whereas spermidine and spermine levels did not differ between sexes. In kidney tissues, testosterone treatment sharply increased ODC mRNA and protein levels as well as ODC activity. Testosterone increased the expression of ODC in PCT and CPST over different time courses, which suggests that ODC activity is differentially regulated in distinct tubules. The expression of AZ1 and N\(^1\)-SSAT mRNA was similar in male and female mouse kidneys. Testosterone treatment enhanced AZ1 and N\(^1\)-SSAT mRNA levels in a time-dependent manner by unknown molecular mechanisms. Putrescine and spermidine levels increased after testosterone treatment in female kidneys. Surprisingly, although ODC protein and activity were undetectable in female kidneys, the levels of AZ1 mRNA and protein were similar to those in males. Therefore, one may propose that ODC protein could be continuously degraded by AZ1 in female kidneys.

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The NATURAL ALIPHATIC POLYAMINES, putrescine (Put), spermidine (Spd), and spermine (Spm), are ubiquitously found in most animal and plant tissues (30). Polyamines are involved in many cellular and physiological processes including cell growth and differentiation (10) and other biological functions that have not yet been fully elucidated. For example, these polycations interact with a great variety of negatively charged entities inside cells, such as nucleic acids, membranes, ribosomes, lipids, and other small molecules.

Biosynthesis of Put, Spd, and Spm requires several enzymes: ornithine decarboxylase (ODC; EC 4.1.1.17), L-methionine adenosyltransferase (EC 2.4.2.13), S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50), spermidine synthase (EC 2.5.1.16), and spermine synthase (EC 2.5.1.22) (30, 38). In the interconversion polyamine pathway, Spm is successively converted into Spd and Put. Polyamine catabolism is controlled by two enzymes, spermidine/spermine-N\(^1\)-acyethyltransferase (N\(^1\)-SSAT) and FAD-dependent polyamine oxidase (PAO; EC 1.5.3.11) (38). Because polyamine accumulation is cytotoxic, the polyamine pathway has to be highly regulated to finely control the intracellular concentration of polyamines. Excesses of acetylated and nonacetylated polyamines are excreted in urine.

Enzymes involved in polyamine metabolism, such as ODC, SAMDC, and PAO, are known to be especially abundant in the male mouse kidney (7, 12, 14, 22). ODC mRNA, protein, and enzyme activity have been clearly localized by various techniques in the renal cortex, particularly in the proximal convoluted tubule (PCT) of the male mouse (7, 18, 21, 31). In contrast, all are extremely low in the female mouse kidney. Recently, in a detailed study, no ODC activity was found in isolated nephron segments dissected from female Swiss mice (20). Therefore, the female mouse kidney constitutes an excellent tool for study...
ing in detail factors involved in the regulation of the polyamine pathway by sex hormones.

Among the numerous factors regulating polyamine biosynthesis (9, 34, 35), androgens such as testosterone dramatically increase ODC mRNA, protein levels, and ODC activity and cause Put to accumulate in kidneys of male and treated female mice (1, 7, 12, 18, 20, 29). Conversely, in castrated males, ODC gene expression decreases and reaches the levels observed in females (29). However, at present, the influence of testosterone on the regulation of the entire polyamine pathway remains unknown.

The polyamine pathway is highly regulated at transcriptional, translational, and posttranslational levels. Regulation of ODC expression has been well documented, including the original feedback controlled by the protein antagonist 1 (AZ1) (15, 27). AZ1 has a high affinity for ODC and binds reversibly to the proline-, glutamic acid-, serine-, and threonine-rich (PEST) region of ODC (11, 26). The PEST region corresponds to a signal for selective proteolysis and is presumed to be involved in the degradation of proteins having rapid turnover. When the ODC-AZ complex binds to the 26S proteasome for degradation, AZ1 molecules are released and recycled. AZ1 plays a central role in the negative-feedback system by accelerating ODC degradation and preventing polyamine overaccumulation. In addition, AZ1 negatively regulates the cellular polyamine levels by inhibiting a specific transporter involved in polyamine uptake (28).

The present study was designed 1) to analyze in detail the time course effect of testosterone on ODC gene expression and ODC activity in single microdissected proximal tubules, 2) to resolve whether renal AZ1 and N1-SSAT gene expression differs between male and female mice, 3) to study the time course influence of testosterone on AZ1 and N1-SSAT gene expression, and 4) to quantify polyamines in kidneys of control mice and testosterone-treated female mice.

MATERIALS AND METHODS

Animals and Treatments

Nine-week-old adult female [30–32 g body wt (BW)] and male OF-1 Swiss (IOPS Caw) mice (35–40 g BW) from Iffa Credo (L’Arbresle sur Orge, France) had free access to tap water and a standard laboratory diet (Souffrat 20% protein, Genthon). The institutional animal care committee approved all experiments.

Female mice were subdivided into six groups: one untreated (control), one oil-treated group, and four androgen-treated groups. Mice subjected to testosterone treatment were injected subcutaneously with 150 μl testosterone propionate (31 mg/ml in sesame oil, i.e., ~155 μg/g BW). Injections were performed at 8:00 A.M., and mice were treated for a period of 1, 2, 3, or 5 consecutive days. Oil-treated mice were injected subcutaneously with 150 μl sesame oil for a period of 5 consecutive days. Untreated male mice were used as positive controls.

Sampling of Kidneys for Northern and Western Blot Analyses

Twenty-four hours after the last injection of testosterone or oil, mice were anesthetized (ip) with 0.1 ml/100 g BW pentobarbital sodium (Nembutal, 6%; Clin Midy, Paris, France) diluted 1:2 in 0.9% NaCl solution. Control mice were similarly anesthetized. The left and right kidneys were rapidly removed and decapsulated. The blood contained in each kidney was immediately removed with blotting paper. The kidneys were placed in a sterilized Eppendorf tube and frozen in liquid nitrogen. They were maintained at −80°C until RNA or protein extractions.

RNA Extraction and Northern Blot Analyses of ODC, AZ1, N1-SSAT, and β-Actin mRNA

Each frozen kidney was homogenized in 3 ml RNAxel solution (Eurobio), and total RNAs were extracted according to the manufacturer’s recommendations and maintained at 4°C. RNAs were rinsed twice with 70% ethanol and dried in a Speed Vac. RNAs were resuspended in cold 10 mM Tris-HCl and 1 mM EDTA, pH 8.0, and their concentrations were determined by absorbance at 260 nm. Fifteen micrograms of RNA samples were submitted to 1.2% agarose gel electrophoresis. After the gel was treated for 20 min in 50 mM NaOH, then for 20 min in a solution containing 0.5 M Tris and 1.5 M NaCl, RNAs were transferred overnight to a nylon membrane (Appligene) and immobilized using a UV cross-linker (Appligene).

Membranes were hybridized with murine 32P-labeled cDNAs corresponding to ODC [pBS-ODC Xho-BamHI (20)], AZ1 [pcDNA3.1 (+) WTAZ, EcoRI-EcoRI (15)], and β-actin [pAL41- cytoplasmic β-actin, PstI-PstI, (8)] and human 32P-labeled N1-SSAT cDNAs [pBluescript SAT9.3, EcoRI- EcoRI (24)]. cDNA were labeled using the RTS RadPrime DNA labeling system (GIBCO BRL, Life Technologies) and α-[32P]dCTP. Hybridization was performed overnight at 65°C. Membranes were washed three times in 2× SSC (0.3 M NaCl and 30 mM sodium citrate), 5 mM phosphate buffer, and 0.1% SDS and three times in 0.5× SSC, 3 mM phosphate buffer, and 0.1% SDS. The amount of radioactivity hybridized to specific mRNA was estimated after scanning densityometry of the membranes using a PhosphorImager SI (Molecular Dynamics, Amersham). Quantitation of β-actin mRNA was used as a control of equal loading and RNA transfer.

Protein Extraction and Western Blot Analyses of ODC and AZ1 Proteins

Each frozen kidney was mixed at 4°C with a Turrax in 1 ml of lysis buffer (19) containing 1 mM protease inhibitor cocktail, 1 mM benzamidine, and 1 mM PMSF and then centrifuged at 10,000 g for 30 min at 4°C. Protein concentrations were determined in the supernatant using the Bradford protein assay (5). Fifty-microgram samples of soluble proteins were subjected to 12% PAGE containing SDS using 6 g/l of glycerol and transferred to a polyvinylidene difluoride membrane (0.45 μm, Immobilon-P, Millipore) at 150 mA for 90 min. Proteins were fixed on the membrane with Ponceau S solution for 15 min. Immunoblots were washed twice in 1× Tris-buffered salt +0.15% Tween 20 (TBST) and immersed in a blocking solution consisting of 5% fat-free milk powder in 1× TBST for 30 min.

The blots were incubated with a polyclonal rabbit anti-human ODC (Eurodianstica), polyclonal rabbit anti-rat AZ1 (a gift of Dr. S. Matsufuji, Tokyo, Japan (23)), or mono-
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clonal mouse anti-β-tubulin primary antibodies in 5% milk-1× TBST. Blots were washed three times for 10 min in 1× TBST and incubated for 60 min with either peroxidase-conjugated anti-rabbit IgG or anti-mouse IgG secondary antibodies or an anti-rabbit IgG secondary antibody conjugated to alkaline phosphatase in 5% milk-1× TBST. Blots were washed three times for 10 min in 1× TBST, and antibody binding was revealed using either an enhanced chemiluminescence (ECL) or ECL Western Blotting Kit. ECL detection was performed using Kodak X-MAT film. Low-exposure film was scanned, and the intensity of the bands was estimated using the ImageMaster Total Lab v1.00 program (Pharmacia, Orsay, France). The intensity of the bands detected by ECF was estimated after scanning densitometry of the membranes with a FluorImager SI (Molecular Dynamics, Amer¬

Kidney Preparation and Microdissection of Different Nephron Segments

Female mice were anesthetized as described above, and the left kidney was prepared for microdissection as previously described (20, 21). The kidney was perfused with 4 ml of medium for incubation and perfusion (MIP; see below) containing 1.63 mg/ml collagenase, removed, and sliced along the corticomedullary axis. Small pyramids containing both cortical and medullary tissue were incubated at 30°C in MIP containing 0.9 mg/ml collagenase, bubbled with O2, and shaken at 6 cycles/min for a period of 5–10 min.

MIP consisted of (in mM) 137 NaCl, 5 KCl, 0.44 KH2PO4, 1 MgCl2, 0.8 MgSO4, 0.33 Na2HPO4, 1 CaCl2, and 20 HEPES, as well as 0.1% BSA, 1% vitamin mixture, 6% dextran, and 0.5–1.0 mM CdCl2, 0.5–1.0 mM mercaptoethanol (205 mosmol/ kgH2O, pH adjusted to 7.40). Triton X-100-treated tubules containing a 2-ml droplet of KOH. Samples were incubated in a water bath at 37°C for 70 min. The KOH droplet containing the 14CO2 was removed, and the amount of radioactivity was estimated by liquid scintillation counting. Control or blank samples contained labeled L-[1-14C]ornithine, but no tubules.

Results are expressed in femtomoles of 14CO2 produced per minute per millimeter tubular length.

Polyamine Extraction and Quantitation of Put, Spd, and Spm

The whole right blood-free kidney was weighed, and polyamines were extracted by homogenizing each frozen tissue in 1 ml of ice-cold 0.4 N HClO4/100 mg tissue. The samples were centrifuged at 4,000 g for 30 min at 4°C, and the clear supernatant was collected and stored at −20°C until further analysis.

Tissue extracts and standards [Put, Spd, Spm, and diami¬noheptane (DAH) as an internal standard] were dansylated according to the procedure described by Seiler (37) and were then treated using the protocol adapted from Besson et al. (2). Dansylation was assessed in glass vials by mixing 40 µl of HClO4 extracts, 10 µl of DAH, and 100 µl of 0.3 M Na2CO3 (Merck). The reaction was initiated by adding 200 µl of freshly prepared dansyl chloride solution (5 mg/ml) in acetone (SDS, spectrosol grade) and allowed to proceed overnight in the dark at room temperature. After dansylation, each sample was diluted with 700 µl H2O, vortexed, and applied to a Waters Sep-Pak C18 cartridge (4). After being washed with 4 ml of 20% methanol, the polyamine-containing fraction was eluted with 2 ml of 100% methanol. The separation and quantification of polyamines were performed by reverse-phase (RP)-HPLC using a Waters system composed of two model 510 pumps, a Wisp 700 autosampler, and an NEC APC4 data module recorder integrator. A Merck 1050 fluorescence spectrophotometer was used to detect fluorescence (350-nm excitation and 495-nm emission). The separations were performed on an RP18 Merck Lichrorcat (25 × 4 mm, 5 µm) precolumn and an RP18, 100 CH Merck column (125 × 4 mm, spherical packing, 5 µm). The solvent system was an acetonitrile/ water gradient at a flow rate of 1 ml/min of 60% acetonitrile in water for 7 min and then 90% acetonitrile for 10 min and a 98% acetonitrile purge for 5 min. The column was reequilibrated to the initial 60% acetonitrile conditions for a 10-min period between successive injections. For each determination, 50 or 100 µl of dansylated samples were injected into the equilibrated column.

The major polyamines were identified by their retention times compared with those of standard (10–70 pmol) polyamines. Peak areas were automatically measured by the integrator and evaluated according to the calibration method (4). The absolute limit of detection per injection was 1 pmol of dansylated Spd and dansylated Spm and 7 pmol of dansylated Put, respectively. Two blank injections were routinely run between calibrations and sample analysis.

Chemicals

Salts, Ponceau S solution, secondary antibodies, Kodak X-MAT film, DAH, and dansyl chloride were purchased from Sigma (St. Quentin Fallavier, France). Collagenase A from Clostridium histolyticum (0.28 U/mg) and protease inhibitor cocktail were from Boehringer Mannheim (Strasbourg, France). L-[1-14C]ornithine (1.85 GBq/mmol) and α-[32P]dCTP (220 TBq/mmol), monocolonal mouse anti-β-tubulin, and ECF and ECL Western Blotting Kits were purchased from Amersham (Buckinghamshire, UK, and Orsay, France).

Statistical Analyses

Results are presented as means ± SE. Statistically signif¬

sicant differences were calculated either by an unpaired stu¬
Results

The purpose of these experiments was to study the influence of testosterone on ODC, AZ1, and N1-SSAT gene expression in the murine kidney. To achieve this goal, female mice were injected with testosterone propionate for a period of 1, 2, 3, or 5 consecutive days. Groups of untreated male and female mice as well as female mice treated with oil for 5 days were used as controls. RNAs, proteins, and polyamines were extracted from the whole kidney and analyzed, whereas ODC activity was quantified in single, isolated proximal tubules.

Effect of Testosterone on ODC Gene Expression and Activity

Time course effect of testosterone on ODC mRNAs in female mouse kidney. ODC mRNA levels were determined to assess the ability of testosterone to regulate ODC gene expression and to establish the time course variation of their induction. Using a specific ODC cDNA probe, two forms of ODC mRNAs (2.2 and 2.7 kb) were detected by Northern blotting in the kidneys of untreated male and female mice and testosterone-treated female mice (Fig. 1) (16). By contrast, the 2.2- and 2.7-kb ODC mRNAs were barely detectable in untreated female kidneys compared with male kidneys. After a single injection of testosterone into female mice, the level of the two renal ODC mRNAs reached that in males. Prolonged testosterone treatment led to a progressive increase in 2.2- and 2.7-kb ODC mRNA levels before a plateau was reached after 2–3 days. Testosterone treatment had no effect on the content of control β-actin mRNA in the kidney (Fig. 1).

Time course effect of testosterone on ODC protein level in female mouse kidney. Western blotting was performed to monitor the amount of ODC protein. An ~53-kDa protein, which corresponds to the predicted size of the ODC polypeptide subunit (Swiss-Prot: mouse P00860), was specifically revealed in kidneys of male and treated female mice using a specific anti-ODC antibody (Fig. 2, left). β-Tubulin, a 55-kDa protein, was used as a control of loading and transfer of proteins (Fig. 2, right). Immunoblots were revealed by ECF to estimate ODC protein levels during the course of treatment. No ODC protein was detectable in kidneys of control untreated female mice. ODC protein levels increased progressively after testosterone injection and reached a peak between days 3 and 5 (Fig. 2). The levels of ODC mRNAs are therefore tightly correlated with those of ODC protein in kidneys of male, female, and testosterone-treated female mice (Figs. 1 and 2).

Time course effect of testosterone on ODC activity in proximal tubules. Numerous investigators have reported an increase in renal ODC activity in mice after testosterone injection (12, 25, 33). However, it has never been determined whether the time course of the testosterone-induced ODC activity differed from one tubule to another. We measured ODC activity in single microdissected PCT and CPST isolated from testosterone-treated female mice (20). In untreated female mice (day 0), ODC activity was undetectable in any tubule (Fig. 3). In contrast, in testosterone-treated females, ODC dramatically increased in both PCT and CPST. However, the time course of ODC activity differed between PCT and CPST. In PCT, ODC activity increased quite linearly during the course of the hormonal treatment (Fig. 3), whereas in CPST it increased sharply from day 1 to day 3 and then remained constant until day 5 (Fig. 3). Although the highest basal level of ODC activity was found in the PCT of male mice (21), in females treated with testosterone for 2, 3, and 5 days, ODC activity was 38% higher in CPST than in PCT (unpaired Student’s t-test, P <0.0001). The proximal straight tubules located in the outer stripe of the outer medulla (OSPST) could not be included in this study because testosterone treatment seems to fragilize this tubule and makes the dissection almost impossible. However, the few data obtained are given for information. In OSPST dissected from female mice treated for 1 and 3 days with testosterone, ODC activity was 17 ± 3
and 189 fmol·min⁻¹·mm⁻¹ (n = 11 and 2 samples, respectively). As a control for the efficiency of Triton X-100 treatment, we veriﬁed that the production of labeled CO₂ in the CTAL was indeed very low (data not shown). In PCT, but mainly in CPST, changes in ODC activity are tightly correlated with the levels of ODC mRNAs and protein in male, female, and testosterone-treated female mouse kidneys (Figs. 1–3).

Effects of Testosterone on Polyamine Content of the Kidney

Quantitation of Put, Spd, and Spm in untreated mouse kidneys revealed sex differences. Put content was sevenfold higher in male than in female kidneys (Fig. 4, 1-way ANOVA, F = 33.8, P < 0.0001). Five days of oil treatment had no inﬂuence on the Put level. A single injection of testosterone into female mice induced a 7.6-fold increase in renal Put content, which therefore reached that in untreated male kidneys within 24 h (Fig. 4, 1-way ANOVA, F = 33.8, P < 0.0001). A prolongation of the hormonal treatment did not signiﬁcantly enhance the renal Put level. Similar levels of Spd and Spm were detected in untreated male and female kidneys (Fig. 4). In contrast, 2, 3, and 5 days after treatment with testosterone, renal Spd content was signiﬁcantly enhanced compared with that in untreated female mice (Fig. 4, 1-way ANOVA, F = 14.2, P < 0.0001). In female mice, renal Spm content was not signiﬁcantly affected by testosterone (Fig. 4).

Influence of Testosterone on AZ1 gene expression

Analysis of the level of AZ1 mRNAs in kidneys of control untreated and testosterone-treated mice. As testosterone dramatically enhanced ODC gene expression and shifted renal polyamine concentrations to high levels, the inﬂuence of testosterone on AZ1 mRNA and protein content was investigated. Indeed, polyamines are involved in AZ mRNA translation by increasing the frequency of the frame shift, which enables the synthesis of the whole AZ protein (27).
Northern blot analyses using a specific AZ1 probe revealed the presence of a single 1.3-kb AZ1 mRNA in the kidneys of untreated male, untreated female, and testosterone-treated female mice (Fig. 5A). Similar levels of AZ1 mRNA were detected in untreated male and female kidneys. However, in kidneys of testosterone-treated female mice, AZ1 mRNA levels progressively increased during the course of the treatment. On day 5 of treatment, the amount of AZ1 mRNA was about twofold higher than that found in untreated female mice (Fig. 5A).

Analysis of the level of AZ1 protein in kidneys of control, untreated, and testosterone-treated mice. Expression of AZ1 protein in kidneys was investigated by Western blot analysis. The specific anti-AZ1 antibody revealed proteins of different sizes (Fig. 5B), 24.1 kDa for the most abundant and 26.5 kDa for the least abundant (27). Both proteins exhibited the same pat-
tern of expression and were abundant in both untreated male and female kidneys. Testosterone treatment slightly increased by ∼1.6-fold AZ1 protein levels in female mice.

Influence of Testosterone on N1-SSAT, a Key Enzyme in the Retroconversion Route

Analysis of N1-SSAT mRNAs in kidneys of control, untreated, and testosterone-treated female mice. The polyamine pathway is tightly regulated to avoid the cytotoxic effect due to polyamine accumulation. As ODC gene expression was dramatically stimulated and polyamine levels were enhanced in testosterone-treated female mouse kidneys, one would expect the retroconversion route, controlled by the enzyme N1-SSAT, to be active. Northern blot analyses using a probe specific for N1-SSAT revealed a single 1.1-kb N1-SSAT mRNA in the kidney of all mice (Fig. 6). In untreated mice, N1-SSAT mRNA was barely detectable, contrasting with our results for ODC and AZ1 mRNAs. In female kidneys, testosterone progressively increased N1-SSAT mRNA levels, which reached a plateau on day 3 of treatment (Fig. 6).

DISCUSSION

It has been known for several years that testosterone regulates the ODC gene in the mouse kidney. In some studies, ODC mRNA, protein, and enzyme activity were analyzed in whole kidney extracts, thereby discarding the notion of the anatomic heterogeneity of the kidney (13). In other reports, the use of histological approaches, e.g., in situ hybridization, immunocytochemistry, and autoradiography, provided more insight into the localization of ODC in the kidney. Within the male nephron, the proximal tubule was identified as the only site containing ODC mRNA (7) and protein (31, 36). Recent data have shown that ODC activity is inequally distributed along the proximal tubule, as it decreased sharply from the pars convoluta toward the terminal portion of the pars recta (21). By contrast, no ODC activity was detected along the female mouse nephron, even when a very sensitive radiolabeling method was used (20). However, in female mice, androgens induced ODC gene expression, specifically in the whole cortex and the outer stripe of the outer medulla, as visualized by in situ hybridization (7, 20) and immunohistochemistry (17).

In this paper, we analyzed for the first time in single, isolated proximal tubules (a few hundred cells corresponding to 0.5- to 1-mm tubule) microdissected from kidneys of female mice, the time course of ODC activity, as induced by testosterone over 1–5 days. This fine approach revealed that the induction of ODC activity differs temporally between the subsegments of the proximal tubule. In PCT, ODC activity increased quite linearly during the course of the hormonal treatment, whereas in CPST it increased sharply and progressively during the first 3 days, then remained constant. Furthermore, ODC activity was higher in CPST than in PCT isolated from androgen-treated females. The increased ODC activity induced by testosterone in the tubules is associated with a concomitant increase in both ODC mRNA (Fig. 1) and protein levels (Fig. 2). This is in agreement with in situ hybridization experiments (20). In female mice treated for 5 days with testosterone, microscopic observations revealed that ODC was exclusively expressed in PCT, CPST, and OSPST cells. The distribution along the proximal tubule of ODC mRNA (visualized by in situ hybridization) and ODC activity in testosterone-treated female mice completely differed from that observed in untreated male mice (20, 21). Indeed, in adult male kidneys, physiological testosterone levels preferentially induce ODC gene expression in the PCT, not in the straight portions of the proximal tubule (20). However, this is clearly not the case in testosterone-treated female mice, raising the question of whether the preferential overexpression of the ODC gene in the PST was due to supraphysiological levels of testosterone.

Physiologically, the high ODC activity detected in PCT and PST totally depends on the availability of the substrate L-ornithine. Several potential sources of

Fig. 6. Analysis of spermidine/spermine-N1-acetyltransferase (N1-SSAT) mRNA levels in kidney of male and untreated and testosterone-treated female mice as determined by Northern blotting. Mice were treated as indicated in the legend to Fig. 1. Left: N1-SSAT mRNAs were detected on the same membrane than that used to probe ODC and β-actin mRNAs (see Fig. 1). Right: quantitation of N1-SSAT mRNA levels, as estimated after scanning densitometry of the membrane (as indicated in the legend to Fig. 2). Values are means ± SE for male (n = 3), untreated female (n = 4), and 5-day testosterone-treated female mice (n = 3); n = 2 for the others.

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L-ornithine can be considered: 1) L-ornithine is present in the blood, which is filtered in the glomerulus and reabsorbed along the PCT; 2) L-ornithine can be taken up via the basolateral carriers of PCT and PST cells; 3) L-ornithine is produced from L-arginine and L-glycine by the enzyme glycine amidino transferase (EC 2.1.4.1), which has been localized in the proximal tubule (unpublished data); and, finally, 4) intracellularly, arginase AII, highly active in the female mouse PST, converts L-arginine into urea and L-ornithine (Levillain O, unpublished observations). It is likely that, in vivo, L-ornithine is supplied to ODC in sufficient amounts from these different sources and that L-ornithine availability does not constitute a rate-limiting factor for Put synthesis. As ODC activity was increased in isolated PCT and PST from androgen-treated mice, a major increase in Put synthesis was also expected in these tubules. Although we measured the three main polyamines in whole kidneys rather than in isolated tubules, variations in renal Put levels are likely to reflect the physiological ODC activity of PCT and PST in vivo. As expected, we observed a drastic increase in Put levels in female kidneys on the first 2 days of hormonal treatment. The lack of a tight correlation between ODC activity and renal Put level between days 3 and 5 could be explained as follows. Put could be exported, then stocked in the blood or excreted in urine, and therefore could be absent from kidney cells (32). In addition, Put could be metabolized into Spd and Spm or decarboxylated into GABA (6). Precise localization along the nephron of the different enzymes involved in polyamine metabolism was essential to elucidate the fate of Put in the proximal tubule.

In the adult male mouse, physiological testosterone levels specifically regulate ODC gene expression in the proximal tubule. Until now, however, whether testosterone also regulates N1-SSAT gene expression has remained unknown. Our data clearly show that the level of N1-SSAT mRNA did not differ in the whole kidney of untreated mice. Because the female mouse does not synthesize testosterone, it seems unlikely that this androgen hormone regulates N1-SSAT gene expression. An alternative possibility might be that testosterone regulates N1-SSAT gene expression in males, whereas, in the absence of testosterone, another hormone or unknown factor controls N1-SSAT gene expression in females. A recent study supports this idea. N1-SSAT gene expression does seem to be regulated by testosterone in male mice, as N1-SSAT mRNA levels decreased dramatically in castrated male mice and were restored to the level of uncastrated male mice after testosterone injection (3). In addition, we show here for the first time that injection of pharmacological doses of testosterone to female mice enhances the levels of N1-SSAT mRNAs in the kidney. Taken together, these results strongly suggest that N1-SSAT gene expression is under the control of testosterone, at least in part.

The main physiological role of AZ1 is thought to be the control of ODC protein levels by feedback regulation (11). However, it is surprising to find similar amounts of both AZ1 mRNAs and protein in untreated male and female mouse kidneys. Indeed, a high level of ODC activity is present in male kidney (3, 12, 33) and nephron (21), whereas no ODC activity is detectable in the nephron of untreated female mice (20). Further experiments are needed to understand why AZ1 levels are the same in male and female mouse kidney. The second surprising result is the progressive increase in AZ1 mRNA and protein levels during the course of hormonal treatment. This result suggests that AZ1 gene expression is regulated either directly by testosterone or indirectly by polyamines resulting from testosterone-stimulated ODC activity. However, because AZ1 synthesis is known to be inhibited by cycloheximide and not by actinomycin D (23), regulation of AZ1 gene expression by testosterone is likely to take place at the posttranscriptional level.

Our study reveals that the N1-SSAT gene was equally expressed in male and female mouse kidney, whereas the main gender difference lay with ODC gene expression. The physiological consequence of a lack of ODC expression in the female nephron is a low content of Put in the kidney. If Put is essential for physiological events and/or for Spd and Spm synthesis in renal cells, it has to be synthesized and carried to renal cells from other tissues. Whether the female mouse nephron metabolizes Put into higher polyamines remains unknown, but if not, Spd and Spm have to be transported into renal cells. One of these two scenarios must clearly occur, because similar high levels of Spd and Spm were found in male and female kidneys. The presence of N1-SSAT and PAO in male and female kidneys (14) indicates that catabolism of Spd and Spm takes place and leads to Put production. However, it seems that the interconversion pathway is not sufficient to compensate for the lack of ODC activity and to supply enough Put to the female mouse kidney.

In conclusion, we have unraveled new mechanisms by which testosterone regulates the renal polyamine pathway. Testosterone induces a differential overexpression of ODC in PCT and PST and probably acts by different mechanisms to regulate ODC gene expression in the two tubules. Testosterone levels (physiological vs. pharmacological) may be a decisive factor in the induction of ODC gene expression in the early and late portion of the proximal tubule. How testosterone enhances AZ1 and N1-SSAT mRNA levels remains to be elucidated.

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