Sex-differential expression of ornithine aminotransferase in the mouse kidney

Olivier Levillain,1 Gabrielle Ventura,2 Henri Déchaud,3 Maya Hobeika,1 Anna Meseguer,4 Christophe Moinard,2 and Luc Cynober2,5

1Université Claude Bernard Lyon I, Faculté de Médecine Lyon RTH Laennec, Laboratoire de Physiopathologie Métabolique et Rénale, Institut National de la Santé et de la Recherche Médicale (INSERM) U 499, Lyon; 2Laboratoire de Biologie de la Nutrition, EA 2488, Faculté de Pharmacie, Paris; 3Hôpitaux Civils de Lyon: Service de Radioanalyse, Centre de Médecine Nucléaire, Hôpital Neuro-Cardiologique and INSERM Equipe Mixte de Recherche 3022, Lyon; 4Grup de Fisiopatologia Renal, Centre d’Investigacions en Bioquimica y Biologia Molecular, Institut de Recerca Vall d’Hebron, Hospital Universitari Vall d’Hebron, Barcelona, Spain; and 5Hôtel-Dieu, Service de Biochimie, Assistance Publique des Hôpitaux de Paris, Paris, France

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Levillain O, Ventura G, Déchaud H, Hobeika M, Meseguer A, Moinard C, Cynober L. Sex-differential expression of ornithine aminotransferase (Oat) in the mouse kidney. Am J Physiol Renal Physiol 292: F1016–F1027, 2007; doi:10.1152/ajprenal.00408.2006.—The mouse kidney expresses the gene of ornithine aminotransferase (Oat). Previous works suggest that Oat is differentially expressed in female and male mouse kidney (Alonso E, Rubio V. Biochem J 259: 131–138, 1989; Levillain O, Díaz JJ, Blanchard O, Déchaud H. Endocrinology 146: 950–959, 2005; Manteuffel-Cymborowska M, Chmuryńska W, Peska M, Grzelakowska-Sztabert B. Int J Biochem Cell Biol 27: 287–295, 1995; Natesan S, Reddy SR. Comp Biochem Physiol B Biochem Mol Biol 130: 585–595, 2001; Yu H, Yoo PK, Aguirre CC. Tsoa RW, Kern RM, Grody WW, Cederbaum SD, Iyer RK. J Histochem Cytochem 51: 1151–1160, 2003). This study was designed to provide a detailed description of the sexual dimorphism of Oat expression in the mouse kidney and to test the influence of sex hormones on its regulation. Experiments were performed on male and female Swiss OF1 mice during their postnatal development, at adulthood, and in orchidectomized and ovariectomized mice. Kidneys, dissected renal zones, and mitochondria were used to analyze OAT mRNA and protein levels and measure OAT activity. The results revealed that before puberty, Oat expression was similar between female and male kidneys whereas from puberty until adulthood Oat expression increased in the female kidney, becoming 2.5-fold higher than in the male kidney. This sex-differential expression of Oat was associated with a sex-specific distribution of Oat along the corticopapillary axis and within the nephron. OAT was three- to fourfold more expressed in the female than the male cortex. In males, Oat was highly expressed in the medulla, mainly in the thick ascending limbs. Renal Oat distribution in orchidectomized mice resembled that in the females. Ovariectomy did not influence Oat expression. Sex differences are explained by the physiological increase in plasma testosterone in males. Expression of medium-chain acyl-CoA synthetase protein confirmed this finding. We report sexual dimorphism of Oat expression in the mouse kidney and show that Oat is naturally downregulated in the presence of testosterone.

The kidney is known to play an important role in whole-body l-amino acid homeostasis. In addition to its essential function in l-amino acid reabsorption which takes place in the proximal tubule, the kidney expresses genes encoding for a multitude of enzymes involved in l-amino acid anabolism and catabolism. These enzymes are differentially expressed in the kidney and exhibit specific distribution patterns along the nephron. The renal expression of several enzymes depends on the species studied and varies in intensity according to sex or nutritional and hormonal balance.

Over the last few years, research has been focused on the metabolism of l-arginine in the rodent kidney. L-Arginine is catabolized by arginase type II (EC 3.5.3.1) into urea and l-ornithine and by glycine:arginine amidinotransferase (GAT; EC 2.1.4.1) to produce l-ornithine and guanidinoacetic acid (Fig. 1). L-Ornithine is an intermediate metabolite that acts as a precursor for the biosynthesis of polyamines and l-amino acids (e.g., l-proline, l-glutamate, and l-glutamine). Polyamine anabolism is controlled by the cytosolic ornithine decarboxylase (ODC; EC 4.1.1.17) that decarboxylates l-ornithine to generate putrescine. In contrast, in another metabolic pathway controlled by the mitochondrial ornithine aminotransferase (Oat; EC 2.6.1.13), l-ornithine is transaminated in the presence of α-ketoglutarate to produce one molecule of l-glutamate and the unstable compound glutamate-γ-semialdehyde that is spontaneously converted into Δ1-pyrroline-5-carboxylate (Fig. 1) (44). This latter molecule is further metabolized by the enzyme pyrrolidine-5-carboxylate dehydrogenase into a second molecule of l-glutamate. Within renal cells, the metabolic behavior of l-glutamate depends on cell requirements and the presence of the cytosolic glutamine synthetase (GS; EC 6.3.1.2) and glutamate decarboxylase (EC 4.1.1.15) and the mitochondrial glutamate dehydrogenase (GLDH, EC 1.4.1.2), which generate l-glutamine, GABA, and α-ketoglutarate, respectively (Fig. 1) (44, 45). Finally, the metabolic behavior of l-ornithine depends on the expression of Odc and Oat in each nephron segment, and more specifically, in each renal cell type.

In the rodent kidney, although the expression of Odc has been abundantly documented in terms of species, sex, localization, and hormonal regulation, there are still few published studies on the expression of Oat mainly in mice. There is a marked sexual dimorphism in the expression of Odc in the kidneys of different mouse strains. Odc is highly expressed in the proximal convoluted tubule (PCT) and to a lesser extent in the renal medulla, mainly in the thick ascending limbs. Renal Oat distribution in orchidectomized mice resembled that in the females. Ovariectomy did not influence Oat expression. Sex differences are explained by the physiological increase in plasma testosterone in males. Expression of medium-chain acyl-CoA synthetase protein confirmed this finding. We report sexual dimorphism of Oat expression in the mouse kidney and show that Oat is naturally downregulated in the presence of testosterone.

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extent in the proximal straight tubule (PST) of male Swiss mice (21), whereas no ODC activity could be detected in the different segments dissected from the female Swiss mouse nephron (19). The lack of constitutive Odc in the female nephron associated with an intense decarboxylation of \( L \)-ornithine in the production of testosterone naturally downregulates the expression of Oat in female and castrated male mice while surgical injection of pharmacological or supraphysiological doses of the hormonal regulation of sexes still remains undocumented. At present, all knowledge of the renal distribution of expression of Oat in the mouse kidney has been reported by different authors who independently measured OAT mRNA and protein levels or OAT activity (1, 18, 27, 31, 46). Taken together, these studies provide strong evidence of a differential expression of Oat in female and male mouse kidneys. However, the renal distribution of Oat in the mouse kidney of both sexes still remains undocumented. At present, all knowledge of the hormonal regulation of Oat in the mouse kidney is restricted to a single hormone: recently, we reported that the injection of pharmacological or supraphysiological doses of exogenous testosterone decreased the renal levels of OAT protein in female and castrated male mice while surgical orchidectomy led to a sharp increase in OAT protein levels (18). However, it remains to be proven that the endogenous production of testosterone naturally downregulates the expression of Oat in the male mouse kidney. Similarly, it is still unknown whether female sex hormones can influence the expression and distribution of Oat in the mouse kidney. In addition, given that the sexual dimorphism in the expression of Odc in the mouse kidney is associated with a sex-specific distribution pattern along the nephron, it is therefore necessary to test whether the expression and distribution of Oat in the mouse kidney are influenced by sexual dimorphism.

The present study was designed to provide a detailed description of the sexual dimorphism of Oat expression in the mouse kidney and to test the influence of male and female sex hormones on Oat expression in the kidneys of both sexes. To achieve these objectives, OAT mRNA, protein, and enzyme activity were analyzed in the kidneys of adult male and female mice, in isolated renal mitochondria, in the different dissected renal zones, during postnatal development, and in orchidectomized and ovariectomized mice. Experiments were performed for the expression of Oat during postnatal development to test the influence of the hormones produced during the important physiological events of weaning and puberty. Finally, an attempt was made to understand and identify the physiological roles of OAT.

MATERIALS AND METHODS

Animals and Treatment

Eight- to nine-week-old adult female [30–32 g body wt (BW)] and male [35–40 g BW] OF-1 Swiss (IOPS Caw) mice from Charles River Laboratories (L’Arbresle-sur-Orge, France) were given free access to tap water and standard laboratory food (Souvifrat, 20% protein, Genthon, France). The animals were housed in a room maintained at 20°C with a 12:12-h light-dark cycle. Mice were anesthetized (ip) using 0.1 ml/30 g body wt pentobarbital sodium diluted 1:2 in 0.9% NaCl solution (Nembutal 6%, Clin Midy, Paris, France). Animal care complied with French regulations for the protection of animals used for experimental and other scientific purposes and with European Community regulations. The author (O. Levillain) is authorized by the French Government to use animals for these experiments (authorization no. 69–33).

Pregnant OF-1 Swiss (IOPS Caw) mice obtained from Charles River Laboratories 3 days before giving birth were housed individually in breeding cages and with free access to tap water and standard laboratory food (Souvifrat, 20% protein). Newborn male and female mice were anesthetized 11, 15, 20, 24, 30, 35, 40, and 49 days after birth. Pups were weaned on day 21, and at least three pups were killed at each time period. The right and left kidneys were collected and decapsulated, and the blood was removed with blotting paper (blood free). Both kidneys were placed in a sterilized Eppendorf tube, frozen, weighed, and conserved in liquid nitrogen. Additional male mice weaned on day 21 after birth were anesthetized at 22–31 days after birth as described above. Blood was sampled from the vena cava to determine the plasma level of testosterone.

Thirty-day-old male mice rather than older adult male mice were used to prevent the accumulation of testosterone in their tissues and plasma (37). Six male mice were subjected to orchidectomy (Charles River Laboratories). Eleven days later (i.e., 41 days after birth), the mice were killed and the right and left kidneys were collected to dissect the different renal zones [superficial cortex (Cs), deep cortex (Cd), outer stripe of the outer medulla (OS), inner stripe of the outer medulla (IS), inner medulla including the papilla (IM)] at 4°C under a stereomicroscope. Samples were frozen.

Twenty-nine- to thirty-one-day-old female OF-1 Swiss mice (18–20 g BW) were used to analyze the influence of ovariectomy on the renal expression of Oat. Eighteen mice were randomized into three groups of six mice: an untreated control group, a sham-operated group, and an ovariectomized group. Surgery was performed by
Charles River Laboratories. All mice were transferred to our laboratory 7 days later. Control and operated mice were killed 3 wk later as described above. The right and left kidneys were collected and treated as described above.

**Sampling of Blood and Plasma for Testosterone Analysis**

Blood was collected in the vena cava of male mice with a 25-G needle (Neolus, Terumo, Limonest, France) mounted on a 1-ml syringe (Terumo). Tubes were heparinized 1 day before the experiment and dried (heparin: Roche, Meylan, France). Blood was immediately transferred to an Eppendorf tube and centrifuged at 8,000 g for 10 min at 4°C. Plasma was stored frozen. Testosterone was measured by RIA after extraction by organic solvent and partition chromatography of the plasma samples as previously described (8).

**Isolation of Mitochondria**

All procedures were carried at 4°C. Mitochondria were isolated by differential centrifugation as previously described (17, 22). Briefly, blood-free kidneys of female and male mice were immersed in a buffer (250 mM sucrose, 10 mM Tris-HCl, pH 7.6, and 1 mM Na2EDTA), homogenized using a Potter-Elvehjem glass homogenizer PTFE pestle (Bellco Glass, Vineland, NJ), and centrifuged at 600 g for 10 min. The supernatant was centrifuged at 11,000 g for 10 min to collect the pellet of mitochondria, which was washed three times in the same buffer. Protein concentrations were determined using the Bradford protein assay (4).

**Tissue Preparation, RNA Extraction, and Northern Blot Analyses**

To analyze the influence of sex on renal OAT mRNA levels, blood-free kidneys of five male and five female mice were collected and frozen. To localize OAT mRNA in the female mouse kidney, blood-free kidneys of six female mice were sliced along the corticomedullary axis to dissect four renal zones (Cs, OS, IS, IM) at 4°C under a stereomicroscope. To obtain a sufficient amount of RNA, the whole IM of three female mice were pooled. The dissected renal zones were frozen.

Each frozen kidney and renal zone were homogenized in RNAxel solution (EuroBio, Courttaboeuf, France), and total RNAs were extracted according to the manufacturer’s recommendations. All steps have been previously detailed and reported (17). Ten-microgram RNA samples from renal zones and 15-μg RNA samples from kidneys were submitted to 1.2% agarose gel electrophoresis.

Membranes were hybridized with rat 32P-labeled cDNA corresponding to OAT (PUC-OAT EcoRI-EcorI, NCBI accession no. NM 022521) (30, 41). The cDNA inserts were labeled using the RTS RadPrime DNA labeling system (Invitrogen, GIBCO BRL, Cergy-Pontoise, France) and [γ-32P]dCTP. The hybridization procedure and detection of the amount of radioactivity hybridized to specific cDNA mRNA have been previously reported (17).

**mRNA Extraction and Quantification by QRT-PCR**

OAT mRNA levels were analyzed in male and female mice kidneys during postnatal development. Blood-free kidneys were collected as described above. Total RNA was extracted from the kidneys with TRizol reagent (Invitrogen) according to the manufacturer’s protocol. mRNAs were analyzed by real-time RT-PCR. RT was performed with 2 μg of total RNA using the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen). mRNA was quantified using the SmartCycler System (Cepheid, Sunnyvale, CA) according to the manufacturer’s procedures. PCR was performed with 1:100 dilution of the RT in the SYBRGreen Kit SmartCycler (Eurogentec, Angers, France) with the specific primers for the cDNA of interest: mouse OAT forward 5′-GGGCTCTTTGTAACCCATT-3′ and reverse primer 5′-AGATGGGTTCTTCCCTCCTT-3′; and 18S forward 5′-GTAACCCGTGGAACCCCAT-3′ and reverse 5′-CCATC-

**CACTCGGATGTCAGG-3′ primer (32). As control for the RNA extraction and RT experiments, 18S primers were used in the same PCR conditions. Results were expressed as the ratio between the levels of OAT mRNA and 18S mRNA. Standard curves were generated by twofold serial dilutions. Curves were drawn on the basis of the log of the input RNA vs. the critical threshold cycle, which is the cycle in which the fluorescence of the sample was greater than the threshold of the baseline. These standard curves allowed for the critical threshold values to be converted to relative RNA concentrations for each sample.**

**Tissue Preparation for Western Blot Analyses**

A series of experiments was performed to analyze the influence of sex, postnatal development, and ovariectomy on renal OAT protein levels. Blood-free kidneys of male and female mice were collected and frozen. An additional series of experiments was performed to localize OAT protein in the mouse kidney. Blood-free kidneys were sliced along the corticomedullary axis to dissect five renal zones (Cs, Cd, OS, IS, and IM) at 4°C under a stereomicroscope. To obtain a sufficient amount of soluble proteins, pieces of Cs, Cd, and OS of two mice and the IS and IM of three male and four female mice were pooled and frozen.

**Protein Extraction and Western Blot Analyses**

Frozen kidneys, renal zones, and mitochondria were mixed at 4°C with a Turrax homogenizer at 100 mg frozen tissue/2 ml of lysing buffer containing protease inhibitor (16). After centrifugation, the concentration of soluble proteins was determined using the Bradford protein assay (4). Samples of soluble proteins (50 or 100 μg) were subjected to 10% PAGE containing 0.1% SDS using 6 W/gel. Protein transfer and equal loading of proteins were visualized on the membranes with Pierce S solution because we observed that β-actin and G3PDH were differentially expressed along the corticopapillary axis of the mouse kidney and in mouse kidneys during postnatal development (data not shown). Blocked immunoblots were incubated with primary polyclonal rabbit antibodies raised against rat/mouse-OAT (dilution 1:1,000), medium-chain acyl-CoA synthetase [Acsm; dilution 1:350 (2)], human-ODC (dilution 1:500, Eurodiagnostica, Paris, France), rat aldose reductase (AR; EC 1.1.1.21, dilution 1:3,000), and then with a peroxidase-conjugated anti-rabbit IgG secondary antibody (dilution 1:10,000) as previously described (17, 22). Antibody binding was revealed using a chemiluminescence (ECL) Western Blotting Kit. Details for ECL detection and quantitation of the bands have been mentioned earlier (17, 22).

**Measurement of OAT Activity**

OAT activity was measured in whole kidneys, dissected renal zones, and isolated mitochondria, and the second kidney of the mice was used to quantify OAT mRNA levels during postnatal development. Samples were mixed at 4°C with a Turrax homogenizer in a buffer (0.33 M sucrose, 5 mM HEPES, 1 mM EGTA, 1 mM DTT, and 0.5% Triton X-100). Homogenates were centrifuged at 10,000 g for 10 min at 4°C. The protein concentration was determined on the supernatant using the Bradford assay (4). OAT activity was determined using the enzyme assay previously described (11, 33). Briefly, the supernatant was incubated with a buffer composed of 75 mM potassium phosphate, pH 7.5, 20 mM t-ornithine, 0.45 mM pyridoxal phosphate, 5 mM α-aminobenzaldehyde, and 3.75 mM α-ketoglutarate for 15 or 30 min at 37°C. Blanks did not contained α-ketoglutarate. The reaction was stopped by adding trichloroacetic acid 40%. Samples were centrifuged 10,000 g for 3 min at 4°C, and absorbance was measured on the clear supernatant at 440 nm on a Hitachi U-1100 spectrophotometer (Meylan, France). Duplicate or triplicate samples and blanks were performed for all experiments. To determine the conditions for assay of OAT activity, the incubation time was tested.

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every 5 min up to 30 min and the protein concentration varied from 0 to 400 μg.

**Chemicals**

Salts and most chemicals, Ponceau S solution, secondary anti-IgG antibodies, and Kodak X-MAT film were purchased from Sigma (St. Quentin Fallavier, France). The protease inhibitor cocktail was purchased from Boehringer Mannheim (Strasbourg, France). Agarose Seakem GTG was purchased from TEBU (Le Perray-en-Yvelines, France). The ECL Western Blotting Kits, [α-32P]dCTP (9.25 MBq/25 μl), ImagerMaster Total Lab v2.01 program, and liquid scintillation counting mixture (Aqueous Counting Scintillant ACS II) were purchased from Amersham (Buckinghamshire, UK).

**Calculations and Statistical Analyses**

To determine the abundance of OAT protein during postnatal development, we proceeded as follows: after scanning of each Western blot and quantitation of the intensity of the bands, a value of 100% was attributed to the band with the highest intensity and thus constituted the reference. For the other bands, we calculated the relative percentage of OAT by comparison with the reference.

To determine the level of OAT protein in ovariectomized mice, the intensity of OAT and G3PDH bands was quantified and expressed as a percentage of the control group.

OAT activity was expressed in absorbance per 15 minutes per milligram soluble protein because we were unable to find a supplier from which buy P5C as a standard to convert the absorbance of P5C into molar units. Depending on the material used or the physiological interest in expressing the data in other units, OAT activity is expressed in absorbance per 15 minutes either per kidney, per milligram soluble mitochondrial proteins, or per gram fresh weight.

When appropriate, results are expressed as means ± SE, and statistical differences were assessed using the Kruskal-Wallis test at a significance level of 95%, and when appropriate this test was followed by the Mann-Whitney U-test at a significance level of 95% (StatView SE+Gr).

**RESULTS**

**Expression of OAT Gene in Female and Male Mouse Kidneys**

Oat expression was analyzed in kidneys of adult female and male Swiss mice by using semiquantitative techniques to measure OAT mRNA and protein accumulation and to determine OAT activity.

OAT mRNA levels were analyzed on Northern blots using a radiolabeled OAT cDNA probe. After hybridization, the OAT mRNA of 2.2 kb was detected that corresponds to the expected size of 2,125 bp [GenBank, access no. NM_016978]. Quantitation of OAT mRNAs in female and male mouse kidneys revealed that OAT mRNAs were significantly more abundant (1.93-fold) in the female than in the male kidney (Fig. 2A, Mann-Whitney U-test, *P* = 0.009). OAT protein levels were estimated by Western blotting. Our purified antibody raised against OAT detected a single band of 48 kDa that corresponded to the predicted size of mouse OAT (Swiss-Prot P29758 mouse: 48,354 Da) (Fig. 2B). OAT protein levels were 2.66-fold higher in the female compared with male mouse kidneys (Fig. 2B, Mann-Whitney U-test, *P* = 0.009) while aldose reductase (AR) protein levels were similar between the two groups (Fig. 2B, Mann-Whitney U-test, *P* = 0.076).

Before quantitation of OAT activity in kidneys, renal zones, and isolated mitochondria, we determined OAT assay linearity with incubation time and proteins. These experiments were performed with the soluble proteins extracted from either the whole male cortex or the female Cs. The spectrophotometric assay for OAT was found to be linear with time for 30 min (*r² = 0.9935 males and *r² = 0.9972 females*) and with protein up to 400 μg for the males (*r² = 0.9981*) and up to 300 μg for the females (*r² = 0.9958*) (data not shown). Using this well-validated OAT assay, soluble proteins extracted from female and male mouse kidneys were incubated to determine OAT activity. The results show that OAT activity was 2.34-fold higher in the female mouse kidneys compared with those of the males (Fig. 2C, Mann-Whitney U-test, *P* < 0.0039). It is useful to bear in mind that male mouse kidneys are naturally heavier than female mouse kidneys (right kidney: 268 ± 13.8 vs. 145.2 ± 5.4 mg, respectively; Mann-Whitney U-test, *P* < 0.0039). Given that an homogenate of each whole kidney was performed to analyze OAT activity, we were allowed to estimate the total OAT activity per kidney. As expected, the total OAT activity in male mouse kidneys was almost equal to that of the female, although OAT activity was still 21% higher in the latter than in the former (Fig. 2D, Mann-Whitney U-test, *P* < 0.0039). Since OAT is imported into the mitochondrial matrix, a single experiment was performed to analyze the expression of OAT in these particles. Mitochondria were isolated from kidneys of female and male mice to quantitate OAT protein level and OAT activity. In mitochondria, OAT protein levels were 2.33-fold higher in the female mitochondria than those of the males (Fig. 2E, left and middle). Similarly, mitochondria isolated from female mouse kidneys exhibited a threefold higher OAT activity that those of the males (Fig. 2E, right, Mann-Whitney U-test, *P* < 0.0012). Taken together, these results provide strong evidence that Oat is expressed more in the female mouse kidney than in male mouse kidney. This sex difference in Oat expression was almost totally compensated for by the higher renal mass in males compared with females.

**Expression of Renal OAT Gene During Mouse Postnatal Development**

These series of experiments were designed to determine the stage of postnatal developmental where the sex-differential expression of Oat reported in the adult mouse kidney occurs. We hypothesized that puberty would be a better candidate period than weaning since an injection of testosterone into female mice downregulates the renal expression of Oat (18). To test our hypothesis, age-matched male and female mice were killed between 11 and 49 days after birth.

**Morphometric parameters.** During the period 10–24 days after birth, male and female mice grew at the same rate since their body weight did not differ. Weaning (21 days after birth) did not modify the rate of growth. In contrast, during the 24- to 30-day period that corresponds to puberty, body weight increased sharply, males growing faster than females. Thereafter, male mice were constantly heavier than the females. The time course of kidney growth strongly resembled that of body weight. Weaning did not induce sex differences in kidney weight. In contrast, from day 24 until adulthood, the kidneys of the male mice grew about twofold faster than the kidneys of the females. The calculations of relative renal mass (left and right
Fig. 2. Ornithine aminotransferase gene (Oat) expression in female (F) and male (M) mouse kidneys. A: renal levels of OAT mRNA were analyzed by Northern blotting. Fifteen-microgram total RNA samples were submitted to 1.2% agarose gel electrophoresis (left). Quantitation of OAT mRNA was performed (right) as described earlier (17, 22). Values are means ± SE; n = 5 mice. *P = 0.009 by Mann-Whitney U-test. B: renal levels of OAT protein were analyzed by Western blotting. Fifty-microgram soluble protein samples were subjected to 10% SDS-PAGE. Representative immunoblot was probed with rabbit anti-OAT and rabbit anti-aldose reductase (AR) antibodies, and binding was revealed as described earlier (17, 22). AR was used as a control for protein loading and transfer (left). Quantitation of OAT protein was performed (right) as described earlier (18, 22). Values are means ± SE; n = 6 mice. *P < 0.0039 by Mann-Whitney U-test. C: OAT activity. Fifty-microliter soluble protein samples were incubated for 15 min at 37°C. Values are means ± SE; n = 6 mice. *P < 0.0039 by Mann-Whitney U-test. D: estimation of total OAT activity per kidney. For each kidney, the OAT activity measured in C was multiplied by the volume of extraction buffer that was proportional to kidney weight. Values are means ± SE; n = 6 mice. *P < 0.0012 by Mann-Whitney U-test. E: expression of OAT in isolated renal mitochondria. One hundred-microgram soluble protein samples of mitochondria were analyzed as described above; n = 1 mouse (left). Middle: quantitation of OAT protein (see above). OAT activity was measured in isolated mitochondria. Twenty-five or 100 μl of mitochondrial extract were incubated for 15–30 min at 37°C. Values are means ± SE; n = 8 samples for each mouse. *P < 0.00012 by Mann-Whitney U-test.

kidney weights/body weight) confirmed that kidneys from males were larger than kidneys from females (data not shown).

Oat mRNA level. The levels of OAT mRNAs were analyzed by QRT-PCR in one kidney of one male and female mouse killed at each time point in postnatal development (Fig. 3A). The steady-state abundance of OAT mRNAs was relativized to that of 18S rRNAs as an internal control. The results showed that OAT mRNAs were already detectable in kidneys 11 days after birth. During the 11- to 24-day period, OAT mRNA levels was fairly similar between male and female mouse kidneys. Thereafter, OAT mRNA levels varied with age, and changes were far more pronounced in the kidneys of female mice than males. In females, OAT mRNA levels increased sharply from day 20 until reaching a plateau in adulthood. In striking contrast, the abundance of OAT mRNA in male kidneys peaked slightly at day 24 before decreasing to plateau at a threefold lower level than that of adult females.

Oat protein level. OAT protein levels were analyzed in the kidneys of three male and two female mice killed at each time point in postnatal development (Fig. 3, B–D). However, the quality of the transfer was constantly checked by staining the gels in Simply Blue Safe Stain (Invitrogen). A significant basal level of OAT protein was detected as early as day 11 after birth in the kidneys of male and female mice. In female mouse kidneys, OAT protein levels increased rapidly and almost linearly between days 11 and 30 to reach the plateau of adult levels (Fig. 3D). In male mouse kidneys, the abundance of OAT protein increased in parallel to that of the females from day 11 to day 24, where it stabilized for an additional 6 days, i.e., until day 30. Thereafter, OAT protein levels progressively decreased until day 40 and stabilized at a level ~60% lower than that of the females (Fig. 3D). The female-to-male ratio of OAT activity was 2.5, in good agreement with the values reported in Fig. 2B.

OAT activity. As previously observed, the level of OAT activity was in the same range in both male and female mouse kidneys during the 11- to 24-day period after birth. Between days 24 and 30, OAT activity sharply increased in the female
mouse kidneys to reach adult levels (Fig. 3E). In male mouse kidneys, OAT activity decreased from day 20 until day 49. At adulthood, OAT activity was about three times higher in the female mouse kidneys than in those of the males.

To test whether changes in Oat expression during postnatal development correlated with puberty in male mice, testosterone levels were measured in plasma of 22- to 31-day-old male mice. The results showed that testosterone levels were below 0.25 ng/ml in the plasma of 22- to 27-day-old mice (Fig. 4). Thereafter, plasma testosterone increased progressively to reach a level 4 and 11 times higher in 30- and 31-day-old male mice, respectively (Kruskal Wallis, P < 0.0019 and Mann-Whitney U-test, *P < 0.0023). Given that testosterone specifically induces the renal expression of several genes, we analyzed the expression of ODC and Acsm during postnatal development to support changes in plasma testosterone levels and prove the efficiency of this hormone. The 62-kDa androgen-dependent Acsm protein was not expressed in kidneys of mice aged 11–24 days (data not shown). A faint expression of Acsm protein was observed 27 and 28 days after birth (Fig. 5, A and B). Thereafter, the level of Acsm protein sharply in-

![Fig. 3. Oat expression during the postnatal development of the female and male mice. Age-matched newborn female and male mice were anesthetized 11, 15, 20, 24, 30, 35, 40, or 49 days after birth. Pups were weaned on the day 21 (arrow). Filled horizontal bar, period of puberty (estimated as days 25–30); ▼, males; △, females. A: level of OAT mRNA in mouse kidneys. Total RNA was extracted from the kidney of 1 mouse at each age using TRizol reagent. The mRNAs were analyzed by real-time PCR and quantified using the SmartCycler System. 18S mRNA was used as an internal control. B and C: representative immunoblot showing the abundance of OAT protein in the kidney of the female (B) and male (C) mice. Fifty micrograms of soluble proteins extracted from the kidney of 1 female and 1 male mouse were simultaneously separated by electrophoresis on 10% SDS-PAGE. Membranes were blotted with the rabbit anti-OAT antibody. Shown are representative results of 1 series of the 2 and 3 experiments for females and males, respectively. D: relative quantitation of OAT protein levels. Three films were analyzed, and the results are expressed as the percentage of the highest value for females (day 49). Values are means ± SE, n = 3. E: OAT activity. OAT activity was measured in the second kidney of the mice used to quantify OAT mRNA levels (see A). Fifty or 100 μl of soluble proteins were incubated for 15 or 30 min at 37°C to determine OAT activity.

![Fig. 4. Time course of plasma testosterone levels in 22- to 31-day-old male mice. Male mice weaned on day 21 after birth were anesthetized from 22 to 31 days after birth. Blood was collected in the vena cava and transferred in an Eppendorf tube, which was then centrifuged at 8,000 g for 10 min at 4°C. Plasma testosterone levels were determined by RIA after extraction by organic solvent and partition chromatography of the plasma samples as previously described (8). Values are means ± SE, with the number of mice given in parentheses. P < 0.0019 by Kruskal-Wallis followed by Mann-Whitney U-test. *P < 0.01, day 26 vs. day 28, 29, 30, and 31, respectively.]
increased from day 29 until day 31 (Fig. 5, A and B). During this period, OAT protein level increased between days 26 and 27 whereas it declined sharply from day 29 to day 31 (Fig. 5, A and B). These results depict the parallel changes in plasma testosterone and Acsm protein levels in the male mouse kidney during postnatal development and strongly support that Oat expression was downregulated in the presence of endogenous testosterone. Similar results were observed with the androgen-inducible Odc (data not shown).

Localization of OAT in Female and Male Mouse Kidneys

Given that expression and regulation of Oat in the mouse kidney revealed a sexual dimorphism, we hypothesized that the distribution of Oat within the nephron could also exhibit a sex-specific distribution pattern. To test this hypothesis, the renal zones were dissected to analyze OAT mRNA and protein levels and measure OAT activity.

Female mouse kidney. Northern blot analysis of total RNA extracted from the renal zones of the female mouse kidney allowed us to detect the 2.2-kb mRNA of OAT in each renal zone (Fig. 6A). The highest level of OAT mRNA was detected in the Cs. In the medullary zones, OAT mRNA levels were about one-half those found in the Cs (Mann-Whitney U-test, P < 0.00275). The data revealed the ubiquitous distribution and heterogeneous level of OAT mRNA within the female mouse kidney. To confirm this result, OAT protein levels were analyzed in the five dissected renal zones by Western blotting. However, the quality of the transfer was verified by staining the gels in Simply Blue Safe Stain. A single band of 48 kDa that corresponded to OAT was visualized in all renal zones (Fig. 6B). OAT protein distribution was heterogeneous within the kidney (Kruskal-Wallis, P < 0.005). A high level of OAT protein was found in the Cs, Cd, OS, and IS. Surprisingly, OAT protein levels in the Cs were not higher than in the OS,
as observed for OAT mRNA (Fig. 6, A and B). This finding was confirmed in another series of experiments (data not shown). OAT protein expression in the IM was particularly low and corresponded to 5% of the whole renal content. AR, a 36-kDa protein essentially expressed in the medullary and papillary collecting ducts, was used as a specific marker of the IS and IM (Fig. 6B) (38). Finally, in two series of experiments, OAT activity was quantitated in the five dissected renal zones. Significant and heterogeneous OAT activity was found in all renal zones (Fig. 6C, Kruskal-Wallis, \( P < 0.005 \)). The highest OAT activity was observed in the Cs and the lowest in the IM. OAT activity was similar in the Cp, OS, and IS, and 25–30% lower than that in the Cs.

**Male mouse kidney.** The levels of OAT mRNAs were analyzed by QRT-PCR (Fig. 7A). The steady-state abundance of OAT mRNAs was relativized to that of 18S rRNAs as an internal control. The results revealed the ubiquitous distribution and heterogeneous level of OAT mRNA within the male mouse kidney. OAT mRNA levels were low in the Cs, Cd, and OS, while the highest level was observed in the IS. Intermediate OAT mRNA levels were detected in the IM. Analysis of OAT protein in renal zones was assessed by Western blotting. The 48-kDa protein that corresponded to OAT was detected in all renal zones (Fig. 7B). The distribution pattern and abundance of OAT protein were similar to that of OAT mRNA (Fig. 7, A–C). OAT protein was distributed as follows: 10–15% of the whole renal content in each of the three zones (Cs, Cd, and OS), 35% in the IS, and 25% in the IM (Fig. 7C, Kruskal-Wallis, \( P < 0.036 \)). The 36-kDa AR was visualized only in the IS and IM, indicating the quality and the purity of the dissected samples (Fig. 7B). In two series of experiments, OAT activity was measured in the five renal zones (Fig. 7D). The results revealed that the distribution of OAT activity along the corticopapillary axis strongly resembled that of OAT mRNA and protein (Fig. 7, A–D). The level of OAT activity varied with renal zone (Kruskal-Wallis, \( P < 0.031 \)). Given that OAT mRNA, protein levels, and activity were highest in the IS, the MTAL might strongly express Oat. Taken together, these results provide strong evidence for a sex-specific distribution pattern of Oat expression in the mouse kidney (Figs. 6 and 7). To prove that the endogenous testosterone could be responsible for the sex-specific distribution pattern of Oat expression, the five renal zones were dissected from orchidectomized mice to quantitate OAT activity. The lack of plasma testosterone 11 days after orchidectomy has been previously reported (19). The results showed that orchidectomy led to a marked change in OAT distribution within the male mouse kidney (Fig. 7E, filled bars, Kruskal-Wallis, \( P < 0.017 \)) compared with that of the controls (Fig. 7E, open bars). The lack of testosterone provoked a sharp increase in OAT activity in the cortex and the outer medulla, and the distribution pattern of OAT activity strongly resembled that observed in the female mouse kidney, even when the results were expressed per gram fresh weight (Figs. 6C and 7E).

**Morphometric parameters.** The body weight of the untreated control and sham-operated mice did not differ (25.2 ± 0.8 vs. 25.1 ± 0.6 g, respectively, \( n = 6 \)). In contrast, orchidectomy enhanced the body weight of female mice by 15% compared with that of the sham-operated mice (28.9 ± 0.6 vs. 25.1 ± 0.6 g, respectively, \( n = 6 \), Mann-Whitney \( U\)-test, \( P < 0.05 \)). The mass of the two kidneys was significantly higher in sham-operated and ovariecctomized mice compared with that of controls (294 ± 8, 311 ± 10, and 266 ± 9 mg, respectively, \( n = 6 \), Mann-Whitney \( U\)-test, \( P < 0.05 \)).

OAT activity was quantitated in the left kidney, while OAT protein levels were analyzed in the right kidney. The relative level of OAT to G3PDH proteins was influenced by neither sham operation nor ovariectomy (Fig. 8, A and B). This result suggests that female sex hormones do not regulate Oat in the mouse kidney. This was confirmed by measuring OAT activity. When OAT activity was expressed per milligram protein, neither sham operation nor ovariectomy modified the level of OAT activity (Fig. 8C). However, because the kidneys of sham-operated and ovariecctomized mice were heavier than those of the controls, total OAT activity per left kidney was significantly higher in the two former than in the latter (Fig. 8D, Kruskal-Wallis, \( P < 0.0179 \) followed by Mann-Whitney \( U\)-test, \( P < 0.0212 \)). However, this small increase in OAT activity cannot be attributed to the absence of female sex hormones since it occurred in the sham-operated mice.

**DISCUSSION**

OAT plays crucial physiological roles in amino acid metabolism. Indeed, this enzyme is at the crossroads of several pathways, including those of L-arginine, L-ornithine, L-glutamate, L-glutamine, and L-proline. Consequently, the level of Oat expression might dramatically disturb several metabolic pathways and physiological functions. Under normal physiological states, several roles of OAT have been indexed (44, 45). This enzyme controls the concentration of L-ornithine and avoids inhibition of glycine:arginine amidotransferase activity due to an accumulation of L-ornithine. In the intestine, OAT produces L-ornithine for the synthesis of L-citrulline whereas in perivenous hepatocytes, the coexpression of OAT and glutamine synthetase contributes to ammonia detoxification (3). It has also been reported that L-proline is a source of L-ornithine in the kidney (42). Diseases are associated with either a defect or an overexpression of OAT. Gyrate atrophy of the choroid and retina is a rare metabolic autosomal recessive disease due to several mutations of the gene encoding for OAT. OAT deficiency leads to a 10- to 20-fold increase in ornithinemia, chorioretinal degeneration, and atrophy of type 2 muscle fibers (for references, see Ref. 10). Hyperornithinemia inhibits the rate-limiting step of creatine biosynthesis controlled by OAT and affects myocytes (34). Moreover, an inappropriate activation of Wnt/b-catenin signaling is implicated in the development of hepatocellular carcinoma and induce an upregulation of OAT, GS, and glutamate transporter genes (7). In addition, OAT might play a role in cancer because it shares the substrate L-ornithine with ODC and, in this way, OAT might affect the level of polyamines which are required for cell proliferation.
Recently, the interplay among OAT, ODC, and polyamines has been documented in kidneys of testosterone-treated mice (18, 20). The role of OAT in L-proline synthesis and thus in collagen production might be crucial in several diseases. In contrast, OAT has been proposed as a potential target for the treatment of hyperammonemia (39). Thus we believe that the sites of OAT expression in the mouse kidney might influence the physiological role of OAT in this organ.

Few studies have been conducted on Oat expression in the mouse kidney. This paper documents in detail the sex-differential expression and localization of Oat in the mouse kidney. The sex-differential expression of Oat in the mouse kidney seems to be due to higher levels of OAT transcripts in females than in males. Our results support the concept that Oat is naturally regulated at the transcriptional level by one or more factors. However, it remains unknown whether the half-life of OAT mRNA differs in kidneys of female and male mice or whether the rate of transcription of Oat was higher in the former than in the latter. Nevertheless, at the physiological level, the low expression of Oat in the adult male mouse kidney was almost completely compensated by the larger size of their kidneys compared with those of the females. The difference in kidney size is mainly due to the morphological modification of the PCT triggered by the endogenous production and action of testosterone (13). Indeed, we have observed that the male mouse PCT epithelium was ~40% thicker than that of the female and this difference was abolished by treating female mice with testosterone (Levillain O, unpublished observations).

Among the life events, it is clear that puberty changes hormonal status. During this period, Oat expression increased in the female mouse kidney whereas it decreased in the male mouse kidney. Changes in the expression of Oat during male puberty coincided with the onset of the endogenous production of testosterone 28 days after birth and the appearance of Ascm protein, which is very sensitive to androgens. This is strengthened by a recent study that reported production of testosterone and increase in renal ODC activity occurring simultaneously in 30-day-old but not 20-day-old CD1 male mice (37). On this basis, we concluded that, in the male mouse kidney, adequate levels of endogenous testosterone provoke a simultaneous downregulation of Oat expression and sharp increase in kidney weight. Both events are independent, but hypertrophy of the male kidney induced by androgens compensates for the low expression of Oat and probably that of other genes. At present, testosterone is an excellent candidate to explain the sex-differential expression of Oat in the mouse kidney. However, it is still unknown whether testosterone downregulates directly or indirectly the renal expression of Oat. This result is probably not restricted to the Swiss OF1 strain because Oat also seems to be differentially expressed in C57BL/6 (43), Swiss CD1 (36), and, to a lesser extent, CFW mice (27). Furthermore, in an attempt to understand whether female sex hormones affect and increase Oat expression in the female mouse kidney during postnatal development, mice were ovariecotomized. Indeed, regulation of Oat by estrogens (including 17β-estrogen) has been demonstrated in female rat kidneys (26, 29, 30). Unfortunately, our results support that female sex hormones do not regulate the expression of Oat in female mouse kidneys.

The sexual dimorphism of Oat expression in the mouse kidney led us to explore whether Oat exhibits a sex-specific
The use of dissected renal zones to analyze the expression of Oat is an excellent approach because each renal zone is characterized by the presence of typical nephron segments (14). The expression of Oat in the mouse kidney revealed an ubiquitous and sex-specific distribution pattern, which suggests that the level of Oat expression in the different nephron segments might vary in intensity. In female kidneys, the highest level of Oat expression occurred in the cortex, suggesting that the proximal tubules (PCT and CPST), which are the most abundant tubules in this zone, highly expressed Oat (35). The presence of OAT activity in the IS and the IM suggests that the MTAL, OMCD, and inner medullary collecting ducts (IMCD) might also express Oat. The deduced distribution of Oat within the female kidney corroborates strongly with the sites of oxidative decarboxylation of L-ornithine (19). In the male kidney, the low expression of Oat in the cortex and OS suggests that the proximal tubule is not the main site of L-ornithine transamination. In contrast, the high level of OAT activity in the IS and the IM suggests that the MTAL, IMCD, and papillary collecting ducts (PCD) express Oat. The sex-specific distribution of OAT protein within the mouse kidney has been confirmed by immunohistochemistry, but this technique did not allow quantitation of OAT protein in each kind of tubule (unpublished observations). Given that in the male mouse nephron, L-ornithine decarboxylation was blocked by difluoromethylornithine only in the proximal tubules, we hypothesized that Oat was expressed in the other nephron segments (21). As expected, Oat expression was essentially found in the medullary zones, suggesting that the thick ascending limbs and the collecting ducts of the male mouse highly expressed this gene. Surprisingly, Yu et al. (46) using in situ hybridization observed a preferential localization of OAT mRNA in the mouse proximal tubule. The discrepancy with our results cannot be explained. Interestingly, the influence of testosterone on the level of expression and the distribution of Oat in the adult male mouse kidney was clearly shown in orchidectomized mice. Not only the size of the kidneys and the thickness of PCT cells but also the distribution pattern of Oat expression within the kidney became similar to those of the females. Although the presence or lack of testosterone influences renal expression of Oat, the physiological regulation of Oat by testosterone in the mouse kidney deserves further comment. The promoter region of mouse and rat Oat has recently been sequenced (40, 41). Unfortunately, the consensus binding site for androgens has not been found on the Oat promoter. These data suggest that testosterone might indirectly regulate Oat expression in the kidney.
OAT is a 49-kDa nucleus-encoded protein imported into mitochondria to give the mature 48-kDa OAT polypeptide, as observed in this study. Cells of the different nephron segments contain mitochondria whose morphology, size, and abundance vary dramatically between tubules (12, 17, 22, 35). Mitochondria of the collecting ducts are very small, and their number decreases as the distance from cortex increases. In the IMCD, mitochondria are randomly scattered throughout the cell (12). The high expression of Oat in the male IM that contains only a few mitochondria appears paradoxical. We hypothesized that the mitochondria of IMCD were extremely enriched in OAT compared with those of the MTAL or female tubules. Alternatively, it is possible that OAT could be localized in another subcellular compartment or there may be an isoenzyme.

A comparative analysis of Oat expression in rats and mice illustrates the species- and sex-specific distribution of Oat in the kidney. In rat kidneys of both sexes, Oat was almost exclusively expressed in the proximal tubules with an increasing intensity of expression from the PCT to the OSPST. In addition, OAT protein levels were about three times higher in the female than in the male Cs, Cd, and OS (22). Two important species differences in the hormonal regulation of Oat should be underlined. Estrogens control Oat expression in the female rat kidney but not in mice whereas testosterone seems to be inactive on Oat expression in the rat kidney (11). It cannot be ruled out that other hormones, nutrients, or factors such as circadian rhythms or pathologies can regulate Oat in the mouse kidney.

The marked sexual dimorphism in the expression of Oat within the mouse kidney might be associated with biochemical and physiological events specific to the nephron segment considered. The metabolic behavior of l-ornithine depends on the expression of other enzymes that belong to an established metabolic pathway. For example, l-ornithine might be a source of l-glutamine in the female proximal tubule because glutamine synthetase, which converts l-glutamate into l-glutamine in the presence of ATP and one ammonium ion, is expressed throughout the proximal tubule (Fig. 1) (15, 25). Although the functionality of this pathway remains to be documented, in female PCT, the mitochondrial glycine:arginine amidotransferase might be the endogenous source of l-ornithine, while in PST, l-ornithine might be provided by the mitochondrial arginase type II. Interestingly, both arginase type II and OAT are three- to fourfold more expressed in the female than in male mouse kidneys (17). In contrast, in male IM that contains only a few mitochondria, OAT is a 49-kDa nucleus-encoded protein imported into mitochondria to give the mature 48-kDa OAT polypeptide, as observed in this study.

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