**Ornithine decarboxylase along the mouse and rat nephron**

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Levillain, Olivier, and Annette Hus-Citharel. Ornithine decarboxylase along the mouse and rat nephron. Am. J. Physiol. 274 (Renal Physiol. 43): F1020–F1028, 1998.—Renal arginase activity is a potent source of ornithine (Orn) for polyamine synthesis. Ornithine decarboxylase (ODC) was localized along the mouse and rat nephron by incubating viable nephron segments isolated by microdissection from collagenase-treated kidneys with or without D,L-2-(difluoromethyl)ornithine (DFMO), a selective inactivator of ODC. Tubules from either control or DFMO-treated animals were incubated with 100 µM L-[1-14C]Orn. In control mice, Orn decarboxylation occurred mainly in the proximal convoluted tubule (PCT). In DFMO-treated mice, Orn decarboxylation was dramatically reduced in PCT and in proximal straight tubules (PST). In rats, Orn decarboxylation also occurred predominantly in the proximal tubule. Addition of 10 mM DFMO to isolated tubules dramatically decreased Orn decarboxylation in PCT and in PST. Thereafter, ODC activity was demonstrated in permeabilized tubules. In Triton X-100-treated tubules from control mice, ODC was exclusively found in proximal tubules (PCT > PST). This ODC activity was strongly inhibited in DFMO-treated mice. In conclusion, the highest ODC activity was found in rat and mouse PCT, a segment devoid of arginase. We hypothesize that the filtered Orn, which is reabsorbed along the PCT, is the main source of Orn for ODC.

polyamines; isolated tubules; localization; proximal tubule; D,L-2-(difluoromethyl)ornithine; permeabilized tubules

ARGinine IS A KEY Amino acid involved in several metabolic pathways, including the formation of urea, nitric oxide (NO), guanidino compounds, and polyamines. It is generally assumed that most metabolic routes involving arginine have greater activity in the liver than in the kidney. By using a specifically designed microtechnique, we have demonstrated that, in rat and mouse, renal arginase synthesis takes place almost exclusively in the proximal tubule, with a progressively decreasing activity from the proximal convoluted tubule (PCT) to its medullary pars recta (18, 20). In both these species, renal arginase (EC 3.5.3.1), an enzyme which deaminates arginine into equimolar amounts of urea and ornithine (Orn), has been localized in the straight, but not the convoluted, part of the proximal tubule (17, 19) and in rat, but not mouse, collecting ducts (17). At present, the physiological role of renal arginase activity is unknown. Orn is not an end product of intermediary metabolism. It can enter three pathways: the urea cycle, polyamine synthesis, and transamination followed by oxidation of the products of transamination in tricarboxylic acid cycle. It can serve also as a precursor for proline synthesis.

In the urea cycle, ornithine carbamoyl transferase activity (EC 2.1.3.3) catalyzes the condensation of Orn with carbamoyl phosphate to form citrulline. This reaction is most active in liver (33) and in the small intestine (46) but does not occur in the kidney. The biosynthesis of polyamines is controlled by ornithine decarboxylase (ODC, EC 4.1.1.17), which decarboxylates Orn to putrescine (Fig. 1) (29, 40). High levels of this enzyme have been found in renal tissue. In the third pathway, Orn reacts with α-ketoglutarate to form glutamic acid–γ-semialdehyde and glutamate (Fig. 1). This reaction is catalyzed by ornithine aminotransferase (OAT; EC 2.6.1.13). Glutamic acid–γ-semialdehyde leads either to glutamate or to proline production (Fig. 1). However, proline formation by pyrroline-5-carboxylate reductase (EC 1.5.1.2) has never been demonstrated in the kidney. Thus, in renal tissue, Orn is a precursor of the polyamines, or it is transaminated by OAT.

Among the mammalian kidneys, those of male mice exhibit a high testosterone-inducible ODC activity (4, 11). Most of the quantitative studies designed to measure ODC activity have been performed on homogenates of the whole kidney (22, 28, 42). Furthermore, ODC has been localized in mouse kidney, using non-quantitative techniques such as autoradiography (30, 47), immunocytochemistry, (32), immunohistochemistry (13), and histochemistry (12). It has been found in PCT, distal convoluted tubules (DCT), and, to a lesser extent, in the medullary collecting ducts (30, 47). Koibuchi et al. (13, 14) observed ODC immunoreactive cells only in the PCT and the cortical proximal straight tubule (CPST or S2). ODC mRNA has been detected exclusively in the mouse PCT (6, 12, 15). Regardless of the different mouse species used, the published results differ not only as far as the distribution pattern of the ODC protein is concerned but also as to the localization of ODC mRNA (12, 13, 15). In none of these studies was ODC activity quantified in isolated viable nephron segments. Such qualitative measurement takes into account cell integrity, ornithine uptake by luminal and/or basolateral carriers, ODC activity, the different metabolic pathways of ornithine, the possible inhibition of ODC by antizyme (10), and the posttranscriptional regulation of ODC mRNA (31).

In the whole adult rat kidney, ODC is highly expressed (4, 43), but, to our knowledge, in this species, the precise localization and quantification of this enzyme along the nephron remain to be determined. In a brief report, Levine et al. (21) observed a higher ODC activity in the medulla than in the cortex. Rat ODC mRNA was expressed in the cortex and the outer medulla, but the highest level was found in the outer medulla.
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LOCALIZATION OF ORNITHINE DECARBOXYLASE IN KIDNEY

Fig. 1. Metabolic pathways of ornithine: 1) arginase, 2) ornithine decarboxylase, 3) ornithine aminotransferase, 4) nonenzymatic step, 5) pyrroline-5-carboxylate reductase, 6) proline oxidase, 7) pyrroline-5-carboxylate dehydrogenase, 8) pyrroline-5-carboxylate synthase, 9) glutaminase, 10) glutamate dehydrogenase, and 11) α-ketoglutarate dehydrogenase.

stripe of the outer medulla (3, 4, 43), a zone rich in proximal straight tubules (OSPST or S3). These data suggest interspecies differences in the localization of renal ODC.

The goal of this study was to investigate the fate of Orn in single, viable nephron segments, to localize ODC activity along the mouse and rat nephron, and to find out whether renal arginase is a source of the Orn used to form putrescine. ODC activity was measured on single, viable nephron segments isolated by microdissection and quantified, using micrometabolic techniques based on the production of 14CO2 (9, 16, 17). Orn transamination was started by adding 0.5 µl of MIP containing L-[1-14C] Orn for ODC.

Materials and Methods

Preparation of the kidney for microdissection of nephron segments. Male OF-1 Swiss mice (35–40 g body wt) from Iffa Credo (L’Arbresle sur Orge, France) had free access to tap water and standard laboratory diet (Souffrat, Genthon, France). Male Sprague-Dawley rats (150 g body wt) from Charles River (St. Aubin-les-Etbeuf, France) had free access to tap water and standard laboratory diet (A-04; Usine d’Alimentation Rationnelle, Epinay sur Orge, France).

Rats and mice were anesthetized with 0.1 ml/100 g body wt ip pentobarbital sodium (Nembutal, 6%; Clin Midy, Paris, France). For the rats, an osmotic diuresis was induced, and, some minutes later, the left ureter was ligated. The left mouse and rat kidneys were then prepared for microdissection, as previously described (17, 18). Kidneys were perfused with 4 ml of medium for incubation and perfusion (MIP, see below) containing 2.5 mg/ml collagenase for the rat and 1.5 mg/ml collagenase for the mouse, removed, and sliced along the corticomедullary axis. Small pyramids containing both cortical and medullary tissue were incubated at 30°C in MIP containing 1 mg/ml collagenase, bubbled with O2, and shaken for 10–20 min for the dissection of cortical segments and for 40–60 min for the dissection of medullary segments.

MIP contained (in mM) 137 NaCl, 5 KCl, 0.44 KH2PO4, 1 MgCl2, 0.8 MgSO4, 0.33 Na2HPO4, 1 CaCl2, 0.1% bovine serum albumin (BSA), 1% vitamin mixture, 20 HEPES, 6% dextran, and energy-providing substrates (5 glucose, 5 lactate, 10 acetate, 1 pyruvate, 2 glutamine). The pH was adjusted to 7.40 with NaOH. The osmolarity was 350 mosmol/kgH2O. Before use, MIP was saturated with O2.

After careful rinsing of the pyramids with MIP, tubule microdissection was performed in MIP at 4°C under a stereomicroscope. The following segments were identified as previously described (18): glomerulus, PCT, CPST or S2, OSPST or S3, descending thin limb (DTL), medullary and cortical thick ascending limb (MTAL and CTAL, respectively), and cortical, outer medullary, and inner medullary collecting duct (CCD, OMCD, and IMCD, respectively).

Glomeruli and tubules were transferred onto siliconized and BSA-coated hollow glass slides with 0.5 µl MIP. The samples were tightly sealed, using a glass coverslip, and the tubules were photographed for subsequent determination of their length. They were kept at 4°C until incubation.

Incubation procedure for viable nephron segments. Incubation was started by adding 0.5 µl of MIP containing l-[1-14C] Orn (230 Bq/sample; 1.85 MBq/µmol) to the tubules and DFMO where specified. The final concentration of labeled Orn was 100 µM, which is within the range of physiological plasma levels. The incubating chamber was again sealed by a glass coverslip containing a 2 µl droplet of KOH (350 mosmol/kgH2O) to trap the 14CO2 formed during the incubation. Samples were incubated in a water bath at 37°C for 70 min. The efficiency of 14CO2 recovery under these conditions has been previously demonstrated by adding a trace amount of 14CO3H– to the incubate and by measuring the kinetics of 14CO2 trapping as a function of pH (16). Then, KOH was removed, and radioactivity was measured.
Identification of nephron segments involved in ornithine metabolism: ODC and the oxidative pathway. For mice, the technique used to quantify Orn decarboxylation was verified by varying the amount of tissue (tubular length), the time of incubation, and the concentration of the substrate (Orn). In a first series of experiments, nine mice were used as a control, and in a second series of experiments, six mice were submitted to pretreatment with DFMO by giving 2% DFMO in tap water for 2 days as the sole drinking fluid. Tubules were incubated in MIP containing L-[1-14C]Orn and energy-providing substrates.

In an initial series of four experiments, rats were used to determine the pattern of Orn decarboxylation. Tubules were incubated in MIP containing L-[1-14C]Orn and the metabolic origins (26). Tubules were incubated in the presence of L-[1-14C]Orn and one of the following concentrations of DFMO (in mM): 0.5, 1, 2.5, 5, 10, 20, 40, and 80. Second, the different nephron segments were incubated in the presence of 10 mM DFMO and L-[1-14C]Orn to quantify the fraction of 14CO2 associated with the ODC activity.

Measurement of ODC activity in permeabilized nephron segments. Experiments were performed on control and 2% DFMO-treated mice, as described above. Animal preparation, kidney perfusion, microdissection, and transfer of the tubules were similar to that described above. The segments were permeabilized, using a detergent (Triton X-100). Each sample received an additional 0.5-µl droplet of the following hypotonic buffer (in mM): Triton X-100 (0.05%, wt/vol), 40 HEPES, 60 NaH2PO4, 0.2 pyridoxal-5-phosphate (PLP), 0.2 EDTA, 10 dithiothreitol (DTT), pH 7.40, and 205 mosmol/kgH2O (9). Triton X-100-treated samples were maintained for at least 60 min at 4°C. Incubation was started by adding 1 µl of a buffer containing (in mM) 20 HEPES, 30 NaH2PO4, 0.1 PLP, 0.1 EDTA, 5 DTT, 105 mosmol/kgH2O and L-[1-14C]Orn (460 Bq/sample; 1.85 MBq/µmol) to the tubules; the pH was adjusted to 7.40 (9). The final concentration of labeled Orn was 100 µM. The incubating chamber was sealed by a glass coverslip containing a 2-µl droplet of KOH. Samples were incubated in a water bath at 37°C for 70 min. The KOH was removed, and the radioactivity was measured.

Calculations. The amount of decarboxylated L-[1-14C]Orn was calculated from the amount of 14CO2 counted in the KOH droplet according to the following formula

\[
\text{cpm in sample} - \text{cpm in blank} = \text{SR} \times \text{tubular length} \times \text{incubation time}
\]

where control or "blank" is the radioactivity measured in samples containing labeled Orn but no tubules. The specific radioactivity (SR, in cpm/µmol) of Orn was measured under the same conditions. Results are expressed in femtomoles of 14CO2 produced per minute and per millimeter tubular length. Where appropriate, statistical differences between different conditions in a given experiment were assessed using the Student’s t-test for paired or unpaired data. Differences were considered when P < 0.05. For correlation analyses, the correlation coefficient r was calculated, and P was determined from Table 1 at the 99% level of significance.

Chemicals. Salts and most chemicals [40 mol dextran, mol wt 40,000; BSA fraction V, EDTA, DTT, PLP, Triton X-100, D-mannitol, L(+)-lactic acid, pyruvic acid, sodium acetate, Table 1. Effect of Triton on ornithine decarboxylation in isolated nephron segments dissected from either control or DFMO-treated mice

<table>
<thead>
<tr>
<th>Nephron segment</th>
<th>No Triton Treatment (Control)</th>
<th>Triton, 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCT</td>
<td>19.21 ± 0.88 [51]</td>
<td>10.80 ± 0.12 [48]</td>
</tr>
<tr>
<td>CPST</td>
<td>4.85 ± 1.03 [21]</td>
<td>2.72 ± 0.19 [30]</td>
</tr>
<tr>
<td>OSPST</td>
<td>3.15 ± 0.51 [15]</td>
<td>2.00 ± 0.12 [9]</td>
</tr>
<tr>
<td>MTAL</td>
<td>1.51 ± 0.08 [35]</td>
<td>0.61 ± 0.08 [41]</td>
</tr>
<tr>
<td>CTAL</td>
<td>2.13 ± 0.11 [25]</td>
<td>0.47 ± 0.08 [32]</td>
</tr>
<tr>
<td>CCD</td>
<td>2.86 ± 0.22 [33]</td>
<td>0.58 ± 0.13 [31]</td>
</tr>
<tr>
<td>OMCD</td>
<td>1.72 ± 0.13 [26]</td>
<td>0.61 ± 0.13 [28]</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SE in femtomoles of 14CO2 per minute and per millimeter tubular length. Number of samples is given in parentheses. For no treatment (control), N = 9 mice; for Triton control, N = 11 mice; for Triton DFMO, N = 4 mice. PCT, proximal convoluted tubule; CPST, cortical proximal straight tubule; OSPST, outer stripe proximal straight tubule; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; CCD, cortical collecting duct; OMCD, outer medullary collecting duct; ND, not done.

L-glutamine, and D-glucose] were purchased from Sigma (Saint Quentin Fallavier, France). DFMO was a gift from Marion Merrell Research Institute (Strasbourg, France). Collagenase from Clostridium histolyticum was bought from Worthington (Freehold, NJ); CLS II, 151 U/mg. Vitamins (100× MBE VT 00) were from Eurobio (Paris, France). L-[1-14C]Orn (1.85 GBq/mmol = 50 mCi/mmol) was purchased from Amersham International.

RESULTS

Orn decarboxylation along the mouse nephron. The validity of the technique used to quantify Orn decarboxylation was demonstrated by the following experiments. Orn decarboxylation increased proportionally with the tubular length in the range of 3–8 mm of PCT (r = 0.831, P < 0.01; Fig. 2A) and with an incubation time of at least up to 60 min (Fig. 2B). A dose-response curve was established with PCT in the presence of increasing concentrations of L-[1-14C]Orn ranging from 0.04 to 0.7 mM. Orn decarboxylation increased proportionally with the concentration of Orn in the range of 50–200 µM (Fig. 3). Thereafter, the production of 14CO2 tended to stabilize.

Because, in previous reports, ODC was found in both the renal cortex and medulla, we dissected proximal tubules, thick ascending limbs, and collecting ducts. The different nephron segments were able to decarboxylate Orn, however, with the higher activity found in PCT and the lower in CPST and OSPST (Fig. 4, solid bars). Because ODC is inactivated by DFMO, mice received 2% DFMO, which was added to the drinking water (pH adjusted to 7.00) for a period of 1, 2, or 3 days. We observed that 14CO2 formation was decreased to the same extent, regardless of the duration of the DFMO treatment (data not shown). The presence of ODC activity was demonstrated by addition of 2% DFMO to the drinking water of mice 2 days prior death. As expected, ODC was inhibited, and 14CO2 formation was decreased by ~80% in PCT and CPST and by 68% in OSPST (P < 0.05; Fig. 4, open bars). 14CO2 formation remained unaffected by DFMO in both MTAL and...
CTAL. In contrast to the above-mentioned segments, DFMO significantly increased Orn decarboxylation in CCD (P < 0.05, Fig. 4). Our results suggest that the renal ODC activity is mainly localized in the PCT. Because our findings were in good agreement with those reported in previous publications, the same experimental conditions were used to localize ODC activity along the rat nephron.

Orn decarboxylation along the rat nephron. Isolated nephron segments were incubated with L-[1-14C]Orn and energy-supplying substrates. We verified that, in rats, the production of 14CO2 was proportional to the incubation time and tubular length (data not shown). The results indicated that all the test segments decarboxylated Orn. The intensity of the reaction (in fmol·min⁻¹·mm or glomerulus⁻¹; means ± SE, n = no. of rats) was low in glomeruli (0.72 ± 0.23, n = 4), DTL (1.67 ± 0.20, n = 3), MTAL (1.33 ± 0.14, n = 4), and CTAL (1.52 ± 0.36, n = 4). Significantly higher values were observed in the collecting ducts with a decreasing gradient from the cortex to the inner medulla (see Fig. 5, solid bars). The highest rate of Orn decarboxylation occurred in the PCT, followed by the CPST and the OSPST (see Fig. 5, solid bars). This suggests that ODC activity might be widely distributed along the rat nephron but is mainly restricted to the proximal tubule.

To confirm that 14CO2 formation was associated with ODC activity, DFMO was added to the above described medium. In a first series of three experiments, we tested the DFMO concentration dependence of the release of 14CO2 from PCT and CCD. These two structures were chosen because of their distinct embryological origins and their high capacity to decarboxylate Orn. DFMO strongly inhibited 14CO2 formation from labeled Orn in PCT (Fig. 6A). At concentrations of 10 and 40 mM DFMO, 14CO2 formation was reduced by 80% and 96%, respectively; this demonstrates the presence of ODC activity in the PCT. In contrast, in CCD, increasing the DFMO concentration from 0.5 to 10 mM induced a parallel enhancement of Orn decarboxylation, which peaked at 10 mM DFMO (+42%, P < 0.05) (Fig. 6B). Higher concentrations of DFMO reduced the release of 14CO2 in CCD (−64% at 80 mM DFMO). To our knowledge, it has never been reported

Fig. 2. Influence of tubular length and incubation time on the ornithine (Orn) decarboxylation rate. Proximal convoluted tubules (PCT) were isolated from control mouse kidneys and incubated with L-[1-14C]Orn (100 µM) and energy-providing substrates. A: each symbol represents a sample; several PCT were pooled to increase the tubular length (n = 34 samples). B: PCT were incubated for 3 successive periods of 30 min, and cumulative results are shown. Data are means ± SE of 15 samples per point.

Fig. 3. Effect of ornithine concentrations on decarboxylation rate. PCT were isolated from control mouse kidneys and incubated with L-[1-14C]Orn and energy-providing substrates. Orn concentrations varied from 40 to 700 µM by increasing the amount of L-[1-14C]Orn; ■, means ± SE of 7 samples.
that DFMO enhances $^{14}$CO$_2$ formation, as was observed in our present experiments.

In a second series of five experiments, the different nephron segments were incubated in the presence of 10 mM of DFMO. This concentration was chosen to determine whether Orn decarboxylation could also be enhanced in OMCD and IMCD, as it was in CCD. Addition of DFMO significantly reduced Orn decarboxylation in all nephron segments tested, except for CCD (Fig. 5). The mean percentage of inhibition varied from one segment to another: 78% in PCT, 71% in CPST, 40% in OSPST, 51% in MTAL, 25% in OMCD, and 21% in IMCD. Because the highest rate of $^{14}$CO$_2$ production occurred in the proximal tubule, we concluded that, in rats, most of the renal ODC was localized in the PCT and, to a lesser extent, in the PST. In these experiments, a $^{14}$CO$_2$ formation was again enhanced in CCD in the presence or in the absence of DFMO correlated well with the data obtained in permeabilized tubules. This result validates the use of the intact tubule model. It is also demonstrated for the first time that the ODC activity can be measured in isolated, permeabilized nephron segments.

Localization of ODC activity along the mouse nephron. Viable nephron segments were incubated with L-[1-$^{14}$C]Orn and energy-providing substrates. Results are means ± SE ($n = no. of samples tested). Control mice ($N = 9$, solid bars) and mice treated with 2% D,L-2-(difluoromethyl)ornithine (DFMO) in drinking water 2 days prior to death ($N = 6$; open bars). CPST and OSPST, cortical and medullary straight part of the proximal tubule, respectively; MTAL and CTAL, medullary and cortical thick ascending limbs; CCD and OMCD, cortical and outer medullary collecting ducts. Percentage values were calculated from the mean values for the control group and DFMO-treated mice. *$P < 0.05$.

Fig. 4. Pattern of ornithine decarboxylase activity along the mouse nephron. Viable nephron segments were incubated with L-[1-$^{14}$C]Orn and energy-providing substrates. Results are means ± SE ($n = no. of samples tested). Control mice ($N = 9$, solid bars) and mice treated with 2% D,L-2-(difluoromethyl)ornithine (DFMO) in drinking water 2 days prior to death ($N = 6$; open bars). CPST and OSPST, cortical and medullary straight part of the proximal tubule, respectively; MTAL and CTAL, medullary and cortical thick ascending limbs; CCD and OMCD, cortical and outer medullary collecting ducts. Percentage values were calculated from the mean values for the control group and DFMO-treated mice. *$P < 0.05$.

Fig. 5. Localization of ornithine decarboxylase activity along the rat nephron. Tubules were incubated with L-[1-$^{14}$C]Orn (100 µM) and energy-providing substrates (control tubules, solid bars) and 10 mM DFMO (treated tubule, open bars). IMCD, inner medullary collecting duct. Data are means ± SE of 5 rats ($n = 5–6$ samples/experiment), except for OSPST (1 rat, $n = 10$ samples). Percentage values were calculated from the mean values of the control group and DFMO-treated tubules. *$P < 0.05$. 

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DISCUSSION

The present study was designed to localize renal ODC along the mouse and rat nephron to determine whether renal arginase and ODC are colocalized. Because most of the published studies have been performed on mice, we validated our technique in mice. Thereafter, ODC was localized along the rat nephron under similar conditions.

Technical aspects. ODC determinations were performed under more varied physiological conditions than those employed in previous studies (12–15, 22, 28, 30, 32, 42, 47), since we used isolated, viable, microdissected nephron segments and plasma concentrations of Orn (100 µM). Steady-state conditions were maintained for 60 min and with a range of 3- to 8-mm lengths of PCT. The small decrease in 14CO2 formation observed at 90 min (Fig. 2) could be explained by the reduction of L-[1-14C]Orn availability. Consequently, we decided to reduce the amount of tissue (e.g., for PCT, mean length = 5.25 mm/sample) and to incubate the samples for a period of 70 min. The use of micro dissected nephron segments guarantees the exact identification of the tubules involved in Orn metabolism and thus the precise localization of ODC along the nephron.

A fairly similar technique was previously used for the localization of arginase and arginine synthase activities along the mammalian nephron (17, 18). The fact that Orn could be metabolized by both ODC and OAT led us to develop a micromethod to measure ODC activity in micro dissected nephron segments, previously permeabilized with a hypotonic detergent solution. This technical approach guarantees the elimination of both the oxidative decarboxylation (Fig. 1) of Orn and the rate-limiting transport of Orn by the carriers located in the cell membranes.

Localization of ODC along the mouse and rat nephron. The present results allow, for the first time, the demonstration of the distribution pattern of Orn metabolism and ODC activity along the mammalian nephron. It is now clear that Orn is decarboxylated in the different nephron segments, including the proximal tubules, thick ascending limbs, and collecting ducts. 14CO2 from labeled Orn can be formed either by the ODC or by a reaction sequence initiated by OAT. However, the use of a selective inactivator of ODC, DFMO, clearly demonstrated that most of the 14CO2 originated from the decarboxylation of Orn by ODC. The uptake of DFMO into various tubular cells has been previously demonstrated by injecting mice intraperitoneally with either [5-3H]DFMO or [5-14C]DFMO. The labeled DFMO was recovered bound within the PCT, distal convoluted tubule (DCT), and collecting duct cells (30, 47, 48). Within the mouse and rat nephrons, 14CO2 production from L-[1-14C]Orn strongly suggests that ODC is predominantly found in the proximal tubule with a decreasing intensity from the PCT to the OSPST (Figs. 4 and 5). This result was confirmed by measuring ODC activity in permeabilized tubules from control and DFMO-treated mice. ODC activity was found exclusively in PCT and CSPT (Table 1). The data obtained for OSPST remains provisional because of the very great difficulty in dissecting this segment. In addition, ODC activity was very low in this segment. This finding correlates with the low density of ODC mRNA found in the outer stripe of the outer medulla of control and testosterone-treated mice (15).

In relatively good agreement with these findings, Pegg et al. (30) and Zagon et al. (47) observed that, in mice, the outer cortical region was more visibly labeled by [5-14C]DFMO than the inner medullary region; the radioactivity was mainly localized in the PCT and, to a lesser extent, in the DCT. In the medulla, the collecting ducts were 60–80% less radioactive than PCT (30, 47). More recently, ODC-immunoreactive cells have been localized in mouse PCT and PST. In one study, ODC seemed more confined to the PST (13); however, in a
second study, ODC immunoreactivity was strong at the luminal surface of PCT and relatively weak and homogeneously distributed in the PST (14). We also found a low ODC activity in the rat MTAL, confirming the findings of Charlton and Baylis (5), whereas the presence of ODC activity in the collecting duct was restricted to the rat OMDC and IMCD. Our findings correlate with the observations reported for the developing rat kidney (7) but are in disagreement with those of Levine et al. (21), who localized 71% of ODC in the rat medulla.

Comparison of ODC mRNA and ODC activity in mouse and rat kidney. An indirect method of localizing ODC is the measurement of the distribution of ODC mRNA. In the kidneys of control or testosterone-treated mice, the ODC gene was highly expressed in the PCT (6, 12, 15), the nephron segment which contained the highest ODC activity (Fig. 3). In contrast, in rats, transcripts of ODC were found in significant amounts in the renal cortex and inner stripe of the outer medulla (43), a result which correlates with the presence of ODC activity in cortical (PCT and CPST or S2) and medullary (MTAL) segments. But the highest level of ODC mRNA was found in the outer stripe of the outer medulla of the rat kidney (43), more precisely, in the OSPST (S3) (3, 4). There is also a discrepancy between the presence (43) and absence (3, 4) of ODC mRNA, at least in the cortical zone of the rat kidney.

It is expected that both protein activity and mRNA ought to be localized in the same nephron segment or cell. The difference in rat ODC protein and mRNA localization is surprising but does not exclude posttranscriptional regulation or inactivation of ODC by antizyme, a specific protein inhibitor of ODC (25). There is convincing evidence that the cellular content of ODC is regulated at the level of translation (31). In many experimental systems, the rate of ODC synthesis does not correlate with the level of ODC mRNA. The two major factors that influence the translation efficiency of ODC mRNA have been identified. The first is the polyamine content. In the case of ODC mRNA, inhibition of translation occurs at relatively low levels of polyamine. The second is the amount of phosphorylated eIF-4E, the active form of eIF-4E that binds to the cap structure of mRNAs (31). The combination of the polyamine content and active eIF-4E in cells is likely to regulate the synthesis of ODC (31).

Amino acid transport. Most of the filtered amino acids are reabsorbed by specific luminal carriers (39, 45) in the early portion of the PCT in the vicinity of the glomerulus (37, 38). Consequently, in later portions of the nephron including the middle of the PCT to the collecting ducts, luminal amino acid concentrations drop rapidly and reach very low levels. Thus peritubular amino acids constitute the major source of these compounds for the essential nutritional needs and metabolic pathways of the cells (38). Amino acid uptake by peritubular and paracellular (“backleak”) paths have not been extensively studied. However, on the one hand, specific peritubular carriers for acid, neutral, and basic amino acids have been demonstrated by means of a double-indicator dilution technique (8), peritubular microinjection of labeled amino acids (2), electrophysiological studies (35, 36), and isolated proximal tubules (1). On the other hand, except for the early PCT, if we consider the difference of amino acid concentrations between the basolateral interstitium and the tubular fluid for each tubule, this would constitute a driving force for the backleak. This path has been demonstrated for glycine (1) and other amino acids (38, 45).

Concerning the renal uptake of Orn, both luminal and basolateral transports have been demonstrated (1, 35).

On the basis of these reports, in our intact dissected tubules, Orn transport might occur preferentially via the basolateral membrane because of the large contact surface of the basal but not the apical membrane with the incubating medium. The luminal uptake of Orn, which takes place mainly in the early PCT, is probably very small unless the backleak worked. Consequently, Orn metabolism has been underestimated in PCT because of the reduced luminal access of Orn. This was confirmed by the twofold increase in $^{14}$CO$_2$ production in Triton X-100-treated PCT (Table 1). Surprisingly, in Triton X-100-treated CPST, the 4.5-fold increase in Orn decarboxylation indicates that 1) Orn availability was the most important rate-limiting factor and 2) ODC activity was only 1.6-fold lower than in PCT. In contrast, permeabilization of OSPST did not enhance the $^{14}$CO$_2$ production, suggesting a low ODC activity.

Comparative study of ODC activity and Orn metabolism. We compared our data with those of different authors who measured ODC activity on whole kidney homogenate in the presence of a high concentration of Orn (400 µM). The results are expressed in the same units (µmol CO$_2$ per h and per g protein), assuming that one millimeter of mouse PCT accounts for 175 ng protein (44). In control male CD1 mice, Tovar et al. (42) found a low ODC activity (0.16–0.25), whereas a higher value of 12.6 has been reported in the male Swiss mouse (22). The higher Orn decarboxylation (intact tubules, 20.9) and ODC activity found in our experiments (permeabilized tubules, 38.5) are easily explained by the fact that we used dissected nephron segments and that we measured ODC activity on permeabilized isolated tubules.

Our study has demonstrated the presence of interspecies differences and similarities in both Orn metabolism and ODC localization, as well as its activity. In both species, Orn was decarboxylated by the different segments: the intensity of the reaction was high in the proximal tubule, low in the thick ascending limb, and seemed to decrease from the cortex to the medulla (e.g., CPST > OSPST, CTA > MTAL, and CCD > OMCD > IMCD). ODC activity was about twofold higher in all rat nephron segments, compared with those of mice. In both species, on the basis of the heterogenous distribution of ODC along the proximal tubule and the dispersion of the data, it seems highly likely that ODC activity progressively declines along the PCT, but this could not be demonstrated. The presence of an ODC activity in the rat but not mouse MTAL and medullary collecting ducts confirmed interspecies differences.
Physiological source of ornithine. In vivo, several sources of Orn might be considered. The first hypothesis was that arginase and ODC were colocalized, as reported for lactogenesis (27). Unfortunately, we were unable to verify the connection between arginase and ODC by incubating tubules with L-[1-14C]arginine, since this amino acid is simultaneously metabolized by the renal arginine decarboxylase (24). However, from our previous results, it is clear that no arginase activity is present in the male mouse and rat PCT (17, 19), a tubule in which ODC activity reached the highest level. In this segment, three other sources of Orn might be considered for the PCT: 1) Orn is filtered in the glomerulus and reabsorbed along the PCT, 2) Orn can be taken up by basolateral carriers, and 3) glycine amidinotransferase (GAT) activity is localized in the PCT (41). In this latter case, GAT catalyzes the formation of guanidinoacetic acid and Orn from arginine and glycine. In contrast to PCT, mice and rat straight proximal tubules exhibit a very high arginase activity, which might provide Orn for ODC (17, 19). In CPST, the data presented in Table 1 clearly indicate that ODC activity was high, but putrescine synthesis was limited by both extracellular Orn concentration and Orn uptake. These limiting factors are probably compensated for by the colocalization of arginase and ODC. Intracellular hydrolysis of arginine by arginase contributes to enhance the local Orn concentration. In OSPST, although the arginine-to-ODC ratio is very high, the contribution of this segment in polyamine production seems negligible because of the very low ODC activity (Figs. 4 and 5, Table 1).

In summary, the different nephron segments of mice and rats metabolize Orn; in both species, the PCT exhibited the highest rate of Orn decarboxylation. The use of DFMO supported the argument that Orn decarboxylation was associated with ODC activity and allows us to confirm that the highest ODC activity is localized in the cortex of the rat and mouse kidney, more precisely, in the PCT followed by CPST, but not the OSPST. Renal arginase might be a source of Orn for the ODC localized in the CPST but not in the PCT.

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REFERENCES


