Localization by Immunohistochemistry of Renal Ornithine Decarboxylase in the Mouse with and without Testosterone Treatment

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Abstract

The immunohistochemical distribution of renal ornithine decarboxylase was studied in male mice both with and without testosterone treatment. Testosterone (1 mg per mouse) induced a marked increase in ornithine decarboxylase activity of the mouse kidney, whereas no significant immunohistochemical difference was observed either in immunoreactivity or its localization. In intact male as well as androgen-treated mice dense ornithine decarboxylase-immunoreactive cells were observed mainly in the cortex, especially many ornithine decarboxylase-immunoreactive cells were observed in the inner portion, while a much weaker immunoreactivity was observed in the medulla. The largest number of ornithine decarboxylase-immunoreactive cells seemed to be localized in the pars recta of the proximal tubule. The immunoreactivity was not detected in all the tubular cells but scattered among them. The renal corpuscles were not immunoreactive. In each ornithine decarboxylase-immunoreactive cell, the cytoplasm showed much denser immunoreactivity than the nucleus.

Ornithine decarboxylase (ODC), which catalyzes the formation of putrescine from ornithine, is the rate-limiting enzyme in the polyamine biosynthetic pathway. This enzyme is quite unique in that its activity fluctuates rapidly and greatly in response to various growth stimuli (Jänne *et al.*, 1978). In the male mouse kidney, a very

high concentration of ODC has been found and the activity of the enzyme is highly dependent on testosterone treatment (Henningsson and Rosengren, 1975; Henningsson *et al.*, 1978). However, it is rather difficult to detect ODC activity in most tissues by immunohistochemistry. Even if ODC activity is induced, the enzyme is only a small part of the cytosolic proteins. Persson *et al.* (1982) first reported the immunohistochemical distribution of ODC in the mouse kidney. Other histological studies report the distribution of ODC with radiolabelled difluoromethylornithine (DFMO) (Pegg *et*

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al., 1982; Zagon *et al.* 1984), which is an enzyme-activated irreversible inhibitor of ODC (Metcalf *et al.*, 1978) and is known to bind specifically to the protein (Pritchard *et al.*, 1981). They showed that ODC was localized mainly in the cortex of the kidney. However, the precise localization of ODC-immunoreactivity inside the cortex is not known, and the immunohistochemical demonstration was done after excess androgen treatment.

In the present series of experiments, we detected ODC-immunoreactivity before and after sc injection of testosterone with antisera raised against the ODC purified from rat liver (Kitani and Fujisawa, 1981), which is also immunoreactive with ODC of mouse kidney (Kitani and Fujisawa, 1988).

Materials and Methods

Animals

Male BALB C mice (8 weeks of age) from the Institute of Experimental Animal Research, Gunma University were used throughout the experiments. They were kept under conditions of controlled temperature ($25 \pm 1^\circ\text{C}$) and illumination (lights on, 0700–1900). Food and water were given *ad libitum*. The animals were separated in two groups as follows: Group 1: one mg testosterone dissolved in 0.2 ml propylene-glycol was injected sc 15 h before sacrifice for the ODC assay or immunohistochemistry and Group 2: the solvent was injected instead of testosterone.

The ODC assay

The mice were killed by decapitation 15 h after the injection. Then their kidneys were removed and homogenized in 4 vol of 50 mM sodium phosphate buffer (pH 7.2) containing 5 mM dithiothreitol. The homogenates were centrifuged at $10,000 \times g$ for 20 min and the supernatants were obtained for the ODC assay. The reaction mixture contained 100 μl of the supernatant, 50 mM phosphate buffer (pH 7.2), 0.5 mM ornithine, 1 mM aminoguanidine, 0.05 mM pyridoxal 5-phosphate and 0.1 mM EDTA in a total volume of 200 μl . The incubation was

Fig. 1. Effect of testosterone on the renal ornithine decarboxylase (ODC) activity in male mice. 8-week-old male mice (BALB C strain) were given a sc injection of testosterone (1 mg per mouse) in 0.2 ml of propylene-glycol (abbreviated "T" in the figure, N=6). The same volume of the vehicle was injected into the control group (abbreviated "C" in the figure, N=6). Each animal was killed by decapitation and ODC activity was measured. Statistical analysis was performed by Student's *t*-test.

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Fig. 2. Comparison of immunohistochemical distribution of ornithine decarboxylase (ODC) in the mouse kidney with (2a) and without (2b) testosterone treatment. No significant difference was observed between the two groups. Note that the inner portion of the cortex (C) showed dense distribution of ODC positive cells. The outer portion of the medulla (M) showed much weaker immunoreactivity. Scale bar length: 200 μm .

Fig. 2a ↑

Fig. 2b ↓

carried out at 37°C for 30 min and the reaction was terminated by adding 10 μ l of 6 N perchloric acid. The amounts of putrescine produced during the incubation period were measured by high-performance liquid chromatography (Matsuzaki *et al.*, 1982). Endogenous putrescine in each sample was assayed at the same time. The enzyme activity was expressed as nmoles of putrescine produced during 1 h per mg wet tissue. The statistical analysis was performed by Student's *t*-test.

Tissue preparation for immunohistochemistry

The mice were perfused under diethylether anesthesia through the left ventricle with cold 0.1 M phosphate buffered saline (PBS) (pH 7.4), containing 4% formaldehyde and 0.2% picric acid. Then the kidneys were removed and post-fixed in the same solution for 2 days at 4°C and 0.1% PBS containing 20% sucrose for 2 days at 4°C. After these treatments, 30 μ m

frozen sections were cut on a cryostat.

Immunohistochemistry

After sectioning, these sections were rinsed in 0.1 M PBS (pH 7.4), for 1 h and were processed for immunohistochemistry. The rabbit anti-ODC serum was obtained by immunization against ODC purified from rat liver (Kitani and Fujisawa, 1981), which also immunoreacts with the ODC purified from mouse kidney (Kitani and Fujisawa, 1988). The site of antigen-antibody reaction in the section was detected with rabbit peroxidase-antiperoxidase complex (PAP) purchased from Dakopatts a/s, Denmark. Methods used for immunohistochemistry were according to Yamada *et al.* (1987) with slight modifications. For immunohistochemistry, sections were preincubated first in 1% H₂O₂ containing 0.1 M PBS (pH 7.4), for 30 min and then in 0.1 M PBS (pH 7.4), containing 1% normal horse serum and 0.4% Triton X-100 for 1 h. These were immersed in ODC antiserum

Fig. 3. Renal corpuscle (RC) showed no immunoreactivity, whereas the surrounding renal tubules showed a positive reaction. Scale bar length: 100 μ m.

diluted 1:2000 for 1 day at room temperature. Then, after further washing, the sections were incubated in goat anti-rabbit serum diluted 1:200 for 3 h. These were incubated in PAP complex diluted 1:100 for 4 h. The sections were rinsed in 0.05 M Tris-HCl buffer, pH 7.4. The site of ODC-immunoreactivity was expressed by incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB), 20 mg/100 ml and 0.05% H_2O_2 in 0.05 M Tris-HCl buffer. After staining, sections were mounted in glycerol.

For control experiments, several sections were incubated in anti-ODC serum which was inactivated by absorption with pure enzyme purified from mouse kidney (Kitani and Fujisawa, 1988).

Results

Fifteen h after the injection of testosterone into the mouse, there was about

a 6-fold increase in renal ODC over the control (Fig. 1). On the other hand, no significant increase in ODC-immunoreactive cells was observed and no change in their distribution was noted (Fig. 2).

In the mouse kidney, dense ODC-immunoreactive cell bodies were observed mainly in the cortex, whereas much weaker immunoreactivity was observed in the medulla (Fig. 2). Most of the immunoreactive cell bodies seemed to be localized in the renal tubule (Fig. 4). On the other hand, no positive reaction was observed in the peritubular tissue (Fig. 4). No immunoreactivity was observed in the renal corpuscle (Fig. 3). More ODC-immunoreactive cell bodies were observed in the inner portion of the cortex. In both the outer and inner portions, the ODC-immunoreactivity was

Fig. 4. Not all the cells in the renal tubule showed a positive reaction. Immunopositive cells were scattered among the immunonegative cells. No positive reaction was observed in the peritubular tissue. Scale bar length: 50 μ m.

not detected in all the tubular cells. The immunopositive cells were scattered among the non-immunoreactive cells (Fig. 4). In the cell, ODC-immunoreactivity was observed mainly in the cytoplasm, while only weak immunoreactivity was observed in the nucleus (Fig. 5). In the present series of experiments it was not established whether ODC-immunoreactive cells mainly localized in the proximal tubule or in the distal tubule. However, judging from the distribution pattern of the ODC-immunoreactive cells, these seemed to be confined mainly to the pars contorta and the pars recta of the proximal tubule. More immunoreactive cell bodies were observed in the pars recta. After the exposure of the antiserum to purified mouse kidney ODC, ODC-immunoreactivity disappeared in both testosterone-treated and non-treated groups.

Discussion

The results of the present series of experiments have shown the immunohistochemical localization of ODC in the kidney of the male mouse. It is likely that the ODC-immunoreactive cells are localized mainly in the pars contorta and the pars recta of the proximal tubule. In previous experiments, with antisera to mouse kidney (Persson *et al.*, 1982) or radiolabelled DFMO (Pegg *et al.*, 1982; Zagon *et al.*, 1984), histological localization of ODC in the mouse kidney has been reported. All these studies were done, however, after inducing ODC activity by administering excess doses of testosterone. After androgen administration, the activity of ODC in the mouse kidney increases markedly (Henningsson *et*

Fig. 5. In each ODC-immunoreactive cell, the cytoplasm showed more intense immunoreactivity than the nucleus. Scale bar length : 20 μ m.

al., 1978). After hyperinduction of ODC, the localization of immunoreactive ODC might not be the same as that in the normal male mouse. Moreover, the precise distribution of ODC has not been demonstrated. We herein confirm that the localization of ODC-immunoreactivity was not different in normal male mice from that in testosterone-treated ones.

ODC is generally considered to be a cytosolic enzyme (Jänne *et al.*, 1987). However, an autoradiographic study with radio-labelled DFMO (Zagon *et al.*, 1984) indicated that ODC is localized in the nucleus as well as in the cytoplasm. Although DFMO binds specifically to ODC purified electrophoretically from the mouse kidney (Pritchard *et al.*, 1981), histological specificity of the autoradiographic localized material remains controversial (Zagon *et al.*, 1984). The present series of experiment with a specific antibody to ODC have revealed that ODC is localized mainly in the cytoplasm.

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