IMMUNOHISTOCHEMICAL DEMONSTRATION OF ORNITHINE DECARBOXYLASE IN DEVELOPING RAT KIDNEY

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Received for publication May 23, 1985

The distribution of L-ornithine decarboxylase was studied in developing rat kidneys by use of peroxidase-antiperoxidase technique. At postnatal day 2 the immunoreaction was exclusively localized in tubule cells. The glomeruli were devoid of immunostaining. A drastic decline of staining intensity occurred during the first two weeks of postnatal ontogenesis.

Ornithine decarboxylase (EC. 4.1.1.17, ODC), the first and apparently, the rate-limiting enzyme in polyamine biosynthesis, catalyzes the conversion of ornithine to the diamine putrescine (4, 15). The activity of the enzyme can rapidly alter in response to various growth-promoting factors (9, 13, 18). In addition to an increased synthesis rate of the enzyme, there exists a posttranslational modification that facilitates the conversion of the active enzyme to an inactive form (4, 14, 21). Furthermore, a macromolecular inhibitor of the enzyme, ODC antizyme, has been found in some mammalian tissues (7, 10, 11). It has also been proposed that ODC is a multifunctional protein (14, 21).

ODC has been detected in nearly all mammalian tissues, as in the ventral prostate gland (5, 24), in the liver (3), in the kidney (17), in the brain (1, 16), and in other organs. The enzyme has been purified from the liver and the kidney (12, 22). ODC could be localized in mouse kidney after an induction by testosterone using both autoradiographic and immunohistochemical approaches (19, 20, 25, 26).

However, no data are yet available as to the cellular localization and regional distribution of ODC in developing rat kidney. This study was aimed at demonstrating ODC immunoreactivity in this organ during postnatal ontogenesis.

MATERIAL AND METHODS

Juvenile Wistar rats of either sex (aged between days 2–14 postnatally) were killed by scissors and kidneys were quickly removed. The tissue was fixed in Bouin's fluid, embedded in paraffin and cut at 4–6 μm serial sections.

For immunohistochemical detection the horseradish peroxidase-antiperoxidase (PAP) technique of Sternberger *et al.* (23) was used. The primary antiserum rabbit anti-mouse ODC, crossreactive with rat antigen (12), was used at a dilution of 1 : 100 for either 1 hr at 20°C or 12 hr at 4°C. The secondary antiserum and the PAP complex were applied as usual (23). The peroxidase activity was revealed with 3,3'-diaminobenzidine (DAB), as described by Graham and Karnovsky (8). Sections were mounted in Canada-balsam and examined under a light microscope.

For purposes of control of the specificity of the immunoreaction, the primary antiserum was replaced by either normal rabbit serum or phosphate buffered saline (PBS). Additionally, control reactions were carried out with antiserum, previously absorbed with the purified enzyme, as described elsewhere in sufficient detail (12).

RESULTS

Judging from the obtained immunostaining patterns, the developing kidney is a rich source of ODC immunoreactivity. However, not all cells of the kidney showed an immunoreaction. ODC immunoreactivity has been observed in most cells of the proximal convoluted, distal convoluted, and collecting tubules. No immunoreactive material was detectable in the glomeruli. The intensity of the reaction altered during postnatal development of the kidney. The strongest immunoreaction was found at day 2 postnatally; the weakest at day 14. Between these stages the intensity of immunostaining permanently declined (Figs. 1-3). In adult kidneys ODC immunoreactivity was nearly ubiquitously distributed, and the staining intensity was quite weak.

All control reactions yielded negative results (Fig. 4).

DISCUSSION

Many experiments have shown that the induction of ODC is an early event in growing tissues (13). Therefore, it was reasonable to expect that developing rat kidney is a rich source of ODC immunoreactivity. Our results confirm this expectation. We have demonstrated that ODC protein is localizable even without previous testosterone treatment of renal tissue.

Furthermore, we have shown that ODC immunoreactivity is unevenly distributed throughout the kidney. The enzyme appeared nearly exclusively in cells belonging to the tubular system. This finding correlates with data reported by Persson *et al.* (19, 20) and Zagon *et al.* (25, 26). These authors revealed that testosterone-induced ODC occurs only in the renal tubules. Though the reason of this location is not yet fully understood, ODC in tubular cells might play roles in the

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Immunohistochemical demonstration of ornithine decarboxylase in the developing rat kidney by use of the PAP-technique.

FIG. 1. Postnatal day 3. $\times 120$

FIG. 2. Postnatal day 6. $\times 250$

FIG. 3. Day 11, postnatally. $\times 120$

FIG. 4. Day 9, postnatally. Control experiment, no ODC immunoreactivity can be observed. $\times 120$

polyamine-mediated energy metabolism of renal brushborder membranes (6). While in the immature kidney the ODC content is relatively high (2, this study), the immunoreaction declines during the first two weeks of postnatal development. Moreover, the distribution patterns alter during maturation of kidney tissue. At the end of the second week, a very weak but ubiquitously distributed ODC immunoreactivity may be recorded. This decline in ODC during ontogenesis is in accordance with biochemical findings made by others (2).

Judging from our own data as well as from findings reported in the literature (2, 4, 6, 12, 13), ODC seems to play an important role in the development and maturation of the renal tubular system of the rat.

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