Immunohistochemical localization of renin in luteinizing hormone-producing cells of rat pituitary

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ABSTRACT The location of renin (EC 3.4.99.19) in rat pituitary was determined by the peroxidase-antiperoxidase immunohistochemical technique. By using antisera prepared with purified rat renal renin, an immunoreactive substance was localized within oviod cells scattered throughout the anterior pituitary. These cells were shown to be luteinizing hormone-producing cells by staining with anti-luteinizing hormone antisera in adjacent sections. By using the double staining method, the renin-containing cells were differentiated from cells containing corticotropin, thyrotropin, growth hormone (somatotropin), and prolactin (mammmotropin). These results suggest a possible local role for renin in the anterior pituitary.

Renin (EC 3.4.99.19) or renin-like activity has been observed in the pituitary gland of rat (1), dog (2), human (3, 4), and hog (5). However, our knowledge of the identity, location, and origin of this activity is incomplete. Slater et al. (4) reported immunoreactive renin in both anterior and posterior lobes of human pituitary by an immunohistochemical technique. Haulica et al. (1) reported that the renin-like activity in the posterior lobe of rat pituitary is three times higher than that in the anterior lobe. Day and Reid (2), and Hackenthal et al. (6), however, suggested that the renin-like activity in pituitary may be cathepsin D.

On the other hand, by devising a method for separating renin and cathepsin, we have demonstrated that practically all of the immunoreactive renin in hog pituitary is localized in the anterior lobe (5), while a large amount of protease with nonspecific renin-like activity is found in both the anterior and the posterior lobes (unpublished data). Furthermore, in our immunohistochemical studies of the pituitary of the mouse (7) and rat (8), renin-specific staining was observed only in the anterior lobes.

A similar lack of agreement is apparent among previous reports with respect to the intraphyophyseal localization of angiotensin (9, 10). The consideration of the physiological function of a renin-angiotensin system in the pituitary has been largely focused on the posterior pituitary, presumably in association with the well-known effect of angiotensin II on vasopressin release (11), though the effect may not be exerted in the pituitary. The effect of angiotensin II on the release of adrenocorticotropic hormone (ACTH; corticotropin) from the anterior pituitary is also known (12), but the physiological role of pituitary renin has remained unclear.

With the objectives of resolving the existing controversies concerning the location of renin and obtaining clues for the physiological significance of renin in the pituitary, we have applied an immunohistochemical staining technique for the identification of the renin-containing cells in the pituitary.

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MATERIALS AND METHODS

Antisera. Specific antibodies to rat renin were produced in Dutch Belted rabbits by using pure rat renin as antigen. The pure renin was prepared by a published method (13). The enzyme preparation satisfied multiple criteria of purity, which included single bands upon polyacrylamide gel electrophoresis, sodium dodecyl sulfate gel electrophoresis, isoelectric focusing, and double immunodiffusion and symmetric chromatographic elution patterns. This preparation (1.0 mg) was conjugated to 0.5 mg of tetanus toxoid with 25 µg of water-soluble carbodiimide and exhaustively dialyzed, and then an aliquot containing 80 µg of renin was mixed with an equal volume of complete Freund’s adjuvant and injected intradermally at multiple sites in the back of each rabbit. After six biweekly boosters, each with the conjugate equivalent of 10 µg of renin, antisera were collected. Tested at dilutions greater than 1:500, these antibodies did not crossreact with human renin or rat cathepsin. Used at 1:2000 dilution for immunohistochemical staining of rat kidney by the unlabeled peroxidase-antiperoxidase method (14), they stained juxtaglomerular cells exclusively (Fig. 1). Antisera to ovine luteinizing hormone (LH; lutropin) (provided by F. Chyttil), antiserum to bovine LH β subunit (provided by J. D. Puett), antiserum to the fragment ACTH-(1-24) (provided by D. N. Orth and W. E. Nicholson), antiserum to human thyrotropin, antiserum to human growth hormone (somatotropin), and antisem to human prolactin (mammmotropin) (provided by the National Pituitary Agency) were also used as primary antibodies.

Tissue Preparation. The pituitary glands were obtained from anesthetized male adult Wistar rats after perfusion by transcardiac puncture with 200 ml of cold saline followed by 200 ml of cold Bouin’s fixative. Pituitaries were removed and postfixed in the same solution for 3–7 hr, dehydrated, and embedded in paraffin. Serial 4-µm-thick sections were cut, mounted on glass coated with 0.1% gelatin/0.01% chrome alum, deparaffinized, and rehydrated.

Immunohistochemical Staining. For identification of renin-containing cells, both the single antigen staining method and the double antigen staining method were used. In the single antigen staining method each section was treated with 10% porcine serum in 0.02% Tween 20 for 2 hr at 25°C, then with appropriately diluted primary antisera or control sera in 10% fetal calf serum for 16–24 hr at 4°C. These sections were further treated with secondary antisera followed by peroxidase-antiperoxidase complex, then with diaminobenzidine tetrahydrochloride with hydrogen peroxide, producing brown stain for the antigen according to the method of Sternberger et al. (14).

Abbreviations: ACTH, adrenocorticotropic hormone (corticotropin); LH, luteinizing hormone (lutropin).
Localization of two different antigens was determined by staining two adjacent sections (4 μm thick) with 1:2000 dilutions of anti-rat renin antiserum or anti-LH antiserum.

To test the specificity of the immunohistochemical reaction, the following control sera were substituted for primary antisera: normal rabbit sera, anti-rat renin antiserum absorbed with purified rat renin, anti-rat renin antiserum absorbed with ovine LH (supplied by the National Pituitary Agency), anti-ovine LH antiserum absorbed with ovine LH, and anti-ovine LH antiserum absorbed with rat renin. All preincubations were done with an excess of the respective antigens for 3 days at 4°C.

To identify two antigens in the same section of a modification of the double staining method of Nakane (15) was employed. After localization of immunoreactive renin by the peroxidase-antiperoxidase method, sections were washed in glycine-HCl, pH 2.2, then anti-ACTH, anti-human growth hormone, anti-human thyrotropin, or anti-prolactin antiserum was applied at an appropriate dilution as primary antiserum in a second staining cycle. The same procedure as the first cycle was repeated except that 4-chloro-1-naphthol was used as peroxidase substrate instead of dianminobenzidine tetrahydrochloride. The two different antigens were localized by two different colors: dianminobenzidine tetrahydrochloride yielded brown reaction products and 4-chloro-1-naphthol yielded purple reaction products.

RESULTS

Renin-specific staining was found in ovoid cells scattered throughout the anterior pituitary. Granular reaction products were generally distributed throughout the cytoplasm and occasionally clustered in perinuclear regions. Generally, nuclei remained unstained. There was no staining observed in the intermediate or posterior lobes (Fig. 2). These results were obtained with several different antisera raised in different rabbits and collected at different stages of immunization.

The cells stained with antisera to rat renin appeared to be gonadotropin-producing cells in morphology. Therefore, an adjacent section was stained with antisera to LH for comparison. The adjacent sections, one treated with antisera to rat renin and the other with antisera to ovine LH or antisera to bovine LH β subunit, revealed that the same cells in the anterior pituitary were immunoreactive with antibody to renin and anti-
body to LH (Fig. 3), indicating the coexistence of the renin and LH in LH-producing cells.

Control staining with normal rabbit serum, anti-rat renin antiserum absorbed with purified rat renin, or anti-ovine LH antiserum absorbed with ovine LH did not show positive staining in these or other cells (Fig. 4a and b). Moreover, preincubation of anti-renin antiserum with ovine LH or preincubation of anti-ovine LH antiserum with rat renin did not affect immunostaining by the respective antibodies, excluding the possible crossreaction of anti-rat renin antibody with LH.

By using the double staining method, ACTH, thyrotropin, prolactin, and growth hormone were stained in cells different from those that exhibited renin immunoreactivity (Fig. 5).

**DISCUSSION**

Renin-like activities have been observed in various endocrine tissues (16, 17). However, whether such activities are due to specific renin or to nonspecific proteolytic activity has been the subject of much discussion. No study has been reported to date in which the type of cell containing the renin-like activity (or containing angiotensin) has been identified. Indeed, whether these renin-like activities are endogenous or are due to plasma renin of renal origin has not been completely clarified.

Our previous studies of the hog pituitary have shown immunoreactive renin activity that is predominantly, if not completely, present in the anterior lobe (5). Moreover, renin in rat was immunohistochemically demonstrated by us to be present in the anterior pituitary (8). The results reported here confirm the anterior localization of rat pituitary renin and, in agreement with our previous results in mouse (7), demonstrate the absence of immunoreactive renin in the intermediate and posterior lobes. Although these results are not in agreement with the observation by Slater et al. (4) of renin immunoreactivity in the posterior lobe of human pituitary, human anterior lobe in that study was shown to contain a far greater number of renin-positive cells than the posterior lobe (4). This difference in renin content in the posterior lobe may be species dependent. The observation of greater renin-like activity in unfractionated extract of rat posterior pituitary than in anterior by Haulica et al. (1) may very well be due largely to the nonspecific action of proteases that under certain assay conditions show much greater angiotensin-generating activity than renin in a given extract (unpublished data).

The present study further reports the localization of immunoreactive renin to the LH-producing cells of the anterior pituitary of rat. This determination of the intracellular presence of immunoreactive renin lends strong support to the notion that renin is not a contamination of pituitary preparations due to entrapped plasma, but is indeed endogenous to the rat pituitary. The presence of renin in LH-producing cells is of further interest in view of the observation by Ganten et al. of angiotensin II immunoreactivity in oviduct cells of rat anterior pituitary (9). Although the cell type was not determined in that study, it was similar in morphology to the LH-producing cells stained in the present study. Taken together, these studies suggest intracellular synthesis of angiotensin II in LH-producing cells. It remains to be demonstrated how the coexistence of LH and renin in the same cell relates biologically.
Fig. 4. Serial 4-μm paraffin sections of a normal rat pituitary stained by the unlabeled immunoperoxidase method. The sections treated with anti-rat renin antiserum absorbed with purified rat renin (a) or anti-ovine antiserum absorbed with ovine LH (b) do not show positive staining. The sections treated with anti-rat renin antiserum preincubated with ovine LH (c) and anti-ovine LH antiserum preincubated with pure rat renin (d) show positive staining (arrows). Preincubation does not affect immunoreactivity, and the same cells are stained by the two antisera. (×380.)
FIG. 5. Four-micrometer paraffin sections of a normal rat pituitary stained by the unlabeled immunoperoxidase double staining method. Cells containing ACTH (a), thyrotropin (b), prolactin (c), or growth hormone (d), stained purple (one arrow), are different from cells containing renin, stained brown (double arrows). (x390.)

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