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Gonadotropin-Dependent Renin in the Rat Testes (41953)

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Abstract. Using specific anti-rat renal renin antibody, the presence of renin in the rat testis was demonstrated by biochemical determination of renin activity. There was no correlation between testicular and plasma renin activity, indicating independent control of testicular and plasma renin levels. Since specific immunohistochemical staining for renin had been observed exclusively in Leydig cells, the effects of hypophysectomy and gonadotropin treatment on the testicular renin were investigated. After hypophysectomy, renin level in the testis decreased significantly, whereas plasma renin was slightly increased. In contrast, testicular renin had remarkably increased through gonadotropin treatment. The results indicate the presence of gonadotropin-dependent renin in the Leydig cells, and suggest a role for it in regulating testicular functions.

Renin-like activity has been found in a variety of extrarenal tissues (1). Subsequently, extrarenal renin was critically examined and much of its activity was attributed to nonspecific action of cathepsins (2, 3). However, recent developments of biochemical research on renin have made it possible to distinguish renin from cathepsin by affinity chromatographic and immunochemical techniques (4–6). Using anti-renin antiserum, we have observed a widespread but uneven distribution of renin in the extrarenal tissues including the pituitary (7, 8) and the adrenal gland (9–12).

Although renin-like activity in the testes has been reported previously (1, 13), it was not certain whether such activity was due to renin or cathepsins. Recently, Parmentier *et al.* (14) localized immunoreactive renin-like materials in the Leydig cells of rat testis and observed its dependency on the pituitary using immunohistochemical methods. The purpose of the present study was to provide additional evidence for the existence of pituitary-dependent testicular renin by determination of antibody-inhibited specific activity.

Materials and Methods. *Protocol for animal experiment.* Male Sprague–Dawley rats weighing 275–300 g were used. The animals were divided into four groups. One group (nine rats) was hypophysectomized by transsphenoidal surgery. A second group (eight rats) was sham-operated. A third group (nine rats) received a subcutaneous injection of 150 IU human chorionic gonadotropin (hCG) every day for 3 weeks to stimulate the interstitial tissue. A fourth group (eight rats) was injected with 0.3 ml of saline subcutaneously once a day for 3 weeks as a control. After 7 weeks after hypophysectomy or 3 weeks of injection, all animals were bilaterally nephrectomized. Twenty four hours later, they were killed by exsanguination under pentobarbital anesthesia, followed by perfusion with saline.

Tissue extraction. Testes obtained were frozen immediately and maintained at −80°C until use. Each testis was homogenized at 4°C in 5–6 ml 0.01 *M* pyrophosphate and 0.1 *M* NaCl, pH 6.5, per gram tissue in a Polytron (Brinkmann Instruments, Westbury, N.Y.) and centrifuged at 39,000g_{av} for 60 min.

Assay of renin activity and suppression by specific antibody. The recovery of angiotensin I (Ang I) added to the extract was extremely low with 7 m*M* Na_2 EDTA alone, 23% with 7 m*M* Na_2 EDTA and 2 m*M* phenylmethanesulfonyl fluoride (PMSF), and only 48% with 7 m*M* Na_2 EDTA, 2 m*M* PMSF, and diisopropyl phosphorofluoridate (DFP). To obtain satisfactory recovery of Ang I, the extracts were pretreated according to the

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method described previously by Hackenthal *et al.* (15) with some modifications; the extracts were dialyzed against 200 vol of 50 m*M* sodium acetate, pH 3.5, containing 0.15 *M* NaCl, 5 m*M* EDTA, and 5 m*M* *N*-ethylmaleimide (NEM) for 24 hr at 4°C, followed by dialysis against 200 vol of 50 m*M* *N*-Tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (TES)/NaOH, pH 7.0, containing 1 m*M* NEM, 5 m*M* Na_2 EDTA, and 0.15 *M* NaCl for 24 hr at 4°C. Obtained after removing the precipitate by centrifugation, the clear supernatant was used for the subsequent assay. Only by the combination of three different inhibitors and subsequent acid treatment described above, was it possible to obtain a complete inhibition of angiotensinase in the extract as tested by greater than 97% recovery of exogenously added Ang I.

The Ang I-generating activity of the extract was determined by a modification of the radioimmunoassay method of Ang I (16). The following conditions were used to minimize the contribution of nonspecific action of cathepsin D. Extracts (100 μl) pretreated as mentioned were incubated with 100 μl of unfractionated plasma of nephrectomized rats in 0.1 *M* TES buffer, pH 7.0, containing 7 m*M* Na_2 EDTA, 2 m*M* PMSF, and 5 m*M* DFP for 2 hr at 37°C. A pH of 7.0 was chosen for Ang I-generation based upon the pH profile of renin and protease activity (17). In the vicinity of this pH, specific renin showed the highest activity while other protease demonstrated less renin-like activity (Fig. 1).

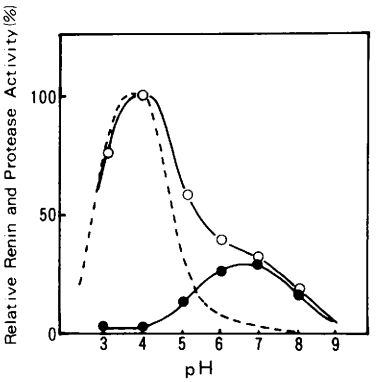

FIG. 1. The pH profile of relative rate of Ang I generation (——) and protease activity (- - -) in the rat testicular extract. Each curve is normalized by setting the peak level of activity as 100%. Specific renin activity (●) was defined as the portion of the total renin-like activity (○) that was inhibited by antibody pretreatment.

The anti-rat kidney renin antiserum has been produced in Dutch-belted rabbits by using pure rat renin as antigen (8, 10, 11). The pure renin was prepared by a published method and satisfied multiple criteria of purity, which included single bands upon polyacrylamide gel electrophoresis, sodium dodecyl sulfate/polyacrylamide gel electrophoresis, isoelectric focusing, double immunodiffusion, and a symmetric chromatographic elution pattern (18). These antibodies did not crossreact with human renin or rat cathepsin D when tested at dilutions greater than 1:500. The specificity of these antisera was further supported by the fact that it stained juxtaglomerular cells exclusively when applied to the immunohistochemical staining of rat kidney sections by the unconjugated peroxidase method (8). Renin inhibition experiments were performed as follows. One hundred microliters of tissue extract were preincubated with 50 μl antiserum (1:1000) for 24 hr at 4°C. After the incubation with renin substrate at 37°C, the Ang I generated was measured as described above. Specific renin activity in the tissue extract was defined as the portion of the total activity that was inhibited by antibody treatment. The protein concentration of the extract was estimated by the Bradford dye-binding method (19) using bovine serum albumin as a standard.

Plasma renin activity was measured by incubating 100 μl of plasma at 37°C and pH 7.0 in the presence of 7 m*M* Na_2 EDTA and 2 m*M* PMSF for 1 hr. The Ang I formed was measured by radioimmunoassay.

Statistical analysis was performed using Student's *t* test.

Results. Renin activity was detected in the testicular extract under the conditions employed and more than 80% of it was inhibited by anti-renin antibody. There was no significant correlation between renin levels in the plasma and testis after bilateral nephrectomy in any of the experimental groups (Fig. 2).

After hypophysectomy, total renin content,

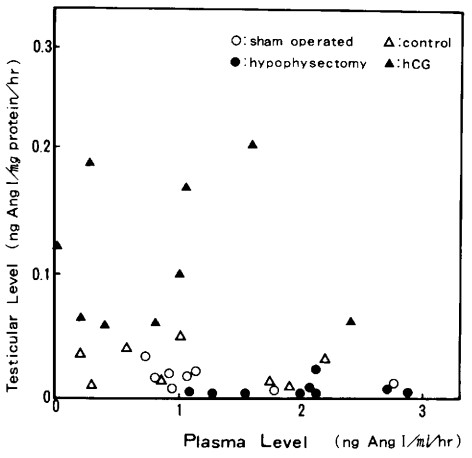

FIG. 2. Plasma renin activity in 34 rats at the time of sacrifice against testicular renin level. There was no significant correlation in any of the experimental groups or as a whole.

total protein content per testes, and specific renin activity per milligram protein decreased significantly. However, plasma renin activity was slightly higher than that of the sham-operated group (Fig. 3). On the contrary, hCG treatment for 3 weeks elicited a significant increase in the total renin content per testes, but not the total protein content. Therefore, the specific renin activity per milligram protein increased significantly. Plasma renin activity did not show significant change (Fig. 4).

Discussion. The presence of renin activity sensitive to anti-renin antibody was demonstrated in the present study. The absence of correlation between plasma and tissue levels indicates the independence of testicular renin from plasma renin. Hypophysectomy induced a significant decrease in the testicular speci-

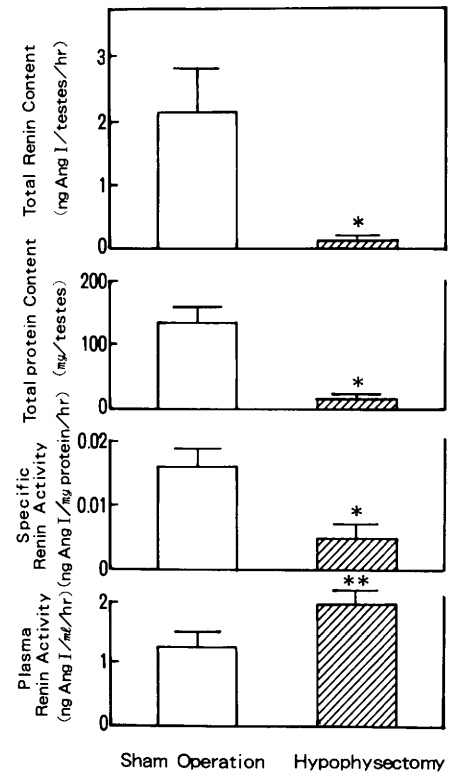

FIG. 3. Effects of hypophysectomy (N = 9) on renin and protein content in the testes and plasma renin activity. Samples were obtained 7 weeks after surgery. Each bar gives the mean ± SE. *, $P < 0.01$; **, $P < 0.05$ compared to sham operation (N = 8).

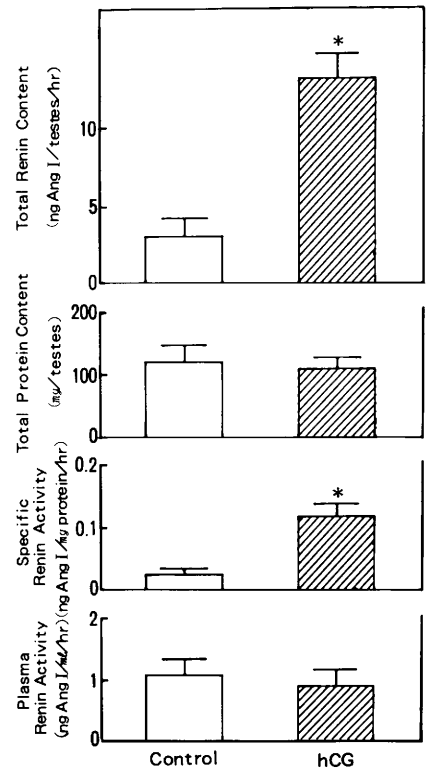

FIG. 4. Effects of hCG treatment (N = 9) on the renin and protein content in the testes and plasma renin activity. Samples were obtained 3 weeks after treatment. Each bar gives the mean ± SE. *, $P < 0.01$ compared to control (N = 8).

fic renin activity. Gonadotropin treatment, however, resulted in a marked increase in renin level, clearly indicating a gonadotropin dependency of testicular renin.

We could also localize the immunoreactive renin in the Leydig cells by the immunostaining using the avidin–biotin-complex method (20) (Fig. 5): results compatible with the previous observation by Parmentier *et al.* (14). They reported the development of renin positive staining in the Leydig cells with the onset of puberty and diminished intensity of the staining after hypophysectomy, indicating pituitary dependence of testicular renin-like immunoreactivity. However, they reported the observation of decreased intensity after gonadotropin treatment. This discrepancy of the results between our renin activity assay and the immunohistochemical study (14) after the hCG treatment may be partly due to the difference in the frequency of the hCG injection; daily for 3 weeks in the present study and every other day administration for 8 days, respectively. It is possible that prolonged treatment may result in accumulation of a greater amount of renin in the testis.

Release of renin from the cells is another possible explanation for the discrepancy. It has been suggested (21) that renin is secreted into human seminal plasma. It is also possible that renin may be secreted locally in a paracrine fashion without affecting plasma renin activity, since plasma renin activity showed no significant change after the hCG treatment. This mode of action may be similar to the mechanism postulated for renin in the anterior pituitary gland (22), in which renin was demonstrated in a particulate structure similar to the secretory granule of the kidney. Renin, thus released, may diffuse into the adjacent tissue and, probably due to reduction of density is unable to be seen by immunohistochemistry.

The role of renin in the testis is not known. If angiotensinogen is present, whether locally or peripherally produced, Ang I could

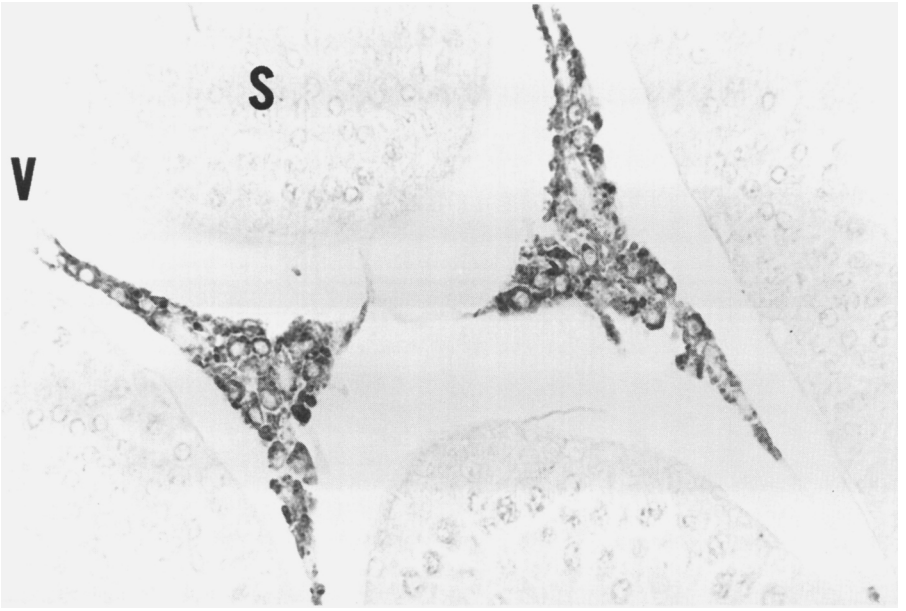

FIG. 5. Paraffin sections of rat testes stained by the immunohistochemical method using anti-rat kidney renin antibody and the avidin–biotin-complex method. The diaminobenzidine tetrahydrochloride was used as peroxidase substrate. The renin-specific immunohistochemical staining was localized exclusively in the Leydig cells, but not in the cells of seminiferous tubules (S) or vascular walls (V). Nuclei of the Leydig cells remained unstained. Control staining with normal rabbit serum or anti-kidney renin antisera absorbed with purified kidney renin did not show positive staining in any cell in the testes.

be generated locally. Ang I-converting enzyme has been found in the testis, epididymis, and vas deferens of rats (23). Therefore, it is possible that the biologically active peptide, angiotensin II (Ang II), is produced locally.

Various kinds of central and peripheral effects of Ang II are well known (24). Ang II is involved in the regulation of pituitary and adrenocortical hormones (24–26). Since renin immunoreactivity has been localized in the cells related to reproduction, such as gonadotrophs of the anterior pituitary (8), inner cortical layer of the adrenal (9), and trophoblasts of the chorion leave (27), it is intriguing to postulate that locally generated Ang II may participate in the regulation of the reproductive system. In view of this, renin can also be involved in the regulation of testicular functions such as testosterone production, spermatogenesis, and sperm mobility (23). The gonadotropin dependency of testicular renin supports its close relationship with testosterone. Increased renin in the testis after gonadotropin treatment may be induced by the increased testosterone level since testosterone has been demonstrated to increase renin in the mouse submaxillary gland (28).

It is not known whether renin and angiotensins exhibit their effects directly on the testicular cells or by changing regional blood flow (23). Further study should be undertaken to elucidate the physiological significance of testicular renin and its mode of action.

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