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IDENTIFICATION OF RENIN AND RENIN MESSENGER RNA SEQUENCE IN RAT OVARY AND UTERUS

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An increase in plasma prorenin during pregnancy suggests that prorenin might be synthesized in the ovary and the secretion of renin or prorenin may be stimulated by an ovarian steroid-mediated process. Recently, renin and angiotensinogen have been identified in human ovarian follicular fluid. However, there is considerable controversy over whether renin is synthesized in the ovary or derived from circulation. In the present study, we confirmed the presence of renin and renin mRNA in rat ovary and uterus by Northern blot analysis with rat renin cRNA as a hybridization probe. Our data show that ovarian or uterine renin is synthesized in the same cells. This suggests that the function of renin might be closely linked to the reproductive process.

A completely inactive renin is present in human plasma(1). This form of renin accounts for more than half of the total renin in normal human plasma. Variations in the proportions of plasma prorenin have already been noticed in various pathological and physiological conditions(2-6). However, the nature and source of plasma prorenin have still been unproved.

Recently, Fernandez et al.(7) and Gloricso et al.(8) reported the presence of prorenin or renin-like activity in human ovarian follicular fluid. The latter reported that prorenin in ovarian follicular fluid was approximately 12 times as high as plasma prorenin and suggested that the ovary could contribute substantially to the high concentration of circulating prorenin after conception or human choricnic gonadotropin (hCG) administration(9).

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The expression of angiotensinogen mRNA was also demonstrated in the rat ovary by Ohkubo et al.(10), using a rat angiotensinogen cDNA. Moreover, angiotensin II, a product of the renin-angiotensin cascade, was found in a sample of follicular fluid(7). This highlights the possibility that angiotensin II may be involved in the events related to reproductive mechanism.

In the present report, we investigated the presence of renin mRNA in rat ovary and uterus with the aid of rat renin cRNA.

MATERIALS AND METHODS

Determination of renin activity - Renin activity was assayed using the partially purified hog angiotensinogen. Prorenin was determined as the increment in activity following activation with trypsin. Details are described elsewhere(11). Angiotensin I generating activity of renin from hog angiotensinogen was determined by radioimmunoassay of angiotensin I generated after incubation(11).

RNA preparation - Female rats of Wister - Imamichi strains (200 - 250 g)were used for the RNA preparation from the ovary, uterus, and kidney. The total RNA was extracted from various tissues from at least 10 animals by homogenization in 5 M guanidine thiocyanate and centrifugation through 5.7 M cesium chloride(12). Poly(A)$^+$RNA was isolated by affinity chromatography on oligo(dT)-cellulose(13). The amount of total RNA and poly(A)$^+$RNA obtained per g of each tissue were as follows; kidney-0.5 mg, ovary-10.0 mg and 640 µg, uterus-0.7 mg and 62µg. In the experiments for RNA blot-hybridization analysis, the total RNA (kidney) and poly(A)$^+$RNA (ovary and uterus) were used.

RNA-blot hybridization Analysis - Poly(A)$^+$RNA or total RNA was denatured with 1 M glyoxal / 50% dimethyl sulfoxide (14), electrophoresed on a 1.5% agarose gel and transferred to diazobenzyloxymethyl paper(15). A 760-base pair fragment derived from the rat renin gene(unpublished data) was subcloned into pSP 64 vector(16). This plamid, pSP RRnRV9, was linearized and transcribed using SP6 RNA polymerase in the presence of a-^{32}P-CTP and used as a hybridization probe(16). After prehybridization overnight at 42°C, the blot was hybridized with the ^{32}P-labeled rat renin cRNA for 16 h, then washed in 0.1 x SSC / 0.1% sodium dodecyl sulfate (1 x SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.4) at 70°C. The size markers used were E. coli rRNAs. The presence of ^{32}P-cRNA / mRNA hybrids was revealed by autoradiography with Fuji X-ray film with intensifying screens at -70°C.

RESULTS

Renin and prorenin activities in ovary and uterus of rats were measured. Active renin was not present in measurable

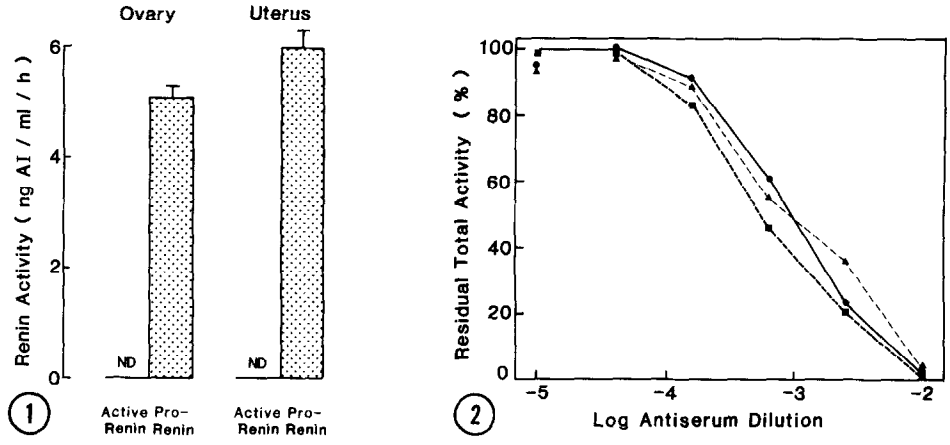

Fig. 1. Active renin and prorenin measurements in cytosol from rat ovary and uterus. Prorenin was calculated from total renin activity minus renin activity before trypsin activation.

Fig. 2. Inhibition of ovarian prorenin, uterine prorenin, and renal total renin by anti-rat renin antiserum. Equimolar quantities of cytosols from ovary(■---■), uterus(▲···▲), and kidney(●——●) were incubated with diluted solutions of antibody for 16 h at 4°C. Following adsorption of immune complexes with protein A-Sepharose, residual renin activity was measured by allowing the supernatants to react with hog substrates.

quantities in the ovary and uterus. However, prorenin activities in ovary and uterus were very high and shcwed 5.1 ± 0.2 ng AI / ml / h, and 6.2 ± 0.3 ng AI / ml / h, respectively (Fig. 1). The immunochemical and biochemical properties of ovarian or uterine prorenin with prorenin and renin from rat kidneys were investigated. Polyclonal antibodies against rat kidney active renin inhibited the renin activities from the kidney, ovary, and uterus, identically (Fig. 2). To identify the renin mRNA in the rat ovary and uterus, total RNA or poly(A)$^+$RNA derived from these tissues was subjected to blot-hybridization analysis with a rat renin cRNA probe. As shown in Fig. 3, the RNA preparations from all the tissues examined exhibited a single hybridization - positive band. Renin mRNAs in the ovary, uterus, and kidneys of rats were indistinguishable by size according to their migration. Interestingly, the size of rat renin mRNA in the tissues examined was slightly larger than that of human kidney mRNA(17,18). Structural analysis of

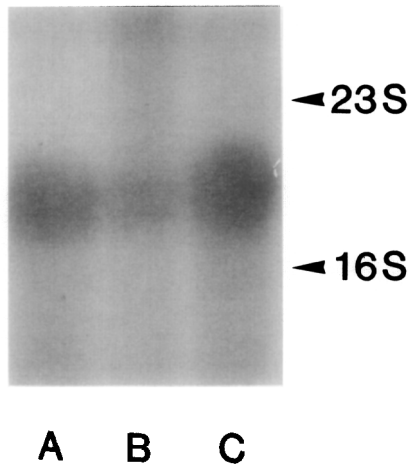

Fig. 3. Blot-hybridization analysis of RNAs from rat ovary, uterus, and kidney with a rat renin cRNA probe. Preparation of total RNA (lane A) and poly(A)$^+$RNA (lanes B and C) were derived from the following tissues; lane A, kidney; lane B, uterus; lane C, ovary. The amount of RNA applied in each lane was 100 µg for kidney and ovary, and 50 µg for uterus. Autoradiographs were performed for 1 h. Other details are described under Materials and Methods.

rat renin gene may explain this size difference. Based on the relative hybridization signal measured in each RNA preparation together with the yield of the poly(A)$^+$RNA fraction obtained from each tissue, the relative levels of renin mRNA in the ovary and uterus varied at around 1 / 100 of that of a rat kidney.

DISCUSSION

Extrarenal sources of renin including the placenta(19), uterus(20,21), and brain(22) have been described previously. Recently, several groups(7,8) reported the presence of prorenin or renin-like activities in human ovarian follicular fluid. They confirmed that follicular fluid prorenin or renin was immunochemically and biochemically identical to that of the human kidney. However, it has not been clear whether ovarian renin is synthesized endogeously or derived from the blood-borne enzyme of another origin.

The presence of renin-angiotensin system in the ovary or uterus is beginning to be understood. Biochemical characterization of the components of the system will be an important step toward understanding the mechanisms of formation and action of angiotensin II, a product of renin-angiotensin cascade, at the molecular level. All of the components, except ovarian renin, have been well characterized. Ohkubo et al.(10) confirmed that angiotensinogen mRNA is expressed in the ovary and Fernandez et al.(11) suggested that the presence of immunoreactive angiotensin II in human follicular fluid.

In this report, we empolyed rat renin cRNA to examine the expression of this protein in rat ovary or uterus. We demonstrated that renin mRNA accumulated in rat ovary and uterus. The ovary or uterus mRNA species were indistinguishable by size from that in the kidney. Our data have provided direct evidence that renin mRNA is expressed in the ovary and uterus, thus supporting the view that renin is locally synthesized in these tissues.

In human, the presence of large proportions of prorenin in the systemic circulation has been known(23,24). It accounts for 70 - 90% of the total plasma renin. The ovary and uterus also contain a large amount of prorenin which comprises well over the total renin(8). Renin or a renin-like enzyme was present in the uterus of all species examined(20,21,25,26). The concentration of renin is low in the non-pregnant uterus and increases during pregnancy(25,26). Most renin in a uterus exists as an inactive form (Prorenin). The enigma of the selective secretion of prorenin in these tissues remains to be resolved. Kidney is known to secrete only a small amount (usually less than 15%) (27) of prorenin. Therefore, the increase of prorenin in plasma during pregnancy or the menstrual cycle might be derived from the

ovary or the uterus. Since prorenin in human plasma was known as an activation intermediate of active renin(28), the presence of specific processing mechanism of renin precursor in the ovary or uterus, different from that of the kidney, may be considered(29). More work is required to identify the role and physiological importance of prorenin in the ovary or uterus. The regional and cellular localization of all of the components of the renin-angiotensin system in the ovary and uterus, suggests that these enzymes and peptides could be closely involved in events related to the reproductive process.

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