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11. All research procedures related to the use of animals (care and maintenance, surgery, and euthanasia) were conducted according to care and use programs accredited by the American Association for the Accreditation of Laboratory Animal Care.
12. The stereotaxic coordinates for each animal were determined by radiographs and computerized tomographic (CT) brain scans; the latter obtained on a General Electric model 8800 CT scanner with a resolution approaching 1 mm. Through the use of a stereotaxic needle carrier, a 50-μl glass micropipette with a pulled tip having an approximate opening of 100 μm was lowered into the brain at the *xyz* coordinates. In each transplantation site, the cells were infused into the brain over a 6-minute period in 4 μl of Eagle's minimum essential medium supplemented with 10% fetal calf serum, gentamicin (50 μg/ml), and Fungizone (2.5 μg/ml). The micropipette was then slowly withdrawn from the injection site.
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Prorenin in High Concentrations in Human Ovarian Follicular Fluid

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Although the kidney is a major source of prorenin, the precursor of renin, there are extrarenal sources for plasma prorenin that have not been identified. The selective increase in plasma prorenin at the time of ovulation suggested that one of these sources might be the ovary. Prorenin was therefore measured in fluid aspirated from 18 ovarian follicles and in plasma collected from three women who were undergoing in vitro fertilization. The follicular fluid contained high concentrations of prorenin that were approximately 12 times higher than plasma prorenin. The prorenin from follicular fluid was immunochemically identical to kidney and plasma prorenin. Thus, the ovary is a likely source for the ovulatory peak of plasma prorenin.

THE ENZYME RENIN AFFECTS BLOOD pressure and electrolyte homeostasis by catalyzing the formation of angiotensin I from circulating angiotensinogen; the active octapeptide, angiotensin II, is then formed by the action of angiotensin-converting enzyme. Angiotensin II causes arteriolar vasoconstriction and stimulates biosynthesis of the adrenal mineralocorticoid aldosterone (*1*). In humans approximately 90% of circulating renin is the large molecular weight, enzymatically inactive, biosynthetic precursor, prorenin (*2–4*). The kidney is probably the only source of circulating active renin because renin disappears from blood after bilateral nephrectomy. The kidney also normally produces close to 90% of plasma prorenin; however, because prorenin persists in plasma after total nephrectomy (*5*), prorenin must also have extrarenal sources. Renin has been identified by biochemical (*6*) and by immunohistochemical techniques (*7*) in several extrarenal tissues. However, no major source of prorenin has been identified in conjunction with the active enzyme in these tissues, with the notable exception of the placenta. Thus, the extrarenal sources of plasma prorenin have remained enigmatic.

Prorenin may be secreted from the female reproductive tract (*8–11*). Thus plasma prorenin, but not active renin, increases acutely during the luteinizing hormone (LH) surge of the menstrual cycle at the time of ovulation (*8*). This increase also occurs in women who receive human chorionic gonadotropin (hCG) to induce ovulation (*9*). Plasma prorenin also increases within a few days after conception (*10, 11*), roughly in parallel with plasma hCG (*11*). We therefore surmised that the ovary might be a source of circulating prorenin and measured the prorenin concentration of human ovarian follicular fluid at the time of ovulation.

Ovarian fluid from 18 different follicles and peripheral venous blood were collected from three women (ages 29 to 32) undergoing in vitro fertilization (*12*). All had received the LH and follicle-stimulating hormone–containing preparation Pergonal, 150 IU per day intramuscularly, from day 3 to day 12 or 13 of their menstrual cycle; hCG (10,000 IU) was administered intramuscularly 36 hours before follicular fluid aspiration on day 13 or 14. One to 4 ml was withdrawn; the follicle was then flushed with 1 to 2 ml of modified Ham F-10 solution; this wash solution was added to the follicular fluid; and the mixture was frozen within 5 minutes of aspiration.

During the follicular phase of the menstrual cycle, plasma prorenin averages 27 ± 8 (SD) ng per milliliter per hour (*8*)—close to ten times the active renin concentration. In these women stimulated with hCG, the concentration of plasma prorenin was elevated markedly (174 to 208 ng per milliliter per hour) and was 20 times that of active renin (Table 1), in agreement with a previous report (*9*). However, follicular fluid prorenin was in turn very much higher than plasma levels, ranging from 730 to 5430 ng per milliliter per hour. This was approximately 100 times the concentration of active renin in follicular fluid. (Fig. 1 and Table 1). The concentration of prorenin in follicular fluid was 11 times greater than that in plasma (ranging from 4- to 26-fold).

Renin substrate (angiotensinogen) in follicular fluid was present at close to 60% of the plasma concentration. In vivo it was probably the same concentration as in plasma because the fluid was diluted approxi-

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mately twofold during collection. Follicular fluid normally contains all but the highest molecular weight plasma proteins (*13*). On the other hand, the concentrations of active renin and angiotensins I and II were much higher than in plasma, in which angiotensin II normally ranges from 5 to 35 pg per milliliter (*14*) (Table 1) and were similar to reported values for follicular fluid (*15*). These concentrations, however, may be artifactually high because inadvertent activation of prorenin can occur in vitro (*2*). Once active renin has been formed, angiotensins could be generated in vitro.

Estradiol and progesterone, which are both synthesized by cells of the maturing follicle (*16*), were an average of 391 and 714 times higher in the ovarian follicular fluid than in plasma, respectively.

We compared the immunochemical and biochemical properties of follicular fluid prorenin with those of partially purified prorenin and renin from human kidney. Polyclonal antibodies against active renin bound renin and prorenin from kidney or from follicular fluid identically (Fig. 2). Polyclonal antibodies specific for prorenin (*4*) failed to bind to active renin from either source but did bind to prorenin from follicular fluid and from kidney identically; after trypsin activation, follicular fluid prorenin was no longer recognized by the antiserum to prorenin (Fig. 2). In addition, when follicular fluid prorenin was activated with trypsin and then incubated with purified angiotensinogen, the enzymatic *p*H optimum was identical to that of active renal renin.

The finding that prorenin is the predominant form of renin in follicular fluid at the time of ovulation is consistent with the observations that prorenin, but not active renin, increases in plasma during the LH surge of the menstrual cycle (that is, just prior to ovulation) (*8*), and with the finding that prorenin levels rise soon after conception (*11*) and after hCG administration (*9*). The high concentrations of prorenin in fol-

Table 1. The components of the renin system and estrogen and progesterone in human ovarian follicular fluid and simultaneously collected plasma. Active renin was measured with an enzymatic assay followed by radioimmunoassay of the generated angiotensin I (*22*). Prorenin was converted to active renin with solid-phase trypsin (*8*). Total renin was then measured and prorenin calculated from total minus active renin. Before measuring total renin, trypsin-activated follicular fluid was diluted 1:30 with 0.05*M* sodium maleate buffer (*p*H 5.7) containing 0.08*M* NaCl, 3 m*M* EDTA, 0.3% lysozyme, and 0.3% bovine serum albumin. Partially purified human renin substrate (*23*) was then added to follicular fluid to a final concentration equal to that of the plasma of that particular patient. Active renin was measured in follicular fluid without adjusting the renin substrate concentration to that of plasma. Because the substrate concentration was above the Michaelis-Menten constant (K_m) (*2*) and was only 39% lower than that of plasma, follicular fluid–active renin was only slightly underestimated relative to the plasma level. Renin substrate (*24*), angiotensin I (*25*), and angiotensin II (*26*) were measured as described. Estradiol and progesterone were measured with Serono Diagnostic Kits. Numbers in parentheses are the SEM of the value immediately above.

		Prorenin (ng/ml/hr)	(FF/P)*	Active renin (ng/ml/hr)	(FF/P)	Pro-renin (%)	Renin substrate (ng/ml)	(FF/P)	Ang. I (pg/ml)	Ang. II (pg/ml)	Estradiol (ng/ml)	(FF/P)	Progesterone (ng/ml)	(FF/P)
Normal subjects†														
Plasma		28		3.7		87								
(n = 9)		(3)		(0.3)		(1)								
Patient 1														
Plasma		182		8.0		96	4880		‡	‡	0.98		2.7	
Follicular	L1§	2100	11.5	20	2.5	99	2780	0.57	630	330	376	384	3072	1138
fluid	L2	1270	7.0	10	1.3	99	1590	0.33	470	105	211	215	1276	473
	L3	2020	11.1	12	1.5	99	1370	0.28	400	200	208	212	303	112
	R1	2080	11.4	25	3.1	99	3280	0.67	870	178	401	409	3096	1147
	R2	2010	11.4	22	2.8	99	2720	0.56	690	200	329	336	2725	1009
	R3	1840	10.1	21	2.6	99	2650	0.54	590	420	444	453	2234	827
	R4	2380	13.1	25	3.1	99	2810	0.58	850	290	333	340	2837	1051
	R5	2830	15.5	37	4.6	99	2540	0.52	800	225	332	339	861	319
	Mean	2066	11.4	22	2.7	99	2343	0.51	663	231	304	336	2051	760
	(n = 8)	(157)	(0.9)	(3)	(0.4)	(0)	(330)	(0.05)	(61)	(40)	(49)	(30)	(385)	(143)
Patient 2														
Plasma		174		13.0		93	3090		‡	‡	3.94		2.8	
Follicular	L1	1100	6.3	11	0.8	99	2170	0.70	1000	113	523	133	1628	581
fluid	L2	1400	8.0	14	1.1	99	2980	0.96	1100	147	552	140	6427	2295
	L3	1300	7.5	14	1.1	99	2400	0.78	980	130	767	195	1756	627
	R1	730	4.2	16	1.2	98	2530	0.82	1100	74	1045	265	1188	424
	R2	920	6.3	19	1.1	98	1960	0.63	820	163	1207	306	1436	513
	Mean	1090	6.3	15	1.1	99	2408	0.78	1000	125	819	208	2487	888
	(n = 5)	(122)	(0.7)	(1)	(0.4)	(0)	(173)	(0.06)	(51)	(15)	(135)	(34)	(990)	(353)
Patient 3														
Plasma		208		5.5		93	4160		‡	‡	0.57		5.3	
Follicular	L1	5430	26.1	63	11.5	99	2550	0.61	880	210	357	626	1627	307
fluid	L2	2490	12.0	42	7.6	98	2710	0.65	740	180	189	332	3613	682
	R1	2510	12.1	21	3.8	99	2360	0.57	730	380	435	763	2529	477
	R2	2710	13.0	29	5.3	99	2260	0.54	1100	320	441	774	2169	409
	R3	3940	18.9	24	4.4	99	2700	0.65	580	240	374	656	3145	593
	Mean	3416	16.4	36	6.5	99	2516	0.60	806	266	359	630	2617	494
	(n = 5)	(570)	(2.7)	(8)	(1.4)	(0)	(90)	(0.02)	(196)	(37)	(46)	(80)	(351)	(66)
All patients														
Plasma		188		8.8		94	4043		‡	‡	1.83		3.6	
(n = 3)		(10)		(3.8)		(1)	(520)				(1.06)		(0.9)	
Follicular		2190	11.4	24	3.4	99	2422	0.63	823	207	494	391	2385	714
fluid		(674)	(2.9)	(6)	(1.6)	(0)	(50)	(.08)	(98)	(42)	(163)	(125)	(171)	(116)
(n = 18)														

*FF/P, follicular fluid/plasma †From (*8*), blood collected during follicular phase of the menstrual cycle. ‡Insufficient plasma was collected to measure angiotensins I and II §L, left ovary; R, right ovary; L1, first aspiration from left ovary.

licular fluid suggest that the ovary could contribute substantially to circulating prorenin in these three situations.

Under most conditions, the majority of the prorenin in plasma is probably derived from the kidney (*2*). In an unpublished analysis of plasma prorenin levels in normal women and men, we have observed no consistent difference between those under age 50 and those over 50 [women under age 50, 15.7 ± 9.6 ng per milliliter per hour (SD; $n = 25$); women over age 50, 12.2 ± 8.4 ($n = 25$) ng per milliliter per hour; men under age 50, 15.7 ± 6.0 ($n = 36$); men over age 50, 17.0 ± 9.7 ($n = 26$) ng per milliliter per hour]. In addition, we have observed that plasma prorenin levels fall within the normal range in postmenopausal women on estrogen-progesterone replacement therapy. In a study demonstrating hCG-dependent renin production by mouse tumor testicular Leydig cells, the majority of the renin was not secreted but was found inside the cells (*17*). Therefore, with the exception of the ovary at the time of ovulation, it seems unlikely that the gonads normally secrete substantial amounts of prorenin into the circulation.

Thus the renin-angiotensin system may have a role in ovarian function. Prorenin might be activated at a specific site, in the ovary or elsewhere, and allow local generation of active renin without increasing its circulating level, thus avoiding unwanted changes in cardiovascular homeostasis. Plasma prorenin could therefore be a marker for an extravascular renin-angiotensin system.

Through its involvement in the production of angiotensin II, prorenin could play several different roles in reproductive function. Angiotensin II can cause vasoconstriction (*1*), aldosterone biosynthesis (*18*), prostaglandin formation (*19*), or angiogenesis (*20*). Thus it could be involved in ovarian contraction and ovum extrusion (*21*), follicular rupture (*22*), steroid biosynthesis, or even neovascularization. Alternatively, prorenin may have undefined physiologic effects, independent of angiotensin II generation. Whatever the role of prorenin, our results suggest that the ovary is a likely source of the ovulatory changes in plasma prorenin. These findings strongly suggest that prorenin is involved in female reproductive function.

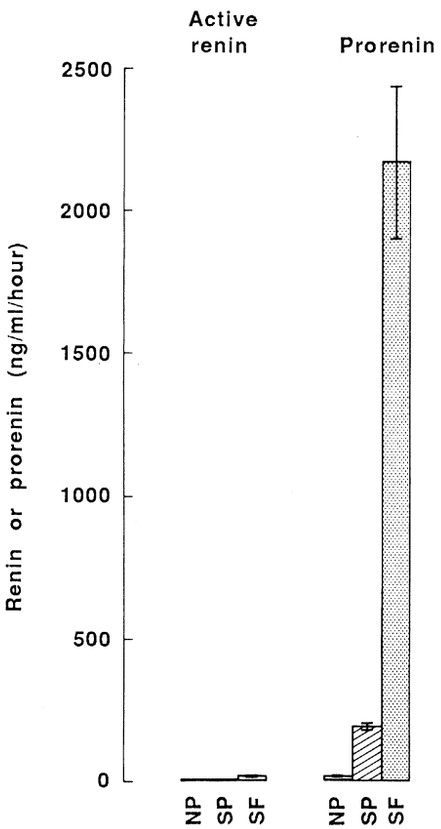

Fig. 1. Active renin and prorenin measurements in plasma from nine cycles in six normal young women during the follicular phase of the menstrual cycle (NP, $n = 9$) (*8*) and in plasma from three women who had been stimulated with gonadotropic hormones prior to follicular fluid aspiration (SP, $n = 3$), and in ovarian follicular fluid aspirated from 18 follicles from these same three women (SF, $n = 18$). The methods are described in Table 1. The data are expressed as mean ± SEM.

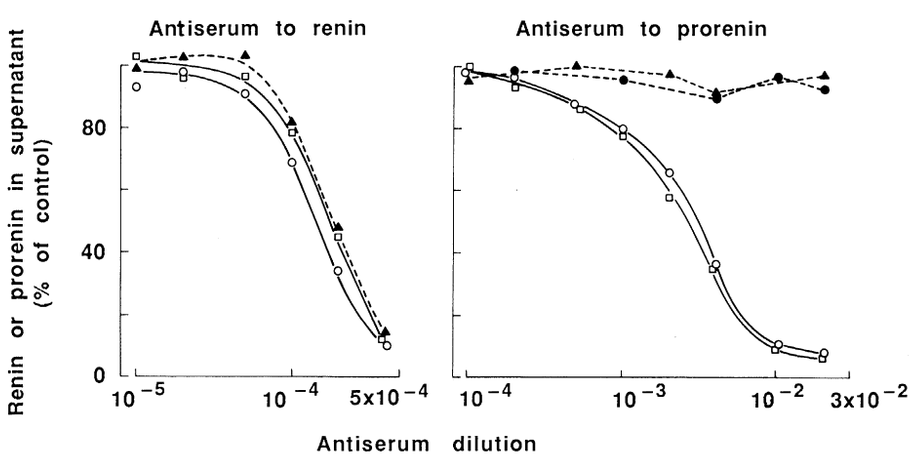

Fig. 2. Immunoprecipitation of follicular fluid prorenin (○), follicular fluid activated prorenin (*8*) (●), renal prorenin (□), and renal renin (▲) by antibodies to active renin and to prorenin. Renal prorenin and renin were purified (*4*, *27*). The antiserum to prorenin was prepared against a synthetic dodecapeptide, corresponding to the COOH terminus of the human renin prosegment (*4*). Immunoprecipitation was performed (*4*) in the presence of 0.21×10^{-3} Goldblatt units of prorenin or renin. After adsorption of immune complexes with protein A–Sepharose, residual active renin or prorenin was measured in the supernatants (expressed as percent of control). The means of two or three assays are presented.

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