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Ovarian Origin of Plasma and Peritoneal Fluid Prorenin in Early Pregnancy and in Patients with Ovarian Hyperstimulation Syndrome*

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ABSTRACT

Prorenin is the major product of renin gene expression in the ovary. Plasma levels of prorenin are elevated in ovarian-stimulated patients and during early pregnancy. To further elucidate the source of the elevated plasma levels of prorenin, we measured prorenin, renin activity, angiotensinogen, and steroid hormone levels in the plasma, luteal fluids (luteal cysts), ascitic fluid, and in ovarian venous samples collected from a patient with severe ovarian hyperstimulation syndrome (OHSS) and ectopic pregnancy. Prorenin/renin was also measured in plasma and in peritoneal fluid obtained during therapeutic paracentesis from four patients with OHSS.

Several corpora luteal fluids were obtained that were rich in estradiol (E_2) and progesterone (P). Ovarian venous E_2 and P were 20-fold higher than in arterial blood and as high or higher than the levels detected in the luteal fluids. The ratios of the hormonal levels in ascitic fluid and plasma were 1.9 for P and 1.4 for E_2. A wide range of prorenin concentrations [1279 ± 918 SD ng/mL/hr, n = 6] were found in corpora luteal fluids, but in each the prorenin concentration was higher than in plasma (494 ng/mL/hr). Prorenin but not renin was higher (+23%) in ovarian venous than arterial blood. Prorenin in the 7 liters of ascitic fluid aspirated (2686 ng/mL/hr) was 5-fold higher than in plasma and similar to the levels measured in the corpora lutea with the highest prorenin concentrations. Renin in luteal cysts and ascitic fluid constituted 3% and 6% of the total renin (renin + prorenin), respectively. Total renin was also higher in peritoneal fluid (1538 ± 925 ng/mL/hr) than in plasma (375 ± 237 ng/mL/hr) of the 4 additional patients with severe OHSS.

These findings indicate that the ovary secretes prorenin during early pregnancy and that its secretion is directed preferentially from the luteal cysts into the peritoneal cavity. In light of recent evidence of an effect of prorenin on the vascular system, the presence of a huge reservoir of prorenin in the peritoneal cavity of patients with OHSS suggests a potential role for prorenin in the pathogenesis of this syndrome. (*J Clin Endocrinol Metab* **82:** 461–464, 1997)

PRORENIN, the biosynthetic precursor of renin, is the major product of renin gene expression in the ovary, placenta, and uterus (1–3). The kidneys are the only organs that both express the renin/prorenin gene and secrete renin into circulation. The role of the very large amounts of prorenin produced in reproductive and other organs is as yet unknown. Recent work indicates that prorenin may have intrinsic vasodilator properties (4). Thus, high prorenin levels are associated with organs with very high blood flows (reproductive organs, kidneys, eyes). Moreover, infusion of prorenin into monkeys (5) and rats (6) is associated with a slight fall in blood pressure in normotensive animals and a marked fall in blood pressure in rats made hypertensive by infusion of angiotensin II (6). It is therefore possible that prorenin works to maintain blood flow to reproductive organs under conditions in which renin and angiotensin II production are increased.

In the ovary prorenin biosynthesis and secretion appears to be regulated by gonadotropins (2, 7, 8). Plasma prorenin increases precipitously within three weeks after conception, normally reaching a peak that is ten times the normal plasma level (9). The early rise of prorenin in pregnancy appears to be largely of ovarian origin because ovarian failure patients who conceive with donated eggs do not have a marked increase in plasma prorenin in early pregnancy (10, 11).

Ovarian-stimulated patients who have multiple follicles and high plasma estradiol (E_2) levels at the time of CG injection are prone to developing the ovarian hyperstimulation syndrome (OHSS). In its severe form, the syndrome is characterized by enlarged ovaries containing multiple corpora lutea, increased vascular permeability, ascites, pleural effusion, electrolyte imbalance, hemoconcentration, and oliguria. The pathogenesis of the syndrome is not known, but it is clearly related to the existence of many corpora lutea and the sustained luteotrophic effect of exogenous or endogenous CG. Markedly elevated prorenin (12) and angiotensin II (13) concentrations in ascitic fluid of patients with OHSS have been reported.

To further elucidate the source of the elevated plasma and peritoneal fluid prorenin levels of early pregnancy and OHSS, we measured prorenin, renin, and steroid hormone concentrations in the plasma, luteal cysts, ascitic fluid, and across the ovary in a patient with severe OHSS and ectopic pregnancy. Total renin (renin + prorenin) was also measured in plasma, and ascitic fluid was obtained during therapeutic paracentesis from four more patients with OHSS. Our results

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provide direct evidence that the ovary synthesizes and secretes prorenin during early pregnancy and demonstrates the existence of very high levels of prorenin of ovarian origin in the ascitic fluid of patients with OHSS.

Subjects and Methods

Patients

Patient with OHSS and ectopic pregnancy. Patient 1, a 23-yr-old woman was admitted to the hospital 11 days after egg retrieval with signs of OHSS that included ovarian enlargement, ascites, pleural effusion, and a weight gain of 5 kg. Her blood pressure on admission was 110/70 mm Hg (similar to the measurement obtained the morning of egg retrieval). Over the next 14 days she gained 8 kg, developed increasing ascites, pleural effusion, and dyspnea. Urinary output ranged from 700–2850 mL/24 h and was maintained by crystalloid infusions. Blood pressure remained stable: systolic pressure was 110–130 mm Hg and diastolic 70–80 mm Hg. Hematocrit ranged from 35–42%, serum sodium from 137–141 mEq/L, and potassium from 4.2–5.2 mEq/L. Serum hCG rose from 100 IU/L–7000 IU/L from day 14 to day 25 after egg retrieval. Transvaginal sonography revealed 2 gestational sacs located in the right fallopian tube. One day later, she underwent laparotomy to remove the right tube. At surgery, 7 L of clear ascitic fluid was drained. Blood from the antecubital vein, brachial artery, and ovarian vein was collected simultaneously before salpingectomy. Ovarian venous blood was collected by phlebotomy of the left ovarian vein with use of 23-gauge needle. No bleeding was observed. Samples were processed at room temperature in tubes containing K_3EDTA. A sample of an ascitic fluid and an aspirate of one gestational sac were also similarly obtained and processed. The concentration of prorenin and renin in this gestational sac has been recently reported (3). All separated samples were stored at −20 C before hormonal measurements. The patient had a prompt postoperative recovery.

Patients with severe OHSS. In four patients (nos. 2, 3, 4, and 5) with severe OHSS, plasma and ascitic fluids (2.5–5.5 L) were collected simultaneously during therapeutic paracentesis into tubes containing K_3 EDTA. The procedure was performed in all patients, under ultrasound control. Three patients had ovarian stimulation for *in vitro* fertilization and one patient for ovulation induction.

Hormonal measurements

Plasma total renin, active renin, prorenin, and renin substrate (angiotensinogen). Plasma renin activity (PRA) was measured by enzymatic kinetic assay at pH 5.7 and 37 C in the presence of 3 mmol/L EDTA and 1.4 mmol/L PMSF (phenylmethylsulfonyl fluoride) (14). Samples were incubated undiluted (plasma) or diluted to 1:30 with phosphate buffer pH 7.4 containing 0.1 M NaCl, 0.1% Na_2 EDTA, 0.5% BSA and 10 mmol/L benzamidine-HCl (cyst or peritoneal fluid), as previously described (3). Purified angiotensinogen (15) was added to ascites or cystic fluid as a source of renin substrate. To avoid cryoactivation of prorenin, samples were thawed rapidly, in front of a fan, to room temperature. After the pH was adjusted to 5.7, they were processed on ice since irreversible cryoactivation does not occur at acid pH. Angiotensin I was generated for 0 (blank) or 3 hours at 37 C and then measured by RIA (14). Results are expressed as ng(angiotensin)/mL/hr or ng/L.s (ng/mL/hr × 0.2778 = ng/L.s) after subtraction of the blank.

Prorenin was measured by cleaving the prosequence during limited proteolysis with trypsin; total renin (prorenin + renin) was then measured as described above. Prorenin was calculated from total renin minus renin (14). Specifically solid phase trypsin (Boehringer Mannheim, Germany) was prepared as recommended by the manufacturer (Pharmacia, Uppsala, Sweden). Fifteen or 30 μL of a 1:1 suspension of gel (in 0.1 M sodium phosphate buffer, pH 7.5 containing 1 mmol/L Na_2EDTA, 76 mmol/L NaCl) was added to 300 μl of diluted fluid or plasma, respectively; and incubated for 18 h at 4 C (final concentration 0.25 or 0.5 mg trypsin/mL). After addition of 1.5 μL, 280 mmol/L PMSF and 36 μL 0.27 M maleic acid, trypsin was removed by centrifugation and the angiotensin I generation step was then performed for 0 (blank) and 1 h at 37 C.

Angiotensinogen was measured as angiotensin I after incubation to exhaustion with excess (40 ng/mL) recombinant human renin (Upjohn, MI). Results are expressed as ng(angiotensin I)/mL (ng/mL × 0.000771 = μmol/L).

Renin and prorenin V_{max} were calculated using the Michaelis Menten equation and a km of 2700 ng/mL, as previously described (14).

Other measurements. Serum estradiol and serum progesterone were measured using RIA kits from Serono Diagnostics (Norwell, MA) and Radioassay Systems Laboratories (Carson, CA), respectively. CG was measured using a Maiaclone immunoradiometric assay from Serono Diagnostics.

Results

Table 1 summarizes prorenin, renin, angiotensinogen, E_2, and P concentrations in body fluids obtained during surgery from patient no. 1. Plasma and peritoneal fluid levels of angiotensinogen and renin collected from four patients with severe OHSS are shown in Table 2. Because samples from patients reported in Table 2 inadvertently cryoactivated during storage, only their total renin levels are reported. The hormonal levels from normal women and at the sixth week of pregnancy (16) are shown for comparison.

As shown in Table 1, a wide range of prorenin levels were found in fluid of individual luteal cysts, but each was equal or higher than in plasma [1279 ± 918 (SD) ng/mL/h]. Renin in luteal cysts constituted 3.3 ± 2.2% of the total renin. Across the left ovary there was a positive (23%) venous-arterial increment in prorenin indicating that the ovary secretes prorenin into the systemic circulation.

The prorenin concentration in the ascitic fluid of patient no. 1 was 5-fold higher than in the arterial blood and was similar to that measured in the corpora luteal fluid with the highest prorenin concentrations. As previously reported (3), the gestational sac (most likely predominantly chorionic fluid) contained 168-fold higher concentrations of prorenin than in their arterial blood.

The ascitic fluid total renin concentration was on the average 5-fold higher than in plasma in the four additional patients with severe OHSS. The angiotensinogen concentration was higher in plasma than in ascites in all of the patients (Tables 1 and 2).

All corpora lutea from patient no. 1 contained fluid rich in E_2 and P, and their concentrations were also higher than in arterial blood. As expected, ovarian vein steroid hormones were 20-fold higher than in arterial blood. However, ovarian vein E_2 and P concentrations were higher than the average level detected in the luteal cysts. The ratios of the steroid hormonal levels in ascitic fluid and plasma were only 1.9 for P and 1.4 for E_2, in contrast to the 5-fold ratio for prorenin.

Discussion

This study confirms the existence of high concentrations of prorenin in ascitic fluid (12) and further demonstrates equally high levels in luteal cysts of patients with OHSS. Ascitic fluid prorenin was 2.7–7.6-fold higher than concurrent plasma level and similar to the concentrations detected in corpora lutea with the highest prorenin concentrations. Apparently, inflow of prorenin into the peritoneal cavity during ovarian hyperstimulation exceeds the peritoneal absorptive capacity, resulting in extraordinarily high concentrations of prorenin.

TABLE 1. Hormone measurements in body fluids collected during surgery for removal of ectopic pregnancy in a patient with ovarian hyperstimulation syndrome (Patient #1)

	Renin (V_{max}) (ng/mL/h)	Prorenin (V_{max}) (ng/mL/h)	Renin %	Aogn (ng/mL)	Estradiol (pmol/L)	Progesterone (nmol/L)
Normal subjects (21) luteal phase	5	65	8	1,810	550	50
Normal pregnancy 6th week (13)	8	350	2	3,300	3,000	80
Patient[a]						
Ascites	165	2,686	6	4,110	29,800	636
Arterial	22	490	4	6,370	20,800	337
Left ovarian vein	18	610	3	6,460	411,200	6,580
Luteal cyst						
R_1	28	2,471	1	5,010	201,900	1,545
R_2	52	2,396	2	3,910	216,600	1,840
L_1	14	508	3	6,690	268,000	6,680
L_2	19	1,040	2	6,280	165,200	2,385
L_3	33	480	7	3,080	27,900	540
L_4	35	778	5	5,120	165,200	2,420
Gestational sac	1,910	82,500	2	335		

Aogn, angiotensinogen (renin substrate); R, Right; L, Left.
[a] Baseline (day 3 of menstrual cycle) plasma levels, prorenin V_{max} = 43 ng/mL/hr, renin activity V_{max} = 2.0 ng/mL/hr, angiotensinogen 3112 ng/mL.

TABLE 2. Renin substrate and total renin in plasma and ascitic fluid of four patients with severe ovarian hyperstimulation syndrome

Patient	No. of oocytes	Day of paracentesis[a]	Pregnancy	Angiotensinogen (ng/mL)		Total renin (ng/mL/h)	
				Plasma	Ascites	Plasma	Ascites
2 (IVF)	18	10	+	7041	2320	462	1270
3 (IVF)	22	13	+	3376	2111	140	1064
4 (OI)		14	+	6008	2903	231	909
5 (IVF)	72	4	−	5450	4589	667	2907

IVF, *in vitro* fertilization; OI, ovulation induction.
[a] From oocyte retrieval or ovulation.

Two mechanisms may account for the higher prorenin concentrations in ascitic fluid than in plasma. There may be direct ovarian-peritoneal transfer of luteal fluid and/or changes in vascular or peritoneal permeability, resulting in a change of inflow and outflow equilibrium at the level of the peritoneum. The similar or even higher concentration of prorenin in the ascitic fluid than in the luteal cysts, and the higher prorenin concentration in the luteal cysts than in the ovarian venous blood, suggests that the secretion of prorenin is directed preferentially from the protruding luteal cysts toward the peritoneal cavity. Direct drainage of luteal fluid into the peritoneal cavity has previously been postulated to explain the higher concentrations of E_2 and P in the peritoneal fluid than in plasma during the luteal phase of the normal menstrual cycle (17).

The biological significance of this huge intraperitoneal pool of prorenin (*e.g.* patient no. 1: 7000 mL × 2686 ng/mL/hr = 18.8 × 10^6 ng/hr) is not clear. Because human prorenin can develop intrinsic renin activity *in vitro* (15), we previously suggested that prorenin may behave like renin *in vivo* at particular target sites without cleavage of its prosegment (1). Our current view is that this does not happen because we have identified highly specific membrane binding sites for prorenin in many tissues (18), and prorenin remains inactive when bound. However, renin binds to the same site and is active when bound. It is therefore possible that prorenin is a natural antagonist of tissue-directed circulating renin. Moreover, we have demonstrated an antihypertensive effect of prorenin in an angiotensin II-mediated hypertensive rat model (6), giving evidence to the postulation that prorenin may have intrinsic vasodilator effects either directly or by competing with angiotensin II.

The presence of high concentrations of prorenin in the peritoneal cavity and in the systemic circulation may have relevance to the genesis of OHSS. A prominent feature of this syndrome is increased vascular permeability (19), augmented filtration of fluid from the vascular to the interstitial compartment, and fluid accumulation in the third space (largely peritoneal fluid). Peripheral vasodilation leading to underfilling of the arterial vascular compartment and hypotension is another feature of severe OHSS (20). Because of its diverse effects on the vascular system, prorenin may be considered a putative mediator in the pathogenesis of the OHSS.

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American Academy of Male Sexual Health Annual Meeting: Erectile Dysfunction: An Interdisciplinary Perspective February 21–23, 1997 San Diego, California

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