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 (E2) and progesterone (P). Ovarian venous E2 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 E2. 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 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)
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 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 right tube, 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 K3EDTA. A sample of an ascitic fluid and an aspirate of one gestational sac were also similarly obtained and processed. The concentration of prorenin in renin in this gestational sac has been recently reported (5). 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 K3EDTA. 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% Na2 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 croyoactivation 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 croyoactivation does not occur at acid pH. Angiotensinogen I was generated for 0 (blank) or 3 hours at 37 C and then measured by RIA (14). Results are expressed as ng(angiotensinogen)/mL/hr or ng/Ls (ng/mL/hr × 0.2778 = ng/Ls) 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 Na2 EDTA, 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 µg trypsin/µL). After addition of 1.5 µL, 280 µmol/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 × 0.00771 = µmol/L).

Renin and prorenin Vmax 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 Maaialone immunoradiometric assay from Serono Diagnostics.

Results

Table 1 summarizes prorenin, renin, angiotensinogen, E2, 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 E2 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 E2 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 E2, 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)

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of oocytes</th>
<th>Day of paracentesis*</th>
<th>Pregnancy</th>
<th>Angiotensinogen (ng/mL)</th>
<th>Total renin (ng/mL/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (IVF)</td>
<td>18</td>
<td>10</td>
<td>+</td>
<td>7041</td>
<td>2320</td>
</tr>
<tr>
<td>3 (IVF)</td>
<td>22</td>
<td>13</td>
<td>+</td>
<td>3376</td>
<td>2111</td>
</tr>
<tr>
<td>4 (OI)</td>
<td></td>
<td></td>
<td>+</td>
<td>6038</td>
<td>2903</td>
</tr>
<tr>
<td>5 (IVF)</td>
<td>72</td>
<td>4</td>
<td></td>
<td>5450</td>
<td>4589</td>
</tr>
</tbody>
</table>

* From oocyte retrieval or ovulation.

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

<table>
<thead>
<tr>
<th>Renin (Vmax)</th>
<th>Prorenin (Vmax)</th>
<th>Renin</th>
<th>Aogn</th>
<th>Estradiol</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ng/mL/h)</td>
<td>(ng/mL/h)</td>
<td>%</td>
<td>(ng/mL)</td>
<td>(pmol/L)</td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>Normal subjects (21) luteal phase</td>
<td>5</td>
<td>65</td>
<td>8</td>
<td>1,810</td>
<td>550</td>
</tr>
<tr>
<td>Normal pregnancy 6th week (13)</td>
<td>8</td>
<td>350</td>
<td>2</td>
<td>3,300</td>
<td>3,000</td>
</tr>
</tbody>
</table>

Aogn, angiotensinogen (renin substrate); R, Right; L, Left.

References


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

The American Academy of Male Sexual Health will be holding its Inaugural Meeting from February 21–23, 1997 in San Diego, California. This year’s meeting will concentrate on Erectile Dysfunction: An Interdisciplinary Perspective and will feature discussions and demonstrations on diagnostic techniques, new medical therapies, the role surgery plays in treating impotence; and, the role of mental health professionals. Featured speakers include renowned specialists Harin Padma-Nathan, M.D. and Carl Montague, M.D. The Academy’s Male Sexual Health Practitioner Accreditation will be offered at the start of the meeting to eligible physicians: those who currently have either 20 hours CME credits in erectile dysfunction within the last 2 years or have completed their residency in urology or a similar comprehensive training program within the last five years. To maintain the certification, each physician must fulfill 20 hours in CME every 2 years.

The American Academy of Male Sexual Health is the only multi-disciplinary organization dedicated to promoting and maintaining high practice standards in medicine and surgery as it relates to the management of male sexual dysfunction. As the accrediting body for male sexual health practitioners, the Academy acts as a public clearing house for information, research, new studies and new treatment options.

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