

Cyclical secretion of prorenin during the menstrual cycle: Synchronization with luteinizing hormone and progesterone

(renin-angiotensin/aldosterone/estradiol)

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ABSTRACT Plasma prorenin, a high molecular weight precursor form of renin, (renin, EC 3.4.23.15; old number, EC 3.4.99.19), was measured three times weekly in normal young women during the menstrual cycle and was related to changes in luteinizing hormone, estradiol, and progesterone. In all subjects a stable baseline level of prorenin occurred during the follicular phase. Then, simultaneously or soon after the luteinizing hormone peak, plasma prorenin consistently increased about 2-fold. Baseline prorenin ranged from 18 to 40 ng per ml per hr, and peak prorenin ranged from 35 to 65 ng per ml per hr. The maximum increase in prorenin averaged 80%. Prorenin remained elevated during the mid-luteal phase of the menstrual cycle and returned to baseline during the late-luteal phase in coordination with the decrease in progesterone. The changes in prorenin were not synchronized with changes in active renin which was significantly increased only during the mid-luteal phase. These findings suggest that prorenin may be involved in reproductive physiology.

The renin-angiotensin system regulates blood pressure and fluid and electrolyte balance by the actions of angiotensin II (1). This octapeptide causes arteriolar vasoconstriction; it also causes stimulation of aldosterone biosynthesis (2) by increasing the conversion of cholesterol to pregnenolone in the adrenal zona glomerulosa cells (3).

An inactive, high molecular weight form of renin (EC 3.4.23.15; old number EC 3.4.99.19), prorenin, found in human plasma (4) has been shown to be the biosynthetic precursor of active renin (5, 6). Unlike most hormone precursors which constitute a minor proportion of the circulating hormone pool, prorenin normally circulates in concentrations close to 10 times that of active form (4). Whereas circulating active renin appears to be exclusively of renal origin, plasma prorenin is derived from the kidney and from other tissues as well (7, 8) but the other tissue source(s) of plasma prorenin are unknown.

Circumstantial evidence suggests that prorenin may be linked to reproductive function, but no clear role has emerged. Prorenin is present in amniotic fluid (9) in concentrations two orders of magnitude higher than in normal human plasma, and it is synthesized in the chorionic cells of the placenta (10). Maternal plasma prorenin increases during pregnancy (11-15); it is increased as much as 10-fold within the first 4 weeks following conception, and it decreases rather slowly postpartum (15). This increase in pregnancy parallels the early increase in human chorionic gonadotropin (15).

The early increase in prorenin following conception and the slow decrease postpartum suggested to us (15) that the increase in plasma prorenin during pregnancy might be maternal, not fetal, in origin and that prorenin might also increase during the luteal phase of the menstrual cycle in

nonpregnant subjects. We, therefore, investigated changes in prorenin in young women during the menstrual cycle in relation to known changes (16) in luteinizing hormone (LH), progesterone, and estradiol.

MATERIALS AND METHODS

Subjects. All of the subjects were normal volunteers from whom informed consent was obtained. They were paid to participate in the study. Six Caucasians and one Oriental were studied throughout a total of 12 cycles. Their ages ranged from 21 to 28 years. They recorded their basal body temperature daily. Blood was usually drawn on each Monday, Wednesday, and Friday throughout two consecutive cycles. In subject 5 the two menstrual cycles were not consecutive but were interrupted by one month in which a normal menstrual cycle occurred. Blood was collected from subject 4 during only one cycle. Subjects 3 and 6 had abnormal menstrual cycles.

Blood Collection Procedures. Blood was drawn between 9 a.m. and noon from seated subjects, who had been ambulatory. Blood (10 ml) was drawn into Vacutainers containing K₃ EDTA. The blood was centrifuged at room temperature, and the plasma was removed and stored frozen at -40°C until assay.

Hormonal Measurements. Active renin in plasma was measured first. Plasma was then incubated with trypsin to convert the prorenin to active renin, and the amount of total renin in plasma was measured. The concentration of prorenin was the difference of these values. A modification of the solid-phase trypsin method of Derkx (17) was used to activate prorenin. Solid-phase trypsin was prepared by coupling trypsin (Boehringer Mannheim) to CNBr-activated Sepharose 4B (Pharmacia) as recommended by the manufacturer. Plasma (300 μ l) was incubated in duplicate at pH 8.0 with 15 μ l of a 1:1 suspension of gel in 0.1 M sodium phosphate buffer, pH 7.5 containing 1 mM Na₂EDTA, 76 mM NaCl (final concentration, 0.25 mg of trypsin/ml) for 18 hr at 4°C. Following addition of 3 mM phenylmethylsulfonyl fluoride and 36 μ l of 0.27 M maleic acid (to adjust the pH to 5.7), samples were centrifuged at 2000 \times g for 30 min at 4°C to remove the trypsin. Renin activity was then measured by incubating the supernatant for 0 (blank) and 1 hr at 37°C, and the angiotensin I formed was then measured by radioimmunoassay (18). Active plasma renin was measured (18) either in fresh plasma or in plasma that had been frozen and thawed only once to reduce the likelihood of inadvertent cryoactivation of prorenin (19). Prorenin activity was determined by subtracting the active renin activity from the mean of the duplicates of the total renin activity minus the blank and expressed as ng of angiotensin I per ml per hr.

Plasma renin substrate was measured as the amount of angiotensin I formed following incubation at pH 5.7 with

Abbreviation: LH, luteinizing hormone.

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excess renal renin (6.6×10^{-2} Goldblatt units/ml) in the presence of the angiotensinase inhibitors, 3 mM EDTA and 6 mM phenylmethylsulfonyl fluoride as described (20).

Progesterone, estradiol, and LH were measured by radioimmunoassay by using kits from Serono Diagnostics.

Statistical Analysis. The significance of changes in active renin or prorenin during the different phases of the menstrual cycle was calculated by using the paired Student's *t*-test. $P < 0.05$ was considered to be significant.

RESULTS

Prorenin During the Menstrual Cycle. Complete hormonal measurements were made during nine apparently normal cycles. Figs. 1 and 2 show the results of two cycles each in subjects 2 and 7. In each cycle the LH peaks were clearly identified. The prorenin level appeared to be quite stable during the follicular phase, but then it increased during or following the LH peak. Thus, for subject 2, mean prorenin during the follicular phase was 25 ± 5 and 22 ± 3 ng of angiotensin I per ml per hr (mean \pm SD) during the first and second cycle, respectively. During the early-luteal phase (days 0–4), prorenin doubled to an average of 46 and 36 ng of angiotensin I per ml per hr, respectively. It decreased slightly thereafter to 33 and 34 ng of angiotensin I per ml per hr during the mid-luteal phases (days 5–11) and to 32 and 35 ng of angiotensin I per ml per hr during the late-luteal phases. In this subject a sharp peak of prorenin was detected immediately after the LH surge that was followed by a plateau.

Subject 7 (Fig. 2) showed a similar pattern. Prorenin averaged 18 ± 1 and 20 ± 1 ng of angiotensin I per ml per hr

during the follicular phase in the first and second cycles, respectively. It then increased to 34 and 32 ng of angiotensin I per ml per hr during the early-luteal phases and then fell to 28 and 29 ng of angiotensin I per ml per hr during the mid-luteal phases and to 19 and 24 ng of angiotensin I per ml per hr during the late-luteal phases. In this subject, no sharp peak of prorenin was detected in either cycle, only a plateau lasting for 5 to 10 days after the LH surge.

For subjects 1, 4, and 5 (Fig. 3) the LH peaks were not so clearly identified (except for the second cycle of subject 1). Nonetheless, the time of the LH peak could be estimated from the slight increase in LH and from the estradiol and progesterone changes. Again, in each case prorenin was clearly higher in the early- and mid-luteal phases than in the other times of the cycle. As already noted, two different patterns of prorenin response were detected, namely a peak followed by a plateau (subject 1, cycle 2; subject 4; subject 5, cycle 2) or only a plateau with no clear peak (subject 1, cycle 1; subject 5, cycle 1). In all of these cycles the decrease in prorenin from the plateau level occurred at the same time as the decrease in progesterone.

The two subjects (3 and 6) who had abnormal cycles had unusual prorenin patterns (not shown). Subject 6 had two abnormal menstrual cycles which lasted 31 and 27 days, respectively. In the first cycle estradiol increased only slightly on day 18, from 10 to 30 pg of estradiol/ml. On day 22 prorenin doubled and then rapidly returned to baseline on day 25. Subnormal peaks of estradiol (95 pg/ml) and progesterone (7 ng/ml) occurred on days 25 to 27. No LH peak was detected. On the 1st day of the next menstrual cycle estradiol and progesterone decreased to undetectable levels and remained there during the entire second cycle. No LH peak was

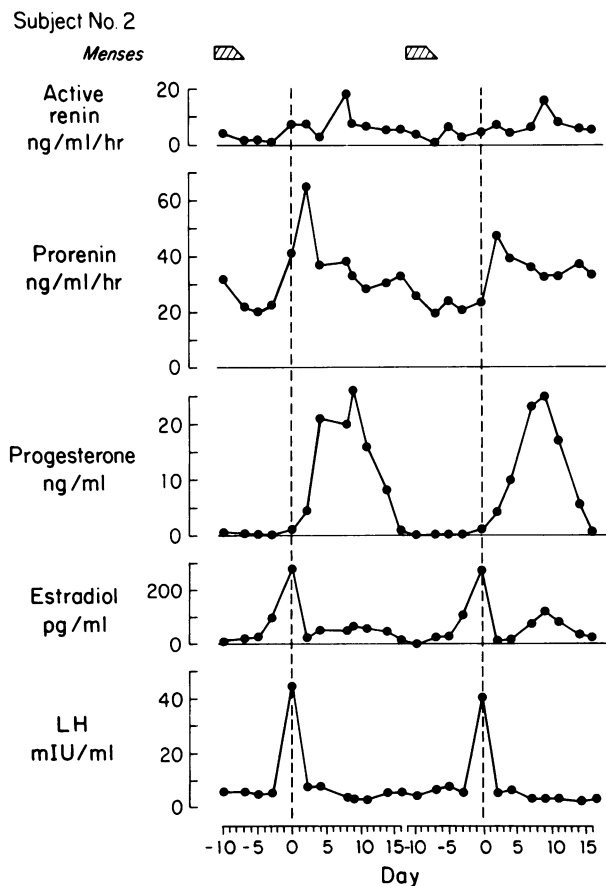


FIG. 1. Cyclical changes in active renin, prorenin, and other hormones during two consecutive menstrual cycles in subject 2. Day 0 coincides with the LH peak. Both cycles lasted 28 days. Blood samples were collected every 2–3 days.

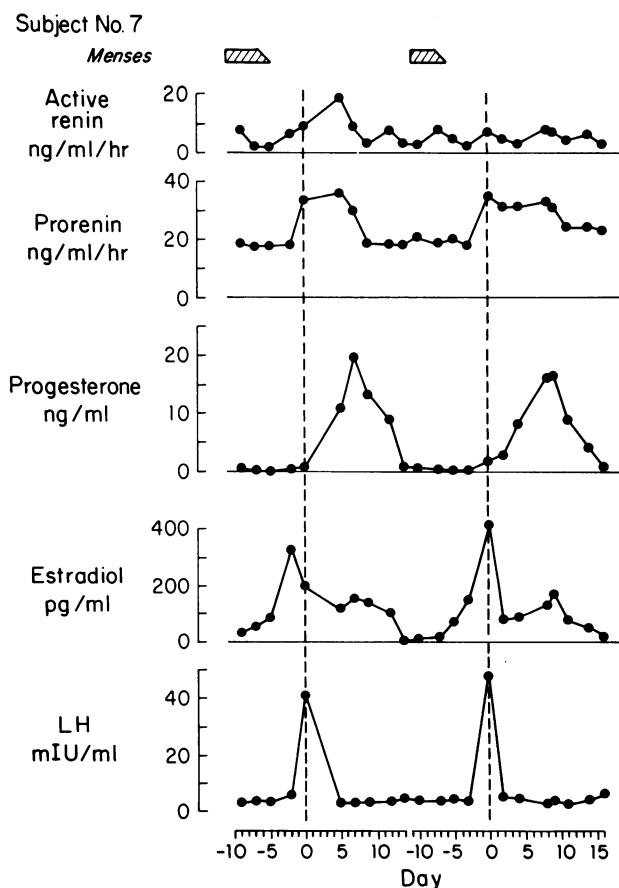


FIG. 2. Cyclical changes in active renin, prorenin, and other hormones during two consecutive cycles in subject 7. Both cycles lasted 28 days. Day 0 coincides with the LH peak.

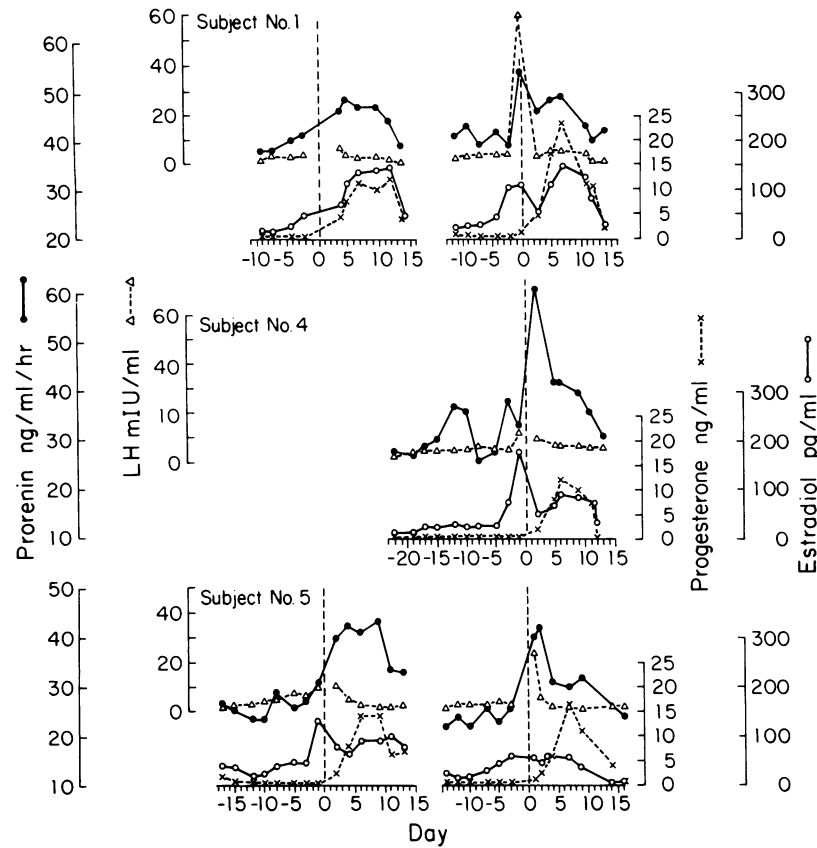


FIG. 3. Cyclical changes in prorenin and other hormones during two consecutive menstrual cycles in subject 1 (each cycle lasted 28 days); during one cycle in subject 4, lasting 40 days; and in two nonconsecutive cycles in subject 5, lasting 34 and 31 days, respectively. Day 0 coincides with the LH peak, which was estimated in four of the cycles from the changes in LH, estradiol, and progesterone on surrounding days, since blood was not collected on the day of the LH surge. Note that in each of the cycles in which LH surge was not detected at least 3 days elapsed between blood sample collections during that period.

detected. Prorenin increased gradually to 175% of baseline by day 14 where it remained for the rest of the cycle. Menstruation occurred on day 28.

Subject 3 menstruated on days 1 to 6 of the study but failed to menstruate again for more than 53 days. On day 9, estradiol level was 153 pg/ml but it ranged between 0 and 15 pg/ml on all other days tested. LH was also slightly high on day 9 at 16 milli-international units/ml. Progesterone was less than 1 ng/ml throughout the study. Prorenin averaged 17 ng per ml per hr, which was lower than all except subject 7. No change in prorenin was observed from days 1 to 31. A 50–75% increase was detected on days 36 and 39, but all other prorenin values ranged between 11 and 19 ng per ml per hr.

Active Renin During the Menstrual Cycle. Changes in active renin were much less consistent. Substantial increases occurred on occasion, usually close to the time when plasma progesterone was at its peak (see both cycles of subject 2 in Fig. 1 and the first cycle of subject 7 in Fig. 2). For this reason, in analyzing the active renin data we averaged the data for the subjects with the normal cycles for the follicular phase—i.e., up to but not including the LH peak (day 0)—for days 0 to 4 (early-luteal phase), days 5 to 11 (mid-luteal phase, i.e., the progesterone peak), and days 12 to menstruation (late-luteal phase). Renin was averaged for each subject and then the mean of the average values was calculated (Fig. 4). During the mid-luteal phase active renin activity was 7.6 ng per ml per hr, 1.4 to 2 times higher ($P < 0.05$ or < 0.01) than at all other periods of the cycle. In contrast, prorenin was highest during the early-luteal phase ($P < 0.001$) at a time when active renin had not changed. Prorenin was also significantly higher than baseline during the mid-luteal phase

($P < 0.001$) when active renin was elevated, and also during the late-luteal phase ($P < 0.05$).

Renin substrate was measured in one cycle in each of three patients (subjects 1, 4, and 5), and it did not change significantly. The mean was 1430 ng of angiotensin I/ml for the follicular phase and 1500, 1530, and 1480 ng of angiotensin I/ml during the early-, mid-, and late-luteal phases, respectively.

DISCUSSION

In this report we have shown that the concentration of plasma prorenin varies predictably during the menstrual cycle. In all subjects a stable and reproducible baseline occurred during the follicular phase. Prorenin then increased in every subject about 2-fold, near the time of the LH peak; the increases ranged from 23 to 160%, with a mean of 83%. In some cycles a clear peak of prorenin occurred within 1–2 days of the LH peak. This was followed by a plateau which was maintained until days 6–10. In other cycles only a plateau was observed that was sustained until days 6–10. The decline of prorenin back to baseline usually began close to the time of the progesterone peak.

Since blood samples were collected only every 2nd or 3rd day in this study, it was not possible to accurately define the magnitude of the increase of prorenin, or the precise relationship between the increase in prorenin and the LH surge. In most subjects, there was no clear dissociation between the increase in LH and the increase in prorenin but in the second cycle of subject 2 (Fig. 1), the peak of LH clearly occurred before prorenin began to increase. This finding suggests that

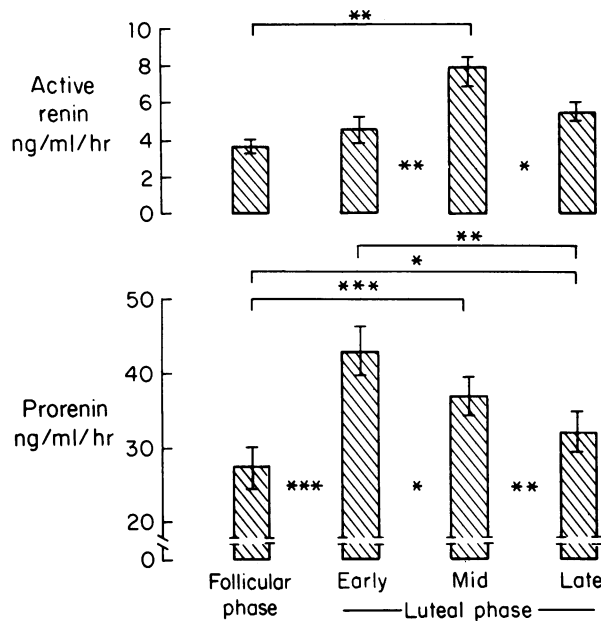


FIG. 4. Changes in active renin and prorenin during nine cycles in five normal subjects. Because active renin fluctuated markedly from day to day, data were averaged from each subject during four phases of the cycle. Follicular phase, from first day of menstruation to day minus 1; early-luteal phase, days 0 to 4; mid-luteal phase, days 5 to 11; late-luteal phase, day 12 to last day before menstruation. The data represent the mean \pm SEM of the average renin or prorenin for each period. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

LH may increase first and that the increase in prorenin occurs slightly later.

The changes in plasma prorenin were not synchronized with the changes in active renin. As has been reported (21–23), active renin was higher on average in the luteal phase but the increases were less consistent than those of prorenin. The increase in active renin occurred only in the mid-luteal phase whereas prorenin was at its highest level during the early-luteal phase. It has been speculated that the increase in active renin is in response to the natriuretic effect of progesterone (21–23), and our data are consistent with such a hypothesis. Since diuretics cause an increase in circulating prorenin (24, 25), it is possible that the diuretic effect of progesterone contributes to the mid-luteal increase in prorenin that may, therefore, be of renal origin. Since the initial rise in prorenin was closely related to the LH surge two independent factors may contribute to the changes in prorenin during the menstrual cycle, the early increase being associated with the LH surge and the later plateau phase being related to the diuretic effect of progesterone. This might explain why subject 6 had only a sharp peak of prorenin and no plateau phase during the first cycle, a cycle in which progesterone was very low (not shown).

Based on these results it is not possible to identify the source of this prorenin. Renin has been identified by immunohistochemical techniques in the same cells as LH in the anterior pituitary (26). Because the antibodies used in that report were unable to distinguish active renin from prorenin, it is possible that prorenin may be released together with LH during the LH surge. Against this hypothesis is the observation in subject 2, second cycle (Fig. 1) that prorenin remained at baseline at a time when LH was at its peak, suggesting that the increase in LH precedes that in prorenin. Alternatively, LH itself or some change related to LH may stimulate the kidney to release prorenin. A third, more likely, possibility is that prorenin is synthesized in the ovary by the cells of the maturing follicle in response to LH. So far, we have been unable to find any reports that renin or prorenin is

present in ovarian cells, but it should be noted that of four subjects we have studied with renin secreting tumors, two had ovarian carcinomas and in each of them plasma and tumor prorenin concentrations were very high (27).

That LH may be the direct or indirect stimulus for prorenin secretion is also supported by our observation in one patient that administration of clomid (a pharmacologic agent that stimulates LH and follicle-stimulating hormone release from the anterior pituitary) followed by administration of the LH/follicle-stimulating hormone-containing preparation Pergonal resulted in a 2.5-fold increase in circulating prorenin. When human chorionic gonadotropin was then given, prorenin increased another 4-fold, reaching levels as high as those during gestation (28). With the observation that prorenin increases rapidly following conception, in parallel with the initial increase in human chorionic gonadotropin (15), the data suggest that gonadotropic hormones from the anterior pituitary and the placenta can stimulate the secretion of prorenin in women.

In comparison to changes in other hormones during the menstrual cycle, the relative increase in prorenin was quite small. Nonetheless, the consistency of the changes and the knowledge that a 10-fold increase in prorenin normally occurs within 4 weeks following conception (15) suggests that the changes have physiological relevance. One explanation for the relatively small increase in prorenin may be that it is synthesized at its site of action—for example, the ovary—and that the plasma increase merely reflects the small fraction of molecules that escapes into the circulation.

The role of prorenin in the menstrual cycle and during gestation is unknown. Renin has also been identified in the Leydig cells of the testis (29) so that prorenin might conceivably be involved in male reproduction as well. Since extrarenal renin is reported to be associated with the same cells as the other components of the renin system, angiotensin II might be formed locally (30, 31). Since angiotensin II affects aldosterone biosynthesis (2) at an early step in the biosynthetic pathway, by augmenting the conversion of cholesterol to pregnenolone (3), perhaps angiotensin II could stimulate other steroids in other target tissues such as the ovaries and testis.

More work is required to identify the source and the reason for the changes in plasma prorenin that occur during the menstrual cycle. Meanwhile, these data strongly suggest that renin and prorenin, besides having important effects on the cardiovascular system, may also play a role in reproductive physiology.

Note Added in Proof. Since submission of this manuscript, Fernandez *et al.* (32) reported the presence of renin-like activity in human ovarian follicular fluid. This observation is consistent with the view that the source of the fluctuations in prorenin during the menstrual cycle is the ovary.

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