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Plasma prorenin response to human chorionic gonadotropin in ovarian-hyperstimulated women: Correlation with the number of ovarian follicles and steroid hormone concentrations

(estradiol/progesterone/renin/ovary)

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ABSTRACT **Plasma prorenin and active renin were measured before and after human chorionic gonadotropin (hCG) administration in two groups of patients undergoing ovarian stimulation for 4–6 days with follicle-stimulating hormone alone or in combination with luteinizing hormone, for *in vitro* fertilization. Baseline total plasma renin (prorenin plus active renin; $n = 12$) averaged 25 ± 8 ng/ml per hr (mean ± SD). Total renin did not change during ovarian stimulation but it increased to 46 ± 16 ng/ml per hr ($P < 0.05$) 1 or 2 days later, just before hCG administration. Thirty-six hours after hCG administration, just before laparoscopy and egg retrieval, total renin was 123 ± 97 ng/ml per hr; a peak of 182 ± 143 ng/ml per hr occurred 2–6 days later—i.e., during the luteal phase of the menstrual cycle. In eight of the patients who did not conceive, total renin returned to baseline 14 days after hCG administration. In four who conceived, a nadir was reached (57 ± 13 ng/ml per hr) 8–12 days after hCG administration and then total renin increased again as the plasma βhCG measurement began to rise. By day 16 it averaged 225 ± 157 ng/ml per hr. In a second group of five patients active renin and prorenin were measured separately. Active renin comprised <20% of the total renin at all times. It was unchanged until day 4 after hCG administration and then increased significantly only when plasma progesterone was high. Thus, the initial response to hCG was entirely due to an increase in prorenin. A highly significant correlation was observed between the number of follicles and the total renin increases on the day of aspiration ($r = 0.93$, $P < 0.001$) and at the peak ($r = 0.89$, $P < 0.001$). After hCG administration, a temporal relationship was observed between the rise in total renin and plasma estradiol and progesterone levels. These results demonstrate that plasma prorenin increases markedly after administration of hCG and that the rise is directly related to the number of ovarian follicles and to plasma estrogen and progesterone levels. The findings suggest that prorenin is produced by the mature ovarian follicle and by the corpus luteum in response to gonadotropin stimulation.**

The renin-angiotensin system plays a key role in blood pressure, fluid and electrolyte homeostasis through the vasoconstrictor action of angiotensin II, and the stimulation of aldosterone biosynthesis by the adrenal zona glomerulosa cells (1). Active renin is synthesized by and secreted from the kidney. Prorenin, its enzymatically inactive precursor, circulates at higher concentrations than active renin (2) and is also mostly derived from the kidneys. Nonetheless, it persists at low levels in the blood of chronically nephrectomized females and males (3–5). Prorenin is also synthesized by the placenta (6–8) and is markedly increased in the plasma of pregnant women (9–12). However, the early pregnancy-related rise in plasma prorenin is apparently not due to placental secretion since it did not occur in a patient with ovarian failure (13).

Recent evidence suggests that prorenin (13–18) and the renin-angiotensin system (14, 19, 20) may be linked to ovarian physiology. Mature human ovarian follicles contain prorenin in high concentrations (14, 18); only 1% is enzymatically active (14). Plasma prorenin, but not active renin, increases at midmenstrual cycle just after the luteinizing hormone (LH) surge (15, 16) and a second but lower rise in prorenin, together with active renin, occurs later (15) when progesterone is high.

Ovarian hyperstimulation with gonadotropins results in the development of multiple follicles and consequently many corpora lutea. Preliminary studies suggest that such patients have a marked rise in prorenin in response to human chorionic gonadotropin (hCG) (13, 14, 17). We therefore examined the relationship of the hCG-induced increase in prorenin to the number of follicles induced by ovarian stimulation and to the changes in plasma estradiol and progesterone that occurred in patients undergoing follicular aspiration for *in vitro* fertilization and embryo transfer.

METHODS

Patients. Two groups of patients undergoing ovarian stimulation for *in vitro* fertilization and embryo transfer were studied. Group A consisted of 12 patients in whom serum samples for routine hormonal measurements were retrospectively analyzed. We selected patients in whom both ovaries had been completely available for follicular aspiration. Ten patients had irreparable tubal disease and in 2 patients the cause of infertility was unknown. Ovarian stimulation was accomplished as reported (21–24). Seven patients were treated with "pure" follicle-stimulating hormone (FSH) [Metrodin, Serono Laboratories (Randolph, MA), containing 75 international units (IU) of human urinary FSH and <1 IU of LH in each ampule] and 5 patients were treated with a combination of "pure" FSH and human menopausal gonadotropin (HMG) (Pergonal, Serono Laboratories, containing 75 IU of FSH and 75 IU of LH). In the FSH-only protocol, four ampules of FSH were injected on days 3 and 4 of the menstrual cycle; this was followed by injection of two ampules of FSH on subsequent days. In the FSH/HMG

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Abbreviations: hCG, human chorionic gonadotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; HMG, human menopausal gonadotropin; IU, international units.

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protocol, two ampules of FSH and two ampules of HMG were injected on days 3 and 4 of the cycle; this was followed by injection of two ampules of HMG on subsequent days. After 5–7 days of treatment, hCG (10,000 IU) was injected as a surrogate LH surge (defined as day 0). hCG was administered 28–52 hr following the last injection of gonadotropins (FSH or HMG). Follicles were aspirated by laparoscopy under general anesthesia about 36 hr later (day 2). All visible follicles were aspirated and the maturation of the oocytes and the granulosa cells from each follicle was determined (24). *In vitro* fertilization of the oocytes and embryo culture were accomplished as reported (24). Starting on day 3, patients were treated with intramuscular injection of progesterone, 25 mg/day, and embryos were transferred to the uterus on day 4.

Group B consisted of five patients studied prospectively in whom plasma samples instead of serum samples were collected so that changes in active renin could be examined. Four patients had irreparable tubal disease and in one patient the cause of infertility was related to male factor. Three patients were treated with FSH only and two patients were treated with a combination of FSH/HMG. Protocols similar to those described for patients in group A were employed. At laparoscopy both ovaries were found to be completely available in three patients. However, in two patients only about 70% of the ovarian surface was available for aspiration.

Blood Collection and Processing. Serum samples (group A) or plasma samples (group B) were collected daily at 0800–0900 a.m. until day 2 and on alternate days thereafter. On the day of laparoscopy blood samples were withdrawn 15 min before the induction of anesthesia. The serum samples from group A were frozen and thawed several times for routine hormonal measurements, which caused cryoactivation of prorenin and falsely high active renin values (2). For this reason only total renin values are reported in group A patients. For group B, blood was collected into K_2EDTA Vacutainers and maintained at room temperature until the plasma was separated. Active renin was usually measured after only one freezing and thawing cycle; however, samples that were collected 6 or more days following hCG administration (see below) were exposed to two freezing and thawing cycles. (From day 6, samples were mailed by the patients to Norfolk on dry ice.)

Hormonal Measurements. Prorenin in serum or plasma was converted to active renin by limited proteolysis with Sepharose-bound trypsin, as described (15). Active renin was measured by enzymatic assay utilizing endogenous plasma renin substrate (25). For serum samples, 3 mM EDTA was added before the enzymatic assay to inhibit angiotensinases and converting enzyme. The angiotensin I formed during the enzymatic assay was measured by RIA (25). Plasma prorenin was calculated as the difference between the endogenous active renin and the total renin measured after trypsin activation. Results are expressed as the rate of angiotensin I formation (ng/ml per hr). Serum estradiol and progesterone were assayed using a commercially available RIA kit (Pantex, Santa Barbara, CA), as reported (22).

Statistical Analysis. The significance of changes in hormone levels during the cycle was calculated by using the paired Student's t test with Bonferroni's correction for multiple measurements (26). Correlations between total renin and number of follicles were determined by regression analysis. $P < 0.05$ was considered to be significant.

RESULTS

Group A. At laparoscopy 10.6 ± 5.6 follicles (mean ± SD; range, 3–20) were aspirated and mature granulosa cells were detected in 88.6% ± 10.1% (range, 66.6–100%) of the aspirated follicles (24).

Total renin levels. The total renin results from two of these patients were reported in a short preliminary communication (13). Baseline total serum renin (day 3 of menstrual cycle) was 25 ± 8 ng/ml per hr (mean ± SD); it remained relatively stable during the administration of FSH and FSH/LH in the follicular phase (Fig. 1). A small but consistent increase was observed on the day of hCG injection (day 0) before hCG was administered ($P < 0.05$) (Fig. 1 and Table 1). Following hCG injection a marked and sustained increase in total renin was observed. Just before follicular aspiration (day 2) it had risen to 6.4-fold baseline (range, 1.7–15.3) and it further increased slightly to reach a peak of 9.0-fold baseline (range, 2.4–22.5), either on day 4 ($n = 5$), day 6 ($n = 6$), or day 8 ($n = 1$). (See Fig. 2 for an illustration of the spectrum of responses.) Thus, the highest level of total renin occurred after follicle aspiration—that is, during the luteal phase of the cycle. In the eight

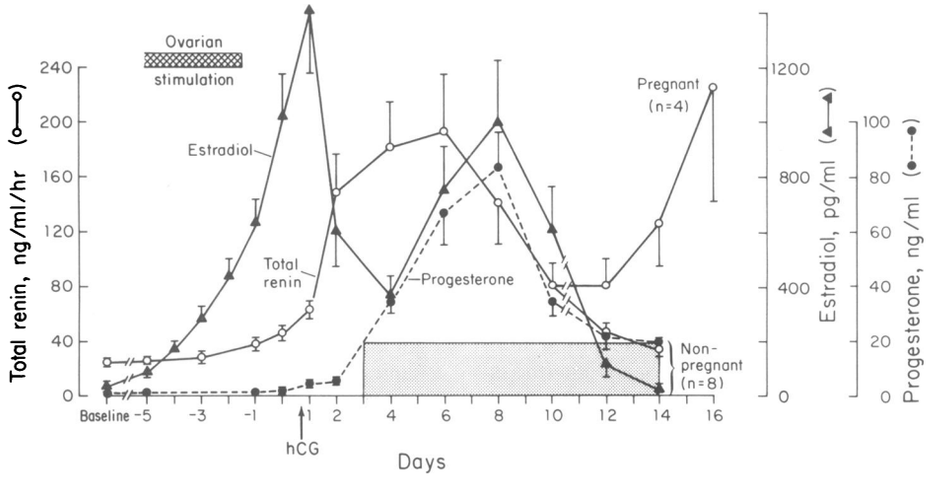

FIG. 1. Serum total renin, estradiol, and progesterone changes (mean ± SEM) in 12 patients undergoing ovarian stimulation with gonadotropins and hCG. Baseline is the third day after the beginning of menstruation. hCG was administered on day 0 and ovarian follicles were aspirated on day 2 after blood sampling. Total serum renin was significantly higher than baseline on day 0 ($P < 0.05$) and was almost back to baseline on day 14 in 8 patients who did not conceive. In 4 pregnant women total renin increased again after day 10. Estradiol was above baseline ($P < 0.05$ or greater) from day −4 until day 12. Progesterone was above baseline on day 1 and thereafter. The shaded area represents the estimated level of serum progesterone (based on the level of progesterone on day 14 in the nonpregnant patients) caused by the administration of exogenous progesterone (25 mg/day), which was given from day 3 until day 14. The serum estradiol and progesterone values after day 10 are from the 8 nonpregnant women.

Table 1. Total serum renin and progesterone before (−) and after (+) hCG injection

Day	Total renin, ng/ml per hr	Progesterone, ng/ml
Baseline*	25 ± 8	0.7 ± 0.3
−1	38 ± 12	0.8 ± 0.3
0	46 ± 16†	1.3 ± 0.9
+1	63 ± 23†	3.8 ± 0.3†
+2	148 ± 97†	5.1 ± 3.6†

Data are expressed as mean ± SD (n = 12).
*Baseline = day 3 of the menstrual cycle.
†$P < 0.05$ compared to baseline levels.

patients who did not conceive, total renin had fallen to 132% of baseline level on day 14 and they had undetectable hCG levels. In the four women who conceived, total renin reached a nadir on days 8–12 (57 ± 13 ng/ml per hr) and then increased to 225 ± 157 ng/ml per hr on day 16 (Fig. 1), when hCG was 424 ± 293 mIU/ml.

Magnitude of increase in total renin in relation to the number of ovarian follicles. Analysis of the relationships between the increase in total serum renin and the number of aspirated follicles on day 2 disclosed a highly significant correlation (r = 0.93, $P < 0.001$, Fig. 3*A*). A somewhat weaker correlation (r = 0.73, $P < 0.01$) was observed on day 1 (8–12 hr after hCG injection). Total renin was also higher than baseline on day 0 but the relationship to the number of follicles was not significant (r = 0.32).

The maximum increase in total renin after hCG administration occurred during the luteal phase and the peak value in each subject was highly related to the number of follicles (r = 0.89, $P < 0.001$, Fig. 3*B*). Fig. 2 illustrates the changes in serum total renin in representative individual subjects with low, intermediate, or high numbers of follicles. For comparison, the levels of total plasma renin throughout a representative natural cycle are also illustrated (16).

Temporal relationship between blood levels of total renin, estradiol, and progesterone. Estradiol increased during the follicular phase during the period of ovarian stimulation by gonadotropins; estradiol fell after hCG injection and fell further after follicular aspiration, but rose again during the luteal phase (Fig. 1). The number of follicles correlated with plasma estradiol on day 0 (r = 0.84, $P < 0.01$), day 1 (r = 0.89, $P < 0.001$), and day 2 (r = 0.87, $P < 0.001$) (Fig. 3*C*). Not surprisingly, a significant correlation between estradiol and total renin was also demonstrated (Fig. 3*D*). Baseline progesterone level (day 3 of the menstrual cycle) was 0.7 ± 0.3 ng/ml; it increased slightly on day 0 (Fig. 1 and Table 1). Following a peak of >80 ng/ml on day 8, progesterone fell to about 20 ng/ml on days 12 and 14. The hormonal level on days 12 and 14 was largely due to the daily injection of exogenous progesterone and so the corpora lutea were apparently contributing an average of 60 ng of progesterone per ml at the peak. Total serum renin disclosed a temporal relationship with serum progesterone levels through the follicular and the luteal phases and with estradiol levels during the luteal phase. However, the rise and fall of both serum estradiol and progesterone during the luteal phase were preceded by the rise and fall of total serum renin (Fig. 1 and Table 1).

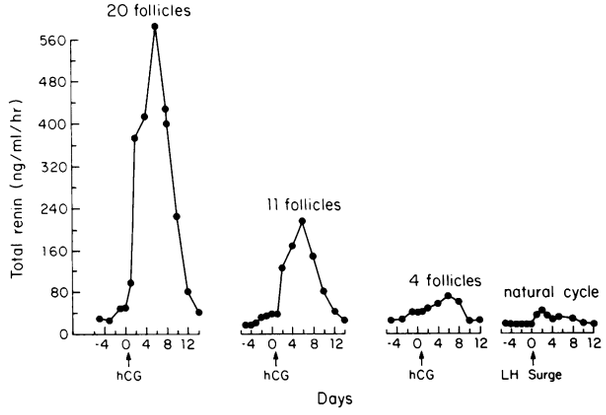

FIG. 2. Total serum renin levels throughout the stimulated cycles of three representative patients with different numbers of ovarian follicles. Shown for comparison are the changes in total plasma renin throughout a natural cycle (16) in which only one follicle usually matures.

Group B. In this second prospectively studied group of patients, 8.2 ± 2.2 (range, 6–11) follicles per patient were aspirated and 73.2% ± 16.4% (range, 45.5–85.7%) were classified as mature follicles.

The changes in plasma prorenin, active renin, and progesterone are shown in Fig. 4. Baseline plasma active renin was 1.9 ± 1.1 ng/ml per hr (mean ± SD); unlike plasma prorenin, active renin remained unchanged until day 6 after hCG administration. On day 6 and thereafter, active renin levels increased significantly ($P < 0.01$), at a time when plasma prorenin was falling and plasma progesterone was at peak levels. Active renin comprised only 8% ± 2% of total renin under baseline conditions and was 11% ± 6%, 4% ± 3%, 5% ± 1%, and 11% ± 4% on days 0, 2, 6, and 8, respectively. The pattern of change in plasma prorenin resembled that of total plasma renin in group A (Fig. 1). The lack of a further increase in plasma prorenin from day 2 to day 4 in this group may be related to the fact that in two patients plasma samples from day 4 were not available for analysis. These two patients had the highest prorenin levels on day 2 (250 and 127 ng/ml per hr).

DISCUSSION

The present results demonstrate that hCG administration following ovarian hyperstimulation with gonadotropins results in marked and sustained increases in circulating prorenin. The levels achieved are much higher than those that occur normally during the spontaneous LH surge of a natural cycle. As in natural cycles, active plasma renin was essentially unchanged until the luteal phase. The height of the circulating prorenin response to hCG was directly related to the number of mature follicles detected at the time of aspiration, suggesting that prorenin is produced and secreted by the ovarian follicles. Furthermore, a temporal relationship between plasma prorenin and corpus luteum steroidogenic activity was also demonstrated.

Relationship of Prorenin Levels to the Number of Mature Follicles. During the follicular phase of the menstrual cycle only one follicle normally matures and ovulates, and plasma prorenin increases an average of 90% after the LH surge for about 2 days (16). In stimulated cycles there was a greater response of prorenin, the magnitude of which was directly related to the number of follicles. The highest prorenin levels seen in nonpregnant women (586 ng/ml per hr, 21-fold baseline) occurred in a patient who had 20 follicles. The average increase in prorenin per follicle (46% on day 2 and 63% on day 6) was similar to that that occurs after the spontaneous LH surge (16). This suggests that the high plasma prorenin levels after ovarian stimulation are due to the presence of multiple follicles and not to abnormally high prorenin secretion by individual follicles. Any small follicles that were not counted either contributed little to circulating levels of prorenin or were proportional to the number of mature follicles. The highly significant correlation between the plasma prorenin concentration during the luteal phase and the number of follicles suggests that prorenin was secreted by the corpora lutea that were formed from the follicles.

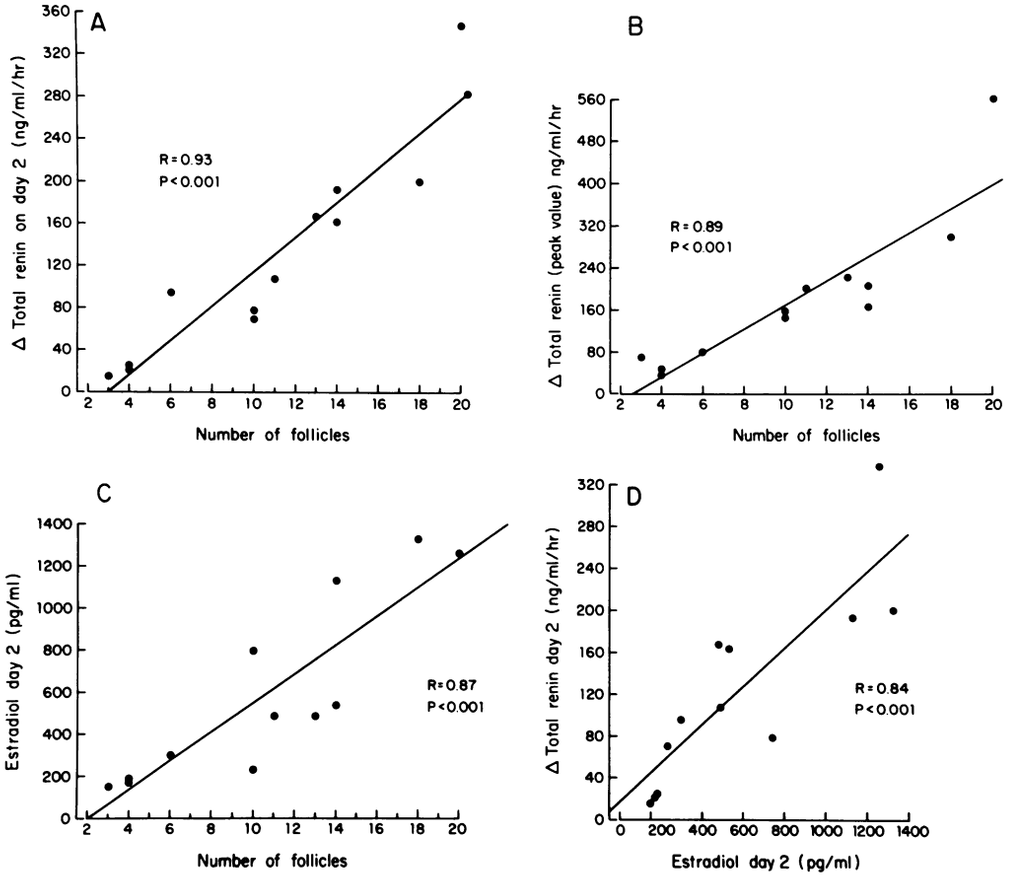

FIG. 3. (*A*) Relationship between the change in total serum renin and the number of follicles on day of ovarian follicle aspiration (day 2). (*B*) Relationship between the maximal change in total serum renin in the luteal phase and the number of ovarian follicles. (*C*) Relationship between serum estradiol levels on the day of aspiration (day 2) and the number of follicles. (*D*) Relationship between the change in total serum renin and serum estradiol levels on the day of ovarian follicle aspiration (day 2).

Time Course of Prorenin and Active Renin Responses. Plasma prorenin does not increase during natural menstrual cycles before the LH surge (15, 16). In contrast, a small but significant increase in prorenin was detected on day 0, before the hCG injection, in the stimulated patients. This rise occurred whether the patients had been stimulated with FSH alone or with a combination of FSH/LH and was therefore not due to the exogenously administered LH. A spontaneous LH surge does not usually occur in hyperstimulated women (27). Presumably, the growing follicle normally secretes small amounts of prorenin, but the secretion of prorenin only increases enough to be detectable when multiple follicles develop.

In the natural menstrual cycle the peak of prorenin is sustained for about 1 day after the LH surge (16). In contrast, hCG injection in stimulated women caused a sustained increase in prorenin that peaked on days 4–6 and did not return to baseline until days 12–14. This prolonged response is most probably related to the longer half-life of hCG (≈32 hr) compared to LH (≈1 hr) (28). The sensitivity of the corpus luteum to hCG is also reflected in the precipitous rise of prorenin after day 12 in the pregnant women (present study and ref. 13).

That the initial increase in prorenin after hCG administration is not associated with an increase in active renin has been clearly demonstrated in this study. The prorenin rise after the LH surge in the natural cycle is also not associated with a significant rise in active renin (15, 16). However, both in natural cycles (15, 29) and in this study, active renin increased during the luteal phase when progesterone was high. This response could be related to the natriuretic effect of progesterone (29) and therefore be of renal origin (Fig. 4). Alternatively, it may be the result of *in vitro* cryoactivation of prorenin (only samples collected during the luteal phase

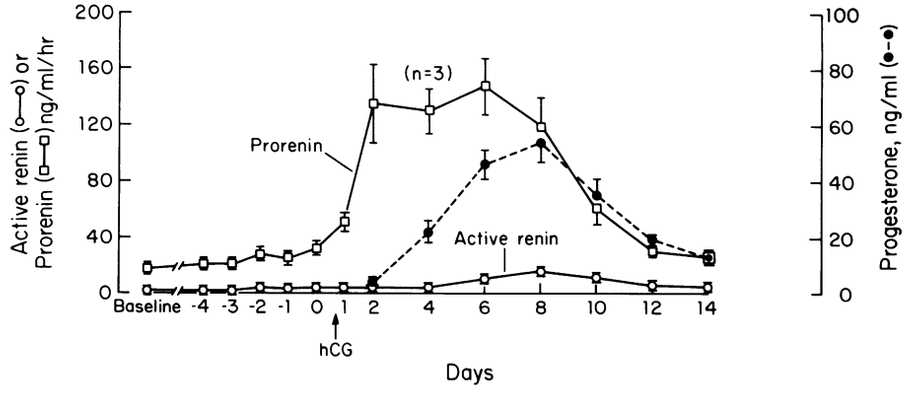

FIG. 4. Plasma prorenin, active renin, and progesterone levels (mean ± SEM) throughout the cycle in five patients treated with gonadotropins and hCG. Patients were treated with progesterone (25 mg/day) from day 3 through day 14.

were frozen and thawed twice and also delivered on dry ice to Norfolk).

Temporal Relationship Between Prorenin and Ovarian Hormones Level. Estradiol is the primary hormone secreted by the granulosa cells of the preovulatory follicle (30–32). Ovarian stimulation was associated with an augmented increase in plasma estradiol during the follicular phase that correlated with the number of follicles. A significant correlation was observed between prorenin and estradiol levels on days 1 and 2 following hCG injection. It is unclear whether the level of each hormone is independently related to the number of maturing follicles or whether this correlation represents a cause and effect relationship. It is possible that estradiol (or its androgen precursors) stimulates ovarian prorenin biosynthesis. Alternatively, locally produced prorenin may affect estrogen levels by causing alterations in thecal/interstitial androgen biosynthesis or metabolism.

The transition between preovulatory follicle and corpus luteum entails a decline in follicular estrogen biosynthesis and an increase in progesterone biosynthesis. Examination of the pattern of prorenin and progesterone levels through the stimulated cycle disclosed a striking temporal relationship between the two hormones. The prorenin rise preceded the increase in progesterone. The idea that ovarian prorenin may be a mediator of the LH/hCG-induced transition between preovulatory follicle and corpus luteum steroidogenic activity is attractive and deserves further study.

Angiotensin II affects intracellular calcium and phosphatidylinositol metabolism (33, 34). Therefore ovarian prorenin, through angiotensin II action, may be involved in other ovarian endocrine events. Putative actions of the ovarian prorenin-renin-angiotensin system include control of ovarian blood flow through its effect on vascular smooth muscle tone (1) and the angiogenic property of angiotensin II (35).

The finding that changes in plasma prorenin are not accompanied by significant changes in active renin has led to speculation that prorenin is activated locally at its site of action (36). That angiotensin II might be formed locally is supported by the reports that extrarenal renin is found in the same cells as the other components of the renin system (37, 38).

In summary, these results are consistent with the view that ovarian prorenin is produced and secreted by the mature follicle and by the corpus luteum in response to stimulation by LH/hCG. Further studies to investigate the role of prorenin in follicular development and corpus luteum function are needed. However, the results from the present study suggest that ovarian prorenin may be related to ovarian steroidogenic activity.

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