Relationship between ovarian steroids, gonadotrophins and relaxin during the menstrual cycle

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The circulating levels of relaxin have been measured and their relationship with the plasma levels of oestradiol (E₂), progesterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) have been investigated during the normal menstrual cycle. In addition, the effect of human chorionic gonadotrophin (hCG) on plasma relaxin levels has been studied. In the first part of the study, blood samples were obtained on days 5, 10 and 15 of the follicular phase and on alternate days from the day of the LH surge (detected in early-morning urine and confirmed by circulating levels of LH) until day 6 of the following follicular phase in nine normally cycling female volunteers. In the second part of the study, a single intramuscular dose of hCG (10 000 IU) was given on day 11 of the menstrual cycle. Relaxin was detectable from the mid-luteal phase until the onset of menstruation. The plasma levels of relaxin on days 10 and 12 of the luteal phase were significantly greater than on day 6. Positive associations between the circulating levels of relaxin and E₂ and negative associations between the plasma levels of FSH and those of both relaxin and E₂ were found on days 8, 10 and 12 of the luteal phase. The relationship between E₂ and FSH was stronger than that between relaxin and FSH. Exogenous hCG had no effect on plasma relaxin levels. The pattern of the relationship between E₂ and relaxin suggests that a common mechanism may regulate their release or that plasma relaxin levels are determined by those of E₂. Furthermore, the absence of any relationship between endogenous LH levels and those of relaxin and the lack of effect of exogenous hCG on plasma relaxin levels suggest that LH does not influence the circulating levels of relaxin directly. The negative relationship between FSH and relaxin is probably indirect, mediated by E₂, although it is possible that relaxin influences FSH release directly.

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Relaxin is detectable in the mid-luteal phase of the normal menstrual cycle, and with pregnancy the levels rise rapidly and peak towards the end of the first trimester (1, 2). Circulating relaxin during pregnancy is derived solely from the corpus luteum (3), and its synthesis is thought to be regulated by luteinizing hormone (LH) in both the non-pregnant (4) and pregnant state (5). However, the demonstration of relaxin mRNA in the placenta suggests that relaxin synthesis may occur in this site (6), although it does not contribute to circulating levels. Relaxin infusions have several neuroendocrine effects: the circulating levels of prolactin and growth hormone are increased in primates (7); and in the rat, LH release is modulated in a steroid-dependent manner (8). In addition, relaxin has been shown to reduce and increase the circulating levels of oxytocin in the pre- and post-partum rat, respectively (9, 10).

The evidence suggesting that relaxin release is regulated by LH or hCG is not conclusive. In non-pregnant women the hCG-induced increase in serum relaxin levels is seen only after a lag of 2–4 days (4), while during pregnancy the effect is delayed still more (5). Furthermore, in vitro relaxin response to hCG is inconsistent, and when present it still shows a lag (11, 12). In contrast, the response of the ovarian steroids to hCG is immediate (5). Thus, the effect of hCG on relaxin levels may be indirect, possibly mediated by increased levels of the ovarian steroids.

We have measured the plasma levels of relaxin during the menstrual cycle and related them to the circulating levels of E₂, progesterone, FSH and LH, and, in addition, we have studied the effect of exogenous hCG on the circulating level of relaxin in order to define which factors may determine the circulating levels of relaxin and to investigate whether relaxin may be involved in the modulation of the hypothalamic–pituitary–ovarian axis.

Subjects and methods

Nine normal women were studied. Blood samples were taken on the following days: follicular phase 5, 10, 15...
(on day 15 only if LH surge had not been detected); luteal phase (LH surge plus 1 day) 2, 4, 6, 8, 10, 12, 14; the following follicular phase 0, 2, 4, 6. The LH surge was detected by urine testing (Clearplan, Unipath, Bedford, UK) and confirmed by plasma levels of LH. Subsequently, ovulation was confirmed by elevated levels of progesterone.

On day 11 of the luteal phase (LH surge plus 12 days) in two successive cycles, subjects received in random order either a single intramuscular injection of 10 000 IU of hCG (Profasi, Serono Laboratories UK Ltd., Welwyn Garden City, Herts, UK) or 1 ml of saline. Blood samples were obtained via an indwelling intravenous cannula sited in anterior cubital fossa, at time 0, 1, 2, 4, 8, 24 and 48 h and on alternate days until day 6 of the following follicular phase.

*Relaxin ELISA*

The concentration of relaxin was measured in unex-
tracted plasma by a non-competitive double-antibody ELISA (13). The polyclonal antibodies were raised in New Zealand White rabbits using synthetic relaxin (hRXN2) as the immunogen. The enzyme used in the assay is horseradish peroxidase. Synthetic human relaxin was used to make up the standards in pooled normal male plasma at concentrations of 0–1250 ng/l. The limit of detection of the assay is 20 ng/l. The samples were assayed in one batch and in this assay the intra-assay variation was 12% (65 ng/l), 2.6% (300 ng/l) and 1.4% (500 ng/l). The cross-reactivity with insulin, nerve growth factor and porcine relaxin was <0.1%.

**Oestradiol, progesterone, LH and FSH assays**

Plasma levels of E₂, progesterone, LH and FSH were measured by non-isotopic immunoassays (Boehringer Mannheim enzyum). Interassay and intra-assay imprecision (coefficient of variation) was less than 10% for E₂, progesterone, LH and FSH.

**Statistics**

The relaxin data were not distributed normally and have been expressed as geometric means. The significance of the differences between time points was determined using a Mann–Whitney U test. Correlation was determined using a simple regression analysis.

**Results**

Relaxin was detectable in the peripheral plasma from day 8 of the luteal phase (geometric mean 24 ng/l; range 20–70 ng/l); mean levels were significantly greater than baseline on days 10 (37 ng/l; 20–103 ng/l) and 12 (38 ng/l; 20–77 ng/l) but not on day 14 (22 ng/l; 20–33 ng/l) (Fig. 1). The mean levels of E₂, progesterone, FSH and LH are shown in Fig. 1b,c.

The circulating levels of relaxin correlated negatively with those of FSH and positively with those of E₂, and FSH correlated negatively with E₂ (Table 1). A weak inconsistent correlation was detected between the levels of LH on day 6 and those of progesterone on successive days. No other consistent correlations between the levels of the analytes were detected.

The single bolus injection of hCG had no effect on the

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**Fig. 1.** The geometric means of the plasma levels of (a) relaxin (○), (b) oestradiol (□) and progesterone (■) and (c) luteinizing hormone (▲) and follicle-stimulating hormone (△) during the luteal phase of the menstrual cycle in nine normal volunteers. * Significant elevation over baseline (p<0.05).

| Table 1. Correlations between the circulating levels of relaxin, follicle-stimulating hormone (FSH) and oestradiol (E₂). |
|------------------------|----------------|----------------|----------------|
| Luteal phase           | Day 8          | Day 10         | Day 12         |
| **Relaxin**            |                |                |                |
| E₂                    | 0.8, p = 0.008| 0.82, p = 0.006| 0.85, p = 0.004|
| FSH                   | −0.6, p = 0.09 | −0.63, p = 0.06| −0.66, p = 0.051|
| **FSH**                |                |                |                |
| E₂                    | −0.84, p = 0.005| −0.74, p = 0.024| −0.67, p = 0.049|

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circulating levels of relaxin. However, the length of the luteal phase was increased in the cycle in which hCG was given (median 4 days; range 0–6).

Discussion

This is the first study to investigate the relationship between relaxin and the circulating levels of the gonadotrophins LH and FSH and of the ovarian steroids E2 and progesterone during the normal menstrual cycle. In addition, it has investigated further the effect of hCG on the circulating levels of relaxin. During pregnancy, relaxin is thought to determine not only the time of onset of labour (14) but also its subsequent progression (15). Furthermore, relaxin has been shown to be important in the normal growth of the uterus and endometrium in primates (16) and rodents (17), and may have a similar role in women. Thus, relaxin is important, although probably not essential, during pregnancy. The factors that regulate the circulating levels of relaxin during pregnancy are uncertain, although animal (18) and some human data (MR Johnson et al., unpubl. data) suggest that they are determined in the cycle of conception.

In the present study no relationship was found between the circulating levels of relaxin and those of LH and there was no effect of exogenous hCG on the plasma levels of relaxin, despite the indirect evidence outlined above, which suggested that LH/hCG regulates the circulating level of relaxin (4, 5). The absence of any relationship between relaxin and LH is surprising because relaxin is derived from the corpus luteum and LH is thought to be the prime regulator of corpus luteum function (19). However, in both studies in which the effect of exogenous hCG on circulating levels of relaxin has been studied, while the effect of hCG administration was immediate on the circulating levels of progesterone and/or E2, it was delayed in the case of relaxin (4, 5). An alternative explanation of these results is that the effect of hCG on relaxin is indirect, perhaps mediated by E2. This would be supported by the strong correlation between the circulating levels of E2 and those of relaxin found in this study and the increase in E2 levels reported following hCG administration (5). The absence of an association between the levels of relaxin and those of progesterone, despite the similarity in the behaviour of the mean levels of each analyte, is unexpected, given their common source. Similarly, the absence of a relationship between E2 and progesterone is surprising. The absence of a relationship between progesterone and either relaxin or E2 is in contrast to the strong association between relaxin and E2, and supports the notion that either their synthesis is linked or E2 is the prime regulator of relaxin synthesis.

The negative relationship between FSH and relaxin is probably indirect, mediated by E2 or another ovarian product, possibly inhibin. However, the evidence that inhibin is important in the regulation of the circulating levels of FSH during the luteal phase of the menstrual cycle is conflicting (20), and, even when demonstrated, is weak (21, 22). Furthermore, the circulating levels of inhibin have been related to those of progesterone (22), and no such relationship was found between relaxin and progesterone in the present study. Indeed, the negative correlations between relaxin and FSH are stronger than those reported for inhibin. Thus, it seems likely that the negative relationship between relaxin and FSH is mediated through E2.

In conclusion, we have demonstrated a strong positive relationship between the circulating levels of relaxin and those of E2. This suggests either that the synthesis of E2 and relaxin is regulated by a common factor or that E2, rather than LH, regulates relaxin synthesis during the menstrual cycle.

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References


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