FSH inhibits the augmentation by oestradiol of the pituitary responsiveness to GnRH in the female rat

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The effect of follicle stimulating hormone (FSH) treatment on the pituitary response to gonadotrophin-releasing hormone (GnRH) was studied in rats in various reproductive conditions. A 3-day treatment of cycling rats with FSH (Metrodin®, 10 IU/injection) lowered the spontaneous pre-ovulatory LH-surge and suppressed the pituitary luteinizing hormone (LH) response to GnRH. FSH also suppressed the LH response of pseudopregnant (PSP) rats on day 8 of pseudopregnancy, but not that of day-8 PSP rats which had been ovariec-tomized on day 4 (OVX–PSP rats). GnRH induced self priming in cycling, PSP and OVX–PSP rats. Oestradiol strongly augmented the pituitary LH-response to GnRH injection in PSP and OVX–PSP rats, but not in cycling rats; probably because in these latter animals the LH response to GnRH was already augmented by endogenous oestradiol. FSH suppressed the LH response to GnRH in oestradiol-treated PSP and cycling rats; in these latter rats the suppression of the LH response was as strong as that in cycling rats not treated with oestradiol. FSH did not suppress the LH response of oestradiol-treated OVX–PSP rats. The effect of FSH was not associated with changes in plasma oestradiol and progesterone concentrations. Analysis of the data revealed that FSH specifically suppressed the augmentative effect of oestradiol, but did not affect the GnRH-self priming effect. It is concluded that under the influence of FSH, the ovaries produce a factor which suppresses the augmentative effect of oestradiol on the GnRH-induced LH response of the pituitary gland. It is suggested that this effect of FSH underlies the suppression of the spontaneous LH-surges of FSH-treated cycling rats. As the present putative ‘oestrogen-antagonizing factor’ did not suppress the GnRH-self priming effect, it is suggested that this factor is not identical to gonadotrophin surge inhibiting factor. Key words: antagonist/GnRH responsiveness/LH/oestradiol

Introduction

In the 1980s, evidence accumulated that in various species, including the rat (Geiger et al., 1980; de Koning et al., 1987, 1989), the monkey (Schenken and Hodgen, 1983; Littman and Hodgen, 1984) and man (Messinis and Templeton, 1986, 1988, 1990a,b), treatment with follicle stimulating hormone (FSH) may attenuate and even completely inhibit the pre-ovulatory gonadotrophin surge. FSH treatment was also shown to suppress the oestrogen-induced pre-ovulatory luteinizing hormone (LH) surge, as well as the gonadotrophin-releasing hormone (GnRH)-induced gonadotrophin secretory response in oestrogen-treated monkeys (Sopelak and Hodgen, 1984; Schenken and Hodgen, 1986); this latter effect of FSH was also seen in women undergoing ovulation stimulation (Messinis and Templeton, 1989, 1990a,b; Messinis et al., 1994a).

These effects of FSH are attributed to the action of one single non-steroidal factor of ovarian origin: gonadotrophin-surge inhibiting or surge-attenuating factor (GnSIF or GnSAF), which suppresses the pituitary responsiveness to GnRH (de Koning, 1995; Fowler and Templeton, 1996). GnSIF/AF is claimed to suppress the gonadotrophin surge by preventing GnRH sensitization of the pituitary gland for its own gonadotrophin-releasing action (Koppenaal et al., 1992; Fowler et al., 1993, 1994; de Koning, 1995; Byrne et al., 1996): the so-called GnRH-self priming effect (Aiyer et al., 1974). However, GnRH self-priming is not the only factor contributing to the increase in pituitary responsiveness to GnRH at the time of the pre-ovulatory GnRH surge; also oestradiol, secreted by the developing follicles, plays a role in this respect (Schuiling et al., 1987, 1990). Attenuation by FSH treatment of the pre-ovulatory gonadotrophin surge, therefore, may also be due to FSH-induced suppression of the augmenting effect of oestradiol. This suggestion was tested in cycling, pseudopregnant (PSP) and ovariec-tomized pseudopregnant rats (OVX–PSP).

Materials and methods

Animals

Female Wistar rats (kept in Macrolon cages) were used (weight ~200 g). Vaginal smears were taken daily; only animals with regular 4-day ovulatory cycles were used. In some of the rats, a luteal phase or pseudopregnancy was induced in 4-day cycling rats by electrical stimulation of the cervix uteri which took place for a period of 30 s on pro-oestrus rats at 17.00 and on oestrus rats at 15.00, with a current of 125 µA and a frequency of 200 Hz. Such a stimulation provokes secretion of prolactin which activates the corpora lutea to produce progesterone. The first day following the last cervix stimulation was denoted day 1 of PSP (Schuiling et al., 1985). Some of the rats were ovariec-tomized under light ether anaesthesia on day 4 of PSP and used for experiments on day 8.

Pretreatment

A number of the rats received two s.c. Silastic implants containing oestradiol (Organon, Oss, The Netherlands) or two sham (empty) implants at times apparent from the ‘experimental protocol’. The
dimensions of the implants were: length 1.0 cm; inside diameter 1.57 mm; outside diameter 3.18 mm. Such implants are known to significantly elevate plasma oestradiol concentrations in PSP rats (Schuiling et al., 1996).

FSH (Metrodin®; Serono Benelux, Den Haag, The Netherlands), 10 IU/injection, dissolved in saline, was administered i.p. at times apparent from the ‘experimental protocol’. FSH injections were given at 09.00. A dose of 10 IU/injection was chosen, based on the results of previous studies (de Koning et al., 1994). On the day of induction of gonadotrophin responses/blood sampling (see ‘experimental protocol’), rats received a cannula in the right carotid artery (09.00).

**Induction of LH response**

Two injections of GnRH (Cryptocur®; Hoechst AG, Frankfurt am Main, Germany) of 0.5 μg/100 g body weight each were administered through the cannula for induction of LH responses; the first one at 13.00 (t = 0); the second one at 14.00. At 1 h before administration of GnRH, rats were anaesthetized with phenobarbitone (Interpharm BV, Meppal, The Netherlands; 80 mg/kg body weight i.p.) in order to block (in pro-oestrous rats) the spontaneous LH surge. Blood samples for assay of LH were taken through the cannula at times apparent from Figure 1. Progesterone in PSP rats and oestradiol in all groups of rats were measured in the t = 0 sample. The plasma samples were stored frozen at −20°C until assay.

**Assays**

Concentrations of LH were measured in duplicate by double antibody radioimmunoassay with anti-ovine LH as antiserum and rat LH as tracer (Welschen et al., 1975); the reference preparation (RP) used was NIDDK-rat LH-RP-3 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The intra- and inter-assay variabilities were <10%. Progesterone and oestradiol were also measured in duplicate. Progesterone was measured as described by de Jong et al. (1974); the sensitivity of the assay was 0.2 nmol/l; the inter- and intra-assay variabilities were <10%. Oestradiol was measured as described by Jurjens et al. (1975). The sensitivity of the assay was 0.02 nmol/l; the inter- and intra-assay variabilities were <8%.

**Parameters**

See Figure 1. LH responses were judged on the basis of the mean maximal increments of the plasma LH concentrations, which did not yield different results when compared with quantification using the area-under-the-curve as the parameter. This parameter was considered to reflect the pituitary responsiveness to GnRH (Schuiling et al., 1988). Increments were calculated by subtracting the basal LH concentration at t = 0 from the maximal LH concentration (i.e. the primed LH response) induced by the second GnRH injection at t = 80 min. The magnitude of the GnRH-self priming effect (‘priming factor’) was calculated by dividing the mean maximal increment of the LH response induced by the second GnRH injection by the mean maximal increment of the LH response induced by the first GnRH injection. The magnitude of the augmenting effect of oestradiol (‘oestradiol-augmenting factor’) was assessed by dividing the mean maximal increment of the LH response induced by the second GnRH injection of oestradiol-treated rats, by that of the corresponding control rats.

**Experimental protocols**

**Experiment 1; cycling rats**

Spontaneous LH surges; effect of FSH: Cycling rats received FSH or solvent injections on oestrus, di-oestrus-1 and di-oestrus-2. On pro-oestrus, blood samples for measurement of the spontaneous LH surges were taken at times apparent from Figure 2.

Pituitary responsiveness to GnRH; effect of oestradiol and FSH: Cycling rats received two oestradiol-implants or two sham implants on di-oestrus-1; rats were treated with FSH or solvent on oestrus, di-oestrus-1 and di-oestrus-2. LH responses were induced on pro-oestrus; blood samples were taken at times apparent from Figure 1.

**Experiment 2; pseudopregnant rats**

Pituitary responsiveness to GnRH; effect of oestradiol and FSH: Pseudopregnant rats received two oestradiol-implants or two sham implants on day 4 of PSP. Rats were treated with FSH or solvent on days 5, 6 and 7 of PSP. LH responses were induced on day 8; blood samples were taken at times apparent from Figure 1.

**Experiment 3: ovariectomized PSP rats**

Pituitary responsiveness to GnRH; effect of oestradiol and FSH: Rats were ovariectomized on day 4 of PSP (OVX–PSP rats). Immediately after ovariectomy, rats received two oestradiol-implants or two sham implants. Rats were treated with FSH or solvent on days 5, 6 and 7 of PSP; LH responses were induced on day 8. Blood samples were taken at times apparent from Figure 1.

**Statistical analysis**

Data are expressed as means ± SEM. Statistical comparisons were made by analysis of variance (ANOVA), followed by Tukey’s HSD test.
FSH, oestradiol and pituitary response

Figure 2. Spontaneous luteinizing hormone (LH) surges of 4-day cycling rats. (A) control rats \((n = 5)\); (B) rats \((n = 5)\) treated with follicle stimulating hormone (FSH), by i.p. injection of 10 IU FSH (Metrodin®) at 09:00 on oestrus, di-oestrus-1 and di-oestrus-2. Control rats received solvent injections. Blood samples were taken at pro-oestrus.

Figure 3. Maximal increments of plasma luteinizing hormone (LH) concentrations (mean ± SEM) as induced by two consecutive equally large gonadotrophin-releasing hormone (GnRH) injections (0.5 µg/100 g body weight; time interval = 60 min) on pro-oestrus of 4-day cycling rats. In all series: \(n = 5\). Left pair of bars: rats not treated with oestradiol; right pair of bars: rats treated with oestradiol. Of each pair of bars: open bars: rats treated with solvent; shaded bars: rats treated with follicle stimulating hormone (FSH). *, **, ***Significantly different \((P < 0.05)\), as determined by analysis of variance followed by Tukey’s HSD test. Pairs of groups were compared using the Mann–Whitney \(U\)-test. The level of significance was chosen at \(P < 0.05\).

Results

Experiment 1: 4-day cycling rats

Spontaneous LH surges; effect of FSH

Figure 2 shows spontaneous pre-ovulatory LH surges of control 4-day cycling rats (left panel) and similar surges of 4-day cycling rats which had been pretreated with FSH (right panel). The mean height of these latter LH surges \((6.5 ± 1.0 \text{ ng/ml plasma})\) was significantly lower than that of the former ones \((15.4 ± 2.5 \text{ ng/ml plasma}; P < 0.05\); Mann–Whitney \(U\)-test). Maximum LH values of the surges of the FSH-treated rats were not delayed as compared with those of the surges of control rats (not significant).

Pituitary responsiveness to GnRH; effect of oestradiol and FSH

Figure 3 shows the effect of FSH treatment on the LH responses, induced by two consecutive GnRH injections in pro-oestrous, phenobarbitone-blocked 4-day cycling rats. Rats were (right panel) or were not (left panel) pretreated with oestradiol; Figure 4 shows that oestradiol treatment significantly elevated the plasma oestradiol concentrations. Figure 3 shows that both before and after oestradiol-treatment, there was a strong GnRH-self priming effect (priming factor (for definition, see Materials and methods and Figure 1): 5.1 ± 0.6 and 8.0 ± 3.1 respectively). There was no additional augmenting effect of oestradiol treatment on the pituitary responsiveness to GnRH (oestradiol-augmenting factor (see Figure 1, 0.8). FSH strongly suppressed the GnRH-induced LH response of both control rats and oestradiol-treated rats \((P < 0.05\); analysis of variance followed by Tukey’s HSD test) by suppressing the augmentative effect of oestradiol (oestradiol-augmenting factor: 0.16). FSH, however, had no effect on the plasma oestradiol concentrations (Figure 4) and did not suppress the GnRH-self priming effect (priming factors of FSH-treated animals before and after oestradiol treatment: 6.9 ± 1.1 and 9.1 ± 3.1 respectively).

Experiment 2: day-8 PSP rats

Figure 5 shows the effect of FSH treatment on the LH responses, induced by two consecutive GnRH injections in
Figure 5. Maximal increments of plasma luteinizing hormone (LH) concentrations (mean ± SEM) as induced by two consecutive equally large gonadotrophin-releasing hormone (GnRH) injections (0.5 µg/100 g body weight; time interval = 60 min) on day 8 of pseudopregnancy. In all series \( n = 5 \). Of each pair of bars: open bars: rats treated with solvent; shaded bars: rats treated with follicle stimulating hormone (FSH). ***Significantly different \((P < 0.05)\), analysis of variance followed by Tukey’s HSD test.

Figure 6. Plasma oestradiol (A) and progesterone (B) concentrations (mean ± SEM) of day-8 pseudopregnant rats. (A) Open bars: control rats \((n = 5)\). Hatched bars: follicle stimulating hormone (FSH)-treated rats \((n = 5)\). Black bars: oestradiol-implanted rats \((n = 10)\). (B) Open bars: control rats; hatched bars: FSH-treated rats; cross-hatched bars: oestradiol-treated rats; black bars: oestradiol/FSH-treated rats. In all series: \( n = 5 \).

Figure 7. Maximal increments of plasma luteinizing hormone (LH) concentrations (mean ± SEM) as induced by two consecutive equally large gonadotrophin-releasing hormone (GnRH) injections (0.5 µg/100 g body weight; time interval = 60 min) on day 8 of pseudopregnancy. Left pair of bars: rats not treated with oestradiol; right pair of bars: rats treated with oestradiol. Of each pair of bars: open bars: rats treated with solvent; shaded bars: rats treated with follicle stimulating hormone (FSH). **Significantly different \((P < 0.05)\), analysis of variance followed by Tukey’s HSD test.

Experiment 3: OVX–PSP rats

Figure 7 shows the effect of combined oestradiol/FSH treatment on the LH response induced by two consecutive GnRH injections in phenobarbitone-treated OVX–PSP rats. Rats were (right panel) or were not (left panel) pretreated with oestradiol; this treatment elevated the plasma oestradiol concentrations to the same extent as in PSP rats (data not shown). Figure 7 shows that there was GnRH-self priming in both control and oestradiol-treated OVX rats (priming factors: 2.0 ± 0.4 and 2.3 ± 0.7 respectively) and a strong augmentative effect of oestradiol on the GnRH-induced LH response (oestradiol-augmenting factor: 6.3). FSH had no effect on any parameter of the GnRH-induced LH response (priming factor in control and oestradiol-treated rats: 2.1 ± 0.7 and 1.8 ± 0.5 respectively; oestradiol-augmenting factor: 7.5).

Discussion

FSH lowered the spontaneous pre-ovulatory LH surge of cycling rats and suppressed the pituitary GnRH-responsiveness of these animals. The suppression by FSH of the GnRH-responsiveness of the pituitary gland may well explain the low pre-ovulatory LH surges of FSH-treated rats. The effect of...
FSH treatment was not associated with suppressed plasma levels of oestradiol (Geiger et al., 1980; Koppenaal et al., 1991). As the effect of FSH on the pituitary GnRH-responsiveness was absent in OVX–PSP rats (the absence of any effect of FSH on gonadotrophin secretion in post-menopausal women; Messinis et al., 1994a), it is probably caused by some factor(s) of ovarian origin.

Oestradiol strongly augmented the pituitary GnRH-responsiveness in PSP and OVX–PSP rats, but not in cycling rats. The lack of an effect of exogenous oestradiol in cycling rats is probably due to the fact that in these animals the pituitary responsiveness is already augmented by endogenous oestradiol (Schuiling et al., 1987; 1990). FSH suppressed the pituitary GnRH-responsiveness of both oestradiol-treated and control PSP-8 rats, but not that of similarly treated OVX–PSP rats, again suggesting that some ovarian factor is involved in the effect of FSH. Analysis of the data revealed that the suppressive effect of FSH treatment in oestradiol-treated cycling and PSP rats was caused by blockade of the augmenting effect of oestradiol; not by blockade of the GnRH-self priming effect or by an effect of oestradiol on the GnRH-self priming effect. This suggests that FSH stimulates the production of some factor which antagonizes the effect of oestradiol.

Suppression by an FSH-induced ovarian factor of the augmentative effect of oestradiol may also explain the suppressive effect of FSH on the pituitary GnRH-responsiveness of non-oestradiol-treated pro-oestrous rats, given the oestradiol-augmented pituitary GnRH-responsiveness of such animals. Similarly, it may explain the suppressive effect of FSH in control PSP rats, as such animals, too, have significant plasma concentrations of oestradiol, although these concentrations are lower than those of pro-oestrous rats. Even the fact that in OVX–PSP rats the augmentative effect of oestradiol is stronger than in intact PSP rats can be explained by the suggestion that the ovaries produce a factor suppressing the effect of oestradiol.

The question arises whether this putative factor is identical to GnSIF/AF. GnSIF/AF has been claimed to antagonize both the GnRH-self priming effect (see Introduction) and to delay the timing of the maximal increase of the blood levels of LH (Koppenaal et al., 1993; de Koning et al., 1994). As far as we know, however, antagonism by GnSIF/AF of the augmentative effect of oestradiol has not been reported, although Hodgen and co-workers (Sopelak and Hodgen, 1984; Schenken and Hodgen, 1986) and Templeton and co-workers (Messinis and Templeton, 1989, 1990a,b; Messinis et al., 1994b), observed in monkeys and women respectively, that GnSIF/AF suppressed the GnRH-responsiveness of oestradiol-treated individuals. In contrast to GnSIF/AF, the present factor did not induce delay of the pre-ovulatory LH-surge and did not antagonize the GnRH-self priming effect; it only antagonized the augmentative effect of oestradiol. GnSIF/AF and the ‘oestrogen-antagonizing factor’ described in this paper, therefore, differ from each other in important aspects.

The present experiments show that under the influence of FSH, the ovaries not only produce oestradiol, but also a factor which antagonizes the effect of oestradiol on the pituitary gland. This putative ‘oestrogen-antagonizing factor’ is not the only factor inhibiting the effect of oestradiol on the pituitary GnRH-responsiveness. In a previous study, it was demonstrated that the effect of oestradiol is also regulated by the plasma concentration of GnRH: the GnRH concentration dose-dependently suppresses the augmentative effect of oestradiol on the pituitary GnRH-responsiveness (Schuiling et al., 1996). On the other hand, by exerting a negative feedback effect on the hypothalamic GnRH secretion oestradiol itself controls the amount of GnRH exposure of the pituitary gland. It thus appears that a complex relationship exists between the effects of GnRH on the one hand and that of oestradiol on the other.

The present data suggest that the control of the effect of oestradiol on the pituitary gland is even more complex. Apparently, the effect of oestradiol is not controlled by one, but by (at least) two factors suppressing the pituitary responsiveness to GnRH: GnRH and the present putative ‘oestrogen-antagonizing factor’. This tight control of the pituitary GnRH-responsiveness with respect to the augmentative effect of oestradiol may be of particular importance in humans. During each ovulatory cycle, in order to avoid multiple pregnancies, the human ovaries should together preferably not produce more than one ovum. This probably demands gonadotrophin secretion rates which do not exceed certain limits. In the fine-tuning of the gonadotrophin secretion, the ‘oestrogen-antagonizing factor’, postulated in this paper, may play a role.

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References


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Welschn, R., Osman, P., Dullart, J. et al. (1975) Levels of follicle stimulating hormone, luteinizing hormone, oestradiol-17β and progesterone and follicular growth in the pseudopregnant rat. J. Endocrinol., 64, 37–47.

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