Role of gonadotrophin releasing hormone baseline concentrations in the control of pituitary gonadotrophin and ovarian steroid secretion in the pseudopregnant rat

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To study the effect of moderately elevated gonadotrophin releasing hormone (GnRH) baseline concentrations during the luteal and follicular phase, pseudopregnant rats were infused s.c. with GnRH at several doses for 5 days. These rats were also treated with oestradiol or sham-treated during the last 3 days of GnRH treatment. GnRH infusions started on day 7 or day 3 of the luteal phase of the ovulatory cycle; in the rat, the luteal phase or pseudopregnancy lasts about 10 days. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responses were induced by i.v. injection of GnRH on day 12 (after expected luteolysis) or on day 8 (before expected luteolysis). In normal rats the LH and FSH responses induced by GnRH on day 12 were higher than on day 8 (~160 and ~50% respectively). In GnRH-infused rats the LH and FSH responses were not increased. In these rats the luteal phase was extended (the plasma progesterone concentrations remained high) and the onset of the follicular phase was postponed (plasma oestrogen concentrations did not increase). Oestradiol increased the day 12 LH and FSH responses; this effect of oestradiol was suppressed by GnRH infusion. On day 8, exogenous oestradiol also increased the LH and FSH responses, but again the effect of oestradiol was suppressed when the animals were concomitantly infused with GnRH. These data may suggest that in the rat, GnRH baseline concentrations participate in the neuroendocrine system controlling gonadotrophin secretion and hence the ovulatory cycle.

Key words: GnRH baseline concentrations/GnRH responsiveness/oestradiol/ovulatory cycle/pseudopregnancy

Introduction

Cyclic rats exhibit 4- to 5-day ovulatory cycles without a luteal phase. However, when the cervix uteri is stimulated around the time of ovulation, secretion of the luteotropic hormone prolactin is provoked and a luteal phase or pseudopregnancy of about 10 days ensues (Bartosik and Szarowski, 1973; Schuiling et al., 1985). After luteolysis a new follicular phase begins.

During the luteal phase, the responsiveness of the pituitary gland to gonadotrophin releasing hormone (GnRH) is relatively low. After luteolysis, around day 10, the responsiveness of the pituitary gland increases under the influence of oestradiol, secreted by the developing follicles (Welschen et al., 1975). On pro-oestrus, on day 12 or 13, the GnRH responsiveness is therefore greatly increased, ensuring the occurrence of a large luteinizing hormone (LH) surge, when the pre-ovulatory GnRH surge is generated by the hypothalamus (Koiter et al., 1979).

Also in long-term ovariectomized rats, oestradiol can augment the pituitary GnRH responsiveness. This augmenting effect of oestradiol is dose-dependently suppressed by elevated baseline GnRH concentrations established by prolonged GnRH infusion (Schuiling et al., 1987, 1991). This suppressive effect of GnRH on oestradiol action cannot fully be explained by the homologous desensitizing effect of GnRH on the gonadotrophs, as the former effect of GnRH is already wholly present at a GnRH infusion dose which only partially desensitizes the gonadotrophs.

Changes in baseline GnRH concentrations in the pseudopregnant rat may also influence the pituitary GnRH responsiveness, in particular at the time of the transition of the luteal phase to the follicular phase, when the declining concentrations of progesterone and the increasing concentrations of oestradiol begin to affect the gonadotrophic cells of the pituitary gland. This assumption was investigated in the present study. Pseudopregnant rats were treated for 5 days with various doses of GnRH. As treatment with GnRH may affect, i.e. suppress, oestradiol production by the follicles, some of the rats were also treated with oestradiol. The effects of these treatments on the GnRH-induced LH and FSH response were studied both before (day 8) and after (day 12) expected luteolysis.

Materials and methods

Animals

Female 3 month old Wistar rats (about 200 g) bred at the Groningen Central Animal Laboratory and kept in animal quarters with lights on from 06.00 to 18.00 h were used. Vaginal smears were taken daily until the end of the experiments; only animals with regular 4 day ovulatory cycles were used.

A luteal phase or pseudopregnancy was induced by electrical stimulation of the cervix uteri, carried out both at 17.00 h on pro-oestrus and at 15.00 h on oestrus. Such stimulation provoked secretion of prolactin which activated the corpora lutea to produce progesterone. The first day following the last cervix stimulation was denoted day 1 of pseudopregnancy. Corpus luteum function was judged according to the plasma concentrations of progesterone; functional luteolysis was considered to have occurred when the plasma progesterone concentrations had decreased (Schuiling et al., 1985).
Pretreatment

Osmotic minipumps (Alzet model 2001; Alza Corp., Palo Alto, CA, USA) or, in control rats, Silastic 'shampumps', i.e. pieces of silicone elastomer (Dow Corning, Midland, MI, USA) with the dimensions of a minipump, were s.c. implanted under ether anaesthesia at 09.00 h on day 7 (experiment 1) or day 3 (experiment 2). Minipumps released GnRH for 5 days at 75, 100 or 150 ng/h and in a volume of 0.1 μl/h. Rats also received two s.c. Silastic implants containing oestradiol (Organon, Oss, The Netherlands) or two sham (empty) implants at 09.00 h on day 8 (experiment 1) or day 4 (experiment 2). The dimensions of the implants were: length 1.0 cm; inside diameter 1.57 mm; outside diameter 3.18 mm.

Induction of LH and follicle stimulating hormone (FSH) responses; assays, parameters and statistics

On the day of induction of LH/FSH responses/blood sampling, at 09.00 h on day 12 (experiment 1) or day 8 (experiment 2), a cannula was inserted into the right carotid artery. Two injections of GnRH of 0.5 μg/100 g body weight each were injected through the cannula for induction of LH and FSH responses; the first one at 13.00 h (t = 0); the second one at 14.00 h. Blood samples for assay of LH, FSH, progesterone and oestradiol were taken through the cannula at (t = 0); the second one at 14.00 h. Blood samples for assay of LH, FSH, progesterone and oestradiol were taken through the cannula at times apparent from Figure 1; progesterone and oestradiol were measured in the t = 0 sample. The plasma samples were stored frozen at -20°C until assay.

Concentrations of LH and FSH were measured in duplicate by double antibody radioimmunoassay with anti-ovine LH and FSH as antiserum and rat LH and FSH as tracer (Welschen et al., 1975). NIDDK-rat LH- and FSH-RP-2 were used as reference preparations. The intra- and interassay 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 intra- and interassay 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%.

LH and FSH responses were judged on the basis of the mean maximal increments of the plasma LH/FSH concentrations. The use of this parameter did not yield different results to those obtained by using area under the curve as the parameter. Increments were calculated by subtracting the basal LH/FSH concentration at t = 0 from the maximal LH/FSH concentration (i.e. the primed LH/FSH response) induced by the second GnRH injection, at t = 80 min (LH) or t = 100 min (FSH); Figure 1. Data are expressed as means ± SEM. Statistical comparisons were generally made by analysis of variance (ANOVA), followed by Tukey's Honestly significant difference (HSD) test (Tukey, 1951). Where appropriate, data were further analysed by linear regression analysis. Pairs of groups were compared using the two-sided Student's t-test. The level of significance was chosen as P < 0.05.

Results

Plasma concentrations of progesterone and oestradiol; basal LH and FSH concentrations and LH and FSH responses on day 8 and day 12

Control rats (no GnRH treatment)

On day 8, the plasma progesterone concentrations were significantly higher than on day 12 [151.8±25.6 nmol/l (n = 5) and 34.0 ± 4.8 nmol/l (n = 5) respectively; P < 0.01; t-test]. The plasma oestradiol concentrations on day 12 were significantly higher than on day 8 [0.33 ± 0.06 nmol/l (n = 5) and 0.05 ± 0.02 nmol/l (n = 5) respectively; P < 0.01; t-test]. The basal concentrations of LH on day 8 and on day 12 were 1.1 ± 0.2 (n = 6) and 1.4 ± 0.1 (n = 5) μg LH-RP₂/l plasma respectively; the basal concentrations of FSH on day 8 and on day 12 were 4.9 ± 0.3 (n = 6) and 4.1 ± 0.2 (n = 5) μg FSH-RP₂/l plasma respectively. The mean maximal increments of the GnRH-induced LH responses on day 12 were significantly higher than on day 8. On day 12 and on day 8 they were 133 ± 32 (n = 5) and 41 ± 5 μg LH-RP₂/l plasma (n = 6) respectively (P < 0.01; t-test). The mean maximal increments of the day 12 and day 8 FSH responses did not differ significantly and were 18.4 ± 3.0 (n = 5) and 12.9 ± 2.3 (n = 6) μg FSH-RP₂/l plasma respectively.

Two Silastic implants containing oestradiol established plasma oestradiol concentrations of 0.55 ± 0.08 nmol/l (n = 42). Oestrogen treatment did not change the time course of gonadotrophin release.

Experiment 1 (day 12)

Figure 2A shows that GnRH dose-dependently increased the plasma progesterone concentrations and that this effect of GnRH was potentiated by oestradiol (ANOVA followed by Tukey's HSD test; P < 0.05). Figure 3 shows that GnRH dose-dependently suppressed the production of oestradiol by the ovaries (regression analysis; P < 0.05). GnRH dose-
GnRH baseline levels and control of the ovulatory cycle

Figure 2. Plasma progesterone (P) concentrations (mean ± SEM) on day 12 (A) and day 8 (B) of pseudopregnancy. Rats were continuously infused with gonadotrophin releasing hormone (GnRH; s.c.) at 0, 75, 100 or 150 ng/h and implanted with sham implants (open columns) or oestradiol-releasing implants (black columns). A: GnRH infusion during days 7-12; oestradiol treatment during days 8-12. B: GnRH infusion during days 3-8; oestradiol treatment during days 4-8. n = 5—7. For reasons of clarity only differences from untreated control values as found by ANOVA followed by Tukey's HSD test, are indicated by an asterisk.

Figure 3. Plasma oestradiol concentrations (mean ± SEM) on day 12 of pseudopregnancy in rats continuously infused with gonadotrophin releasing hormone (GnRH) at 0, 75, 100 or 150 ng GnRH/h during days 7-12. n = 5.

dependently increased the basal LH concentrations (regression analysis; P < 0.05); there was no independent effect of oestradiol on the basal LH concentrations (Figure 4A). Except at 150 ng GnRH/h, there was no significant effect of GnRH, alone or in combination with oestradiol, on the basal concentrations of FSH (Figure 4C).

Figure 4, B and D shows that infusion with GnRH suppressed the LH and FSH responses induced by the test doses of GnRH; the suppression of the LH/FSH responses was already maximal at 75 ng GnRH/h. In rats not infused with GnRH, exogenous oestradiol further increased the LH and FSH response; in GnRH-infused rats, on the other hand, the LH and FSH responses of oestradiol-treated rats were still maximally suppressed (ANOVA followed by Tukey's HSD test).

Experiment 2 (day 8)

Figure 2B shows that GnRH stimulated the production of progesterone only at 150 ng/h in the presence of oestradiol. There was no effect of GnRH on the production of oestradiol [0.05 ± 0.02 nmol oestradiol/l plasma in control rats (n = 5) versus 0.05 ± 0.02 nmol/l in 150 ng GnRH/h-treated rats; n = 5; data not shown]. Figure 5A shows that oestradiol increased the basal concentration of LH at 0 and 75 ng GnRH/h, indicating that the higher infusion rates of GnRH prevented this effect of oestradiol. Figure 5C shows that the basal concentration of FSH was only significantly increased at 150 ng GnRH/h; there was only an effect of oestradiol on the basal concentration of FSH in the control group, not treated with GnRH (ANOVA followed by Tukey's HSD test).

Figures 5B and D show that oestradiol increased the LH and FSH responses significantly; these responses were as high as those of day 12 control rats (cf. Figure 4B and D). Figure 5B and D also shows that in GnRH-treated rats this augmenting effect of oestradiol was suppressed in a dose-dependent manner; the suppressive effect of GnRH was maximal at 150 ng GnRH/h.

Discussion

The normal transition of the luteal phase to the follicular phase around day 10 of pseudopregnancy did not occur in GnRH-treated rats. In these animals plasma progesterone concentrations remained high while oestradiol concentrations remained low. This study does not reveal exactly how these effects of GnRH are brought about. However, the elevated plasma progesterone concentrations of GnRH-infused rats may have played a role in the postponement of the follicular phase, even in the face of unchanged or increased (as was the case at the highest GnRH infusion rate) basal concentrations of FSH, since it is known that elevated concentrations of progesterone may prevent follicular development and increase of the oestradiol production (Koiter et al., 1991). In the present experiments the production of progesterone by GnRH-infused rats may have been stimulated by the increased basal LH concentrations of these animals (Rothchild, 1965; McLachan et al., 1989). However, it is also possible that the GnRH infusion caused luteinization of the developing follicles, resulting in increasing progesterone and decreasing oestradiol secretion Smith et al. (1975).

Normally, the GnRH-induced LH responses are about 160% higher than here observed on day 8 and the FSH responses about 50% higher. In the GnRH-infused rats, however, both LH and the FSH responses were not increased on day 12; this
demonstrates that the LH and FSH secretory systems of the gonadotrophs responded in the same way to GnRH-pretreatment. The failure of the LH and FSH responses to increase in GnRH-infused rats may at first sight be attributed to lack of oestradiol. However, the present data also show that the LH responses still did not increase when the animals were treated with oestradiol. Clearly, the direct augmenting effect of oestradiol was also inhibited in GnRH-infused rats. Although this might partly be due to the desensitization of the gonadotrophs by GnRH action, in day 8 pseudopregnancy rats, the qualitative, augmenting effect of oestradiol was already completely suppressed at a dose of GnRH at which the quantitative effect of desensitization of the gonadotrophs was not yet maximal.

It is unlikely that this blockade of the effect of oestradiol was due to the high concentrations of circulating progesterone, as experiment 2 shows that such high concentrations of progesterone do not preclude augmentation of the pituitary responsiveness to GnRH by oestradiol. Apparently, as in GnRH/oestradiol-pretreated ovariectomized rats (see Introduction), a suppressive effect of elevated GnRH concentrations on the GnRH responsiveness of the pituitary gland (desensitization) was present on day 12 in all groups of GnRH-infused rats. On day 8, i.e., during the luteal phase, a similar suppressive effect of GnRH was observed, although a higher concentration of GnRH appeared to be necessary to suppress fully the augmenting effect of oestradiol. Therefore, it appears that during the luteal phase, the pituitary gland is to some degree protected against the desensitizing effect of GnRH. After the expected time of luteolysis, the pituitary gland becomes more sensitive to this effect of GnRH.

Intravenous infusion of GnRH at 100 ng/h establishes a GnRH concentration of about 50 pmol/l in the plasma (Schuiling et al., 1987). Such a concentration is within the physiological range and well below maximal values as measured in the portal vessel blood at the time of the pre-ovulatory LH surge of cyclic rats (about 130 pmol/l; Sarkar et al., 1976) or at the time of the oestrogen-induced LH surge in ovariectomized rats (about 400 pmol/l; de Gref et al., 1986). Still, as the present experiments show, these moderate GnRH baseline concentrations profoundly affect the ovarian and pituitary function.

A direct effect of GnRH on ovarian function may, however, play a role, as high affinity GnRH receptors are present in the rat ovary (Harwood et al., 1980a). A number of studies, generally performed with high concentrations of superpotent

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Figure 4. Basal luteinizing hormone (LH) (A) and follicle stimulating hormone (FSH) (C) concentrations and maximal increments of plasma LH (B) and FSH (D) concentrations (mean ± SEM) as induced by two consecutive equally large injections of GnRH (0.5 μg/100 g; time interval = 60 min) on day 12 of pseudopregnancy. Rats were continuously infused (s.c.) with gonadotrophin releasing hormone (GnRH) at 0, 75, 100 or 150 ng/h during days 7-12 and implanted with sham implants (open columns) or oestradiol-releasing implants (black columns) on day 8. n = 5. For reasons of clarity only differences from untreated control values, as found by ANOVA followed by Tukey's HSD test, are indicated by an asterisk.

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GnRH agonistic analogue, have demonstrated both stimulatory (Mori et al., 1994) and inhibitory effects on ovarian oestradiol and progesterone production, depending on gonadotrophin priming of ovarian cells (Wickings et al., 1990; Mori et al., 1994). Working with pregnant mare's serum gonadotrophin/human chorionic gonadotrophin (PMSG/HCG) primed immature rats which were infused with 140 ng GnRH/h (which is in the range of the present GnRH infusion rates), Harwood et al. (1980b) observed suppression of the serum progesterone concentrations; this suppression was associated with loss of LH and prolactin receptors. In the present experiments, therefore, one would expect a direct inhibitory effect of GnRH on ovarian progesterone production, rather than the observed stimulatory effect. We therefore believe that the ovarian steroidal response of GnRH treated rats observed in this study is executed via the pituitary gland and is not due to a direct stimulatory action of GnRH on the ovaries.

The present data show that baseline GnRH concentrations, when exceeding a certain value, stimulate the production of progesterone by the corpora lutea. They apparently control in this way the transition of the luteal phase to the follicular phase of the ovulatory cycle. Indirectly, therefore, GnRH baseline concentrations control the onset of follicular development and regulate the production of oestradiol. In addition, the GnRH baseline concentrations may desensitize the pituitary gland to the LH/FSH-releasing effect of GnRH and suppress the augmentation by oestradiol of the pituitary GnRH responsiveness. It therefore appears to be of physiological importance that the GnRH baseline concentrations be low during the follicular phase of the ovulatory cycle in order to ensure normal follicular development. Such low baseline GnRH concentrations are normally caused by suppression by oestradiol of the hypothalamic secretion of GnRH. The present results, therefore, show the interdependence of the effect of oestradiol on the pituitary gland and the effect of oestradiol on the hypothalamus; this means that the feedback effects of oestradiol at the hypothalamus and the pituitary gland must act in concert.

These experiments may suggest that the GnRH baseline concentrations are an essential part of the neuroendocrine system controlling the ovulatory cycle of the rat. A similar view is advocated by Clarke and coworkers, working with ovariectomized ewes (Clarke and Cummins, 1982). It demonstrated that GnRH pulses (cf. Lincoln et al., 1985) are superimposed upon a low basal, non-pulsatile concentration of GnRH. Recently this group of investigators provided evidence...
for a physiological function of this baseline secretory component of GnRH secretion. The baseline GnRH input, if sufficiently high, may substitute for lack of pulsatile GnRH input (Phillips et al., 1990). They may even cause the generation of oestrogen-induced LH surges in ovariectomized ewes (Clarke, 1991).

The present data may be of clinical relevance. Women with fertility disorders referred to as 'hypothalamic amenorrhoea' exhibit a suppressed pituitary response after treatment with oestradiol (Schuiling et al., 1990). In the light of the present findings, this observation may suggest that the pituitary gland of such women is exposed to elevated GnRH concentrations, e.g. due to insufficient feedback inhibition by oestradiol of the hypothalamic GnRH secretion and not, as is the more common view, to unexplained very low GnRH concentrations (Lachelin and Yen, 1978; Barkan et al., 1985; Spratt et al., 1987). Also in the polycystic ovary syndrome, hypersecretion of GnRH may play a role (Berga, 1994). However, the precise role of GnRH secretion in the control of the ovulatory cycle and in the genesis of endocrinopathies of ovarian cyclicity can only be assessed with certainty when reliable methods for direct measurement of hypothalamic GnRH secretion in the human are available.

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