Suppressive effect of prolactin on oestrogen-induced secretion of LH by sequentially perifused rat hypothalamus-pituitary

Jin-Woo Lee¹, Akira Miyake², Keiichi Tasaka, Shirou Otsuka, Toshihiro Aono and Keiichi Kurachi

Department of Obstetrics and Gynecology, Osaka University Medical School, Osaka 553, Japan

Abstract. The effect of prolactin (Prl) on oestrogen-induced gonadotrophin secretion was examined in vitro in a sequential double chamber perifusion system. As control groups, mediobasal hypothalamus (MBH)-pituitary pairs or pituitaries without the MBHs were perifused with Medium 199. As an experimental group, MBH-pituitary pairs were perifused with Medium 199 containing 1 µg/ml of rat Prl. These groups were stimulated with 10⁻⁷ M oestradiol-17β (E₂) for 30 min, and luteinizing hormone (LH) in the serial fractions of effluent was measured.

In the control group of MBH-pituitary pairs perifused with medium without Prl, secretion of LH began to rise within 30 min after the beginning of stimulation, reached a peak 30 min after the end of stimulation and then remained at a plateau for the rest of the experimental period, whereas in the control group of pituitaries alone no significant response was observed. In the experimental group perifused with medium containing Prl, LH-secretion showed peaks 20 and 80 min after the end of E₂-stimulation, respectively, and the first peak was significantly (P < 0.01) less than the level in the control group.

These data demonstrate that Prl at this concentration suppressed the rapid LH release induced by E₂. Its site of action is suggested to be at the hypothalamic level, and its possible mechanism of action is discussed.

Possible mechanisms by which prolactin (Prl) suppresses gonadotrophin release reported by others are as follows: first, Prl may suppress hypothalamic luteinizing hormone releasing hormone (LRH) release (Gil-Ad et al. 1978; Grandison et al. 1977; Smith 1980). The mechanism of this action has been postulated by many investigators to be that increased Prl increases dopamine (DA) turn-over via a short loop feedback mechanism and the increased DA level, in turn, suppresses LRH release (Gudelsky et al. 1976; Chatani et al. 1983; Esquifino et al. 1984). Second, Prl can directly suppress the pituitary responsiveness to LRH in vivo (Vasquez et al. 1980; Carter & Whitehead 1981) and in vitro (Cheng 1983) by decreasing LRH receptors in the pituitary gland (Clayton & Bailey 1982; Marchetti & Labrie 1982).

On the other hand, there are several reports about the suppressive effect of Prl on oestrogen-induced gonadotrophin secretion in patients with the galactorrhoea-amennorrhoea syndrome (Aono et al. 1976), and drug-induced hyperprolactinaemia (L’Hermite et al. 1978; Anderson et al. 1982), restoration of oestrogen positive feedback by bromocriptine treatment (Aono et al. 1979) and by surgical removal of a pituitary adenoma (Koike et al. 1982) have also been reported.

However, the mechanism and site of action of Prl for this suppressive effect is uncertain. Therefore, we examined the mechanism of the suppressive effect of Prl on luteinizing hormone (LH) release in an in vitro perifusion system.

¹ Present address: Department of Obstetrics and Gynecology, Catholic Medical School, 505 Banpodong, Kangnamku, Seoul 135, Korea.
² To whom all correspondence should be addressed.
Materials and Methods

Female Wistar-Imamichi rats (Nihon Laboanimal Co., Osaka, Japan) weighing 220–250 g in dioestrus were used. They were housed under controlled lighting conditions (lights on from 06.00 to 21.00 h) at 25°C and given free access to water and food. The rats were killed at 12.30 h and their MBH and/or pituitary was removed. The MBH and pituitary were placed in the first and second chamber, respectively, of a sequential double-chamber perifusion apparatus (Miyake et al. 1982). The MBH tissue block excised was demarcated by the hypothalamic sulci laterally, the caudal aspect of the optic chiasm rostrally and the rostral aspect of the mamillary bodies caudally. The whole pituitary glands were used without hemisection.

In the first study, 8 MBH-pituitary pairs in sequence were perifused with Medium 199 (Handai-Biken, Japan) containing antiserum to LRH obtained from a rabbit at 1:100 dilution or normal rabbit serum at the same dilution. In the second study, 6 MBH-pituitary pairs in sequence and 7 pituitaries without MBHs were perifused with Medium 199 as a control group. In an experimental group, 8 MBH-pituitary pairs were perifused with Medium 199 containing 1 µg/ml of rat prolactin (NIAMD-rPrl-B-3). Perfusion was started immediately after sacrifice at a flow rate of 3 ml/h with medium saturated with 95% O2−5% CO2 at 37°C. The perifusion system was equilibrated for 2.5 h and samples of 0.5 ml each were collected at 10 min interval from 15.00 h. Six samples were collected over a 1-h period, and then 10⁻⁷ M oestradiol-17β (E₂) in Medium 199 was perifused for the next 30 min. Eighteen fractions in one experiment were collected for 3 h and stored at −20°C until assayed. Rat LH in these fractions was measured by radioimmunoassay (Hayashi et al. 1976). The sensitivity of the LH assay and its intra-assay coefficient of variation were 2 ng NIADDKD-rat LH-RP-2/tube and 7.5%, respectively. In each experiment the mean concentration of LH in the 6 fractions collected during 1 h before each treatment was used as the basal value, and values during experiments were calculated as percentage changes from the mean basal value in each group. Two-way analysis of variance was used for evaluation of the statistical significances of differences in the stimulatory effects of oestrogen and suppressive effects of Prl, respectively.

Results

In the first experiment on MBH-pituitary pairs perifused with normal rabbit serum, LH release began to increase within 30 min after the beginning of E₂ stimulation (P < 0.01), reaching peak of 147.7% over the basal value 30 min after the beginning of E₂ stimulation (P < 0.01), and then undulating at the plateau level for the rest of the experimental period (Fig. 1). On the contrary, as shown in Fig. 1, no significant LH release after E₂ stimulation was observed in the group perifused with antiserum to LRH. LH changes in the second experiment using Prl are shown in Fig. 2. In the group of MBH-pituitary pairs without Prl, E₂ administration caused significant LH release (130.4% increase over the basal value) as observed in the first experiment of perifused MBH-pituitary pairs without antiserum to LRH. No significant change in LH release was observed with pituitaries alone. The second experiment of MBH-pituitary pairs perifused with Prl showed two peaks: a first lower peak of 47.8% increase over the basal value (P < 0.01) was noted 20 min after the end of E₂ stimulation, and a second higher peak of 71.5% increase over the basal value (P < 0.01) was seen 80 min after the end of stimulation. The first peak was significantly less (P < 0.01) than that of the control group, but the second peak was not significantly different from that of the control.

![Fig. 1.](image-url)
Discussion

In the present first study, LH secretion began to rise 30 min after the beginning of E2 stimulation of MBH-pituitary pairs perfused with medium containing normal rabbit serum, but this rise was not observed under perifusion with antiserum to LRH. These results suggest that this LH secretion is probably due to stimulation by E2 of LRH release from the MBH.

Although the site of the inhibitory effect of E2 on LH release has been reported to be at the pituitary gland in vivo (Negro-Vilar et al. 1973), the acute inhibition of LH release from the pituitary gland by E2 has been observed in vitro neither in the previous studies (Turgeon & Waring 1981; Miyake et al. 1982) nor in the present study. The mechanism of difference in LH release following E2 administration between in vivo and in vitro studies is not clear at present.

An important finding in the present study was the rapidity of the LH response to oestrogen i.e., LH started to rise within 30 min after stimulation. This suggests that the LRH was ‘released’ rather than newly ‘synthesized’, since a period of 30 min was too short to be compatible with the time needed for synthesis of the peptide (McEwen et al. 1982; Drouva et al. 1984).

Synaptosomal release of LRH without cell bodies has been mentioned in several reports (Tytell et al. 1980; Deyer et al. 1980; Warberg 1982; Drouva et al. 1984). Drouva et al. (1984) reported that 1) E2 appears to be selectively and specifically involved in process coupling, nerve ending depolarization and, in turn, LRH release, and 2) the effect of E2 is receptor-mediated and does not appear to require nuclear translocation of the steroid or transcription procedures, since it can be readily elicited upon simple addition of E2 to nerve endings disconnected from their cell bodies. Furthermore, Warberg (1982) found that LRH, TRH and α-MSH are concentrated in synaptosome-riched fractions where they are present in granules, and that they are released in a Ca++-dependent manner by stimuli considered to depolarize the neural membranes. In addition, the depolarization procedure in synaptic transmission shows only 0.5 msec of ‘synaptic delay’ (Berne & Levy 1983). Consequently this LRH release is concluded not to result from new synthesis of LRH but rapid and direct release of LRH from stores, as in the synaptosomal experiments described above.

In the present study, Prl suppressed oestrogen-induced LH release only partially. For the effect of Prl, its concentration and its duration of reaction are important (Cheng 1983), because the suppressive effect of Prl is dose-dependent. In the present experiment, we added Prl at 1 µg/ml, which corresponds to about the upper level in severe hyperprolactinaemia in humans. This concentration may be relatively low considering that we examined its effect in vitro, and that the perifusion time before E2 stimulation was 3 h. However, although the concentration of Prl and the duration of treatment in the present experiment may not have been sufficient to cause complete suppression, we observed significant suppression of the first peak.

Prl is known to suppress gonadotrophin secretion by increasing DA turnover (Gudelsky et al. 1976; Esquifino et al. 1984; Chatani et al. 1983), but this mechanism does not seem to explain the present results adequately. Moreover, this process has been noted to be very slow, being apparent about 12–16 h after increase of Prl (Moore et al. 1980), and so it is unlikely that DA increase by short-loop feedback of Prl control operates during
a short period of within 1 h. Thus in our experiment Prl may have had a direct suppressive effect on the responsiveness of the pituitary to LRH.

In the present experiment, the pituitary without MBH did not respond to oestrogen and was therefore not perfused with Prl. But a direct suppressive effect on the MBH cannot be disregarded, because gonadotrophin secretion is the result of a response of the pituitary to LRH. Indeed there are many reports of this direct suppressive effect both in vivo (Vasquez et al. 1980; Carter & Whitehead 1981) and in vitro (Cheng 1983). However, the time sequences of results in these reports were very different from that in the present experiment. Furthermore, as mentioned above, we used a concentration of Prl of less than one twentieth of that used in a previous in vitro study on the pituitary without the MBH (Cheng 1983). In that experiment, Prl at 20 μg/ml significantly suppressed the response of the pituitary gland to LRH. Therefore, caution is required in concluding that this direct suppression did not occur in the present experiment.

From the above considerations, and since there was not another possible influencing factor and the reaction time was very short, it appears likely from the present experiment that Prl directly inhibited the oestrogen-induced gonadotrophin releasing mechanism at the hypothalamic level. Further studies seem necessary on the effect of Prl on LRH and DA releases in longer observation periods.

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