Epidermal growth factor stimulates secretion of rat pituitary luteinizing hormone in vitro

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Abstract. The effects of epidermal growth factor (EGF) on pituitary luteinizing hormone (LH) release and on the releases induced by oestradiol (E2) and LH-releasing hormone (LRH) were examined in a sequential double chamber perifusion system. In this system the mediobasal hypothalami (MBH) and/or pituitaries excised from normally cycling female rats in dioestrus were perifused with test media.

Perifusion with EGF at 1 ng/ml for 30 min induced significant release (80–100% increase, P < 0.05) of LH from hypothalamo-pituitary pairs, but not from the pituitary alone. Perfusion of the pituitary alone with medium containing 1 ng/ml EGF, resulted in significant release of LH (70–140% increase, P < 0.05) after administration of 10^-7 M E2, but did not significantly influence LH release in response to 20 ng/ml LRH.

These findings suggest that EGF may be involved in the regulation of pituitary gonadotrophin secretion by a direct effect on the hypothalamus and indirectly by increasing the pituitary responsiveness to E2.

Epidermal growth factor (EGF) was first isolated from mouse salivary gland (Cohen 1962). Many studies have shown that EGF has activities to stimulate cell growth and increase DNA, RNA and protein syntheses in cells (Cohen 1972; Armelin 1973). There are reports showing that tissues of human placenta and choriocarcinoma have specific receptors for EGF (O’Keefe et al. 1974; Benveniste et al. 1978), and that EGF stimulates secretion of human chorionic gonadotrophin (hCG) and the hCG-α-subunit from these tissues (Benveniste et al. 1978; Takemori et al. 1981).

In this study we examined the effect of EGF on LH release from the pituitary of normally cycling female rats in an in vitro sequential double chamber perifusion system.

Materials and Methods

Cycling female Wistar-Imamichi rats of 200–250 g weight were used. They were kept in a temperature-controlled room with 15.5 h light a day (from 06:00 to 21:30 h). Vaginal smears were examined daily for 1–2 weeks. Rats in dioestrus of the oestrous cycle were decapitated at 12.30 h and their mediobasal hypothalami (MBH) and pituitary were removed and perifused in the serial double chamber perifusion system described previously (Miyake & Yen 1981; Miyake et al. 1982). In this system, two 0.1 ml chambers connected serially are perifused with Medium 199 (Handai-Biken, Japan) saturated with 95% O2–5% CO2 at 37°C and after equilibration for 2.5 h, the effluent is collected in 0.5 ml fractions at 10 min intervals. The following 3 groups of experiments were made. In the first, the pituitary alone or in sequence with the MBH was perifused. Six samples of effluent were collected over a period of 1 h for determination of basal values. Then medium containing EGF at 1 or 100 ng/ml was perifused for the next 30 min, and fractions of effluent were collected over a 3 h period. In the second and third groups of experiments, the pituitary alone was perifused with Medium 199 containing 1 ng/ml EGF, and 6 fractions of effluent were collected. Then the pituitary was perifused with or without 20 ng/ml LH-releasing hormone (LRH) (Tanabe Pharm. Co., Japan) (second group) or 10^-7 M oestradiol (E2) (third group), and 18 fractions of effluent were...
collected over a 3 h period and stored at 
-20°C for assay. 
Eight experiments were done in each group. 
LH in effluent fractions was measured by radioim¬
unoassay as described previously (Hayashi et al. 1976). 
In this assay, the lower limit of detectable LH was 2 
ng/tube and the intra- and inter-assay coefficients of 
variation were 7.5 and 12.4%, respectively. The statistical 
significance of differences in hormone concentrations 
before and after each treatment was examined by two-
way analysis of variance.

Results

Effect of EGF on basal secretion of LH

The mean (±SE) basal concentrations of LH in the 
eluant from the pituitary alone and in series with 
the MBH in the 6 fractions of effluent collected in 
1 h before treatment were 140.4 ± 10.2 and 127.0 ± 29.0 ng/ml, respectively. The difference between 
these values was not significant. Changes of LH 
in the perifusion effluent from the pituitary are 
shown as per cent changes from the basal levels in 
Fig. 1. Perfusion with medium containing 1 or 100 
ng/ml EGF for 30 min resulted in 80–100% 
increase in LH release from the pituitary in series 
with the MBH (significant at P < 0.05), but not 
significant increase in LH secretion from the pitui-
tary alone.

Fig. 2.

LH changes in the effluent from the pituitary perifused 
after administration of 20 ng/ml LRH in Medium 
199 with or without 1 ng/ml EGF. Other explanations are 
as for Fig. 1.

Effect of EGF on LRH-stimulated LH release

The pituitary LH responses to perifusion with 
medium containing LRH alone or with 1 ng/ml 
EGF are shown in Fig. 2. The LH concentration in 
the effluent increased 50–180% (significant at 
P < 0.05) on perifusion of 20 ng/ml LRH. But 
there was no significant difference between the 
increases in LH with and without EGF.

Effect on EGF on E2-induced LH release

Changes of LH in the perifusion effluent from the 
pituitary in dioestrus after 10-7 M E2 infusion are

![Graph showing LH changes in the effluent from the pituitary with and without hypothalamus, with and without EGF.](image-url)
**Fig. 3.**

LH changes in the effluent from the pituitary alone after infusion of $10^{-7} \text{M} \cdot \text{E}_2$ in Medium 199 with or without 1 ng/ml EGF. Other explanations are as for Fig. 1.

shown in Fig. 3. Perfusion of E2 caused 70–140% increase in LH concentration in the effluent (significant at $P < 0.05$) in the presence of EGF, but no significant increase in the absence of EGF.

**Discussion**

To our knowledge this is the first report that EGF increases LH release from the rat pituitary in dioestrus in series with the hypothalamus, but not from the pituitary alone. These findings suggest that EGF induces release of LH via the hypothalamus, although we did not measure changes in LRH concentration in this study.

Human placental tissue also has many receptors for EGF (O'Keefe et al. 1974), and EGF stimulates the secretion of hCG and the hCG-α subunit (Take-mori et al. 1981; Hirata & Sueoka 1982). The mechanism of hCG secretion from the placenta is unknown, but one possibility is that LRH in cytotrophoblasts of the placenta may regulate hCG secretion from the syncytiotrophoblasts. This mechanism may be similar to that by which hypothalamic LRH stimulates pituitary gonadotrophin secretion. These findings suggest that EGF induces LRH release from cytotrophoblasts, resulting in increase in secretion of hCG and the hCG-α subunit. From the present data, it is conceivable that there is a receptor for EGF in the rat hypothalamus.

Westendorf & Schonbrunn (1982) observed that EGF caused 40% increase in prolactin (Prl) release over the basal value in GH4C1 cells, a clonal strain of functional rat pituitary cells in culture. This finding indicates that EGF increases Prl release by a direct action on the pituitary gland. This is in contrast to the present findings on gonadotrophin secretion. The difference in the effects of EGF on the releases of Prl and LH may be ascribe to a difference in the regulatory mechanisms of the two hormones. Westendorf & Schonbrunn (1982) also observed that EGF had an additive effect with bombesin in inducing Prl release. In the present study, we found that EGF induced LH release from the pituitary by E2, but did not influence the release induced by LRH. These findings indicate that EGF may modify the actions of other hormones on the pituitary gland.

In summary, the present in vitro results suggest that EGF is involved in the regulation of gonadotrophin secretion through an effect on hypothalamic LRH and/or by the enhancing the gonadotrophin response to E2 at the pituitary level. The physiological significance of EGF in gonadotrophin regulation is still unknown. There are no reports on EGF binding sites or the EGF contents of the hypothalamus and pituitary and further studies on the precise mechanism of the effect of EGF on gonadotrophin secretion are necessary.

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**References**


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