Opiate receptors and anterior pituitary hormone secretion in man. Effect of naloxone infusion

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Abstract. The role of endogenous opioid receptors on anterior pituitary hormone secretion was evaluated by the administration of the opioid receptor antagonist naloxone. The infusion of naloxone (8 mg iv followed by 4 mg/h for 3 h) did not alter basal growth hormone (GH), prolactin (Prl) and thyrotrophin (TSH) secretion but produced a significant rise in cortisol and gonadotrophins in normal man. The infusion of the opiate antagonist appeared to increase the rate and amplitude of luteinizing hormone (LH) pulsatility.

Naloxone pre-medication (10 mg iv 30 min before testing) did not alter the pituitary response to TRH and LRH stimulation.

These results demonstrate that naloxone can modify basal anterior pituitary hormone secretion and strongly suggest an endogenous opioid modulation of some of these hormones.

It has been long known that morphine and related compounds can influence pituitary hormone secretion by virtue of action on the central nervous system (Meites et al. 1979). The recent discovery of opiate receptors in the brain of mammals as well as the isolation of endogenous opioid-like substances (Hughes et al. 1975), has led to considerable speculation regarding their physiological role in the control of the hypothalamus-pituitary-axis. Exogenously administered endorphins and enkephalins have been shown to affect pituitary hormone secretion in experimental animals (Bruni et al. 1977) and the reversal or attenuation of the effect by naloxone, an opiate antagonist, is taken as neuropharmacological evidence for the involvement of opiate receptors in this process.

In man, morphine raised basal prolactin (Prl) levels, but did not alter basal growth hormone (GH) and cortisol secretion (Tolis et al. 1975), whereas a long-acting analogue of met-enkephalin released Prl, GH and decreased gonadotrophins and cortisol (Von Graffenried et al. 1978; Stubbs et al. 1978). The prior administration of naloxone blunted these effects (Stubbs et al. 1978).

To further investigate the role of opiate receptors and, possibly, identify an action of endogenous opiate receptors on anterior pituitary function, we have studied the effect of naloxone, a potent opiate antagonist compound, on basal and TRH/LRH stimulated pituitary hormone secretion in man.

Materials and Methods

Naloxone infusion

Six normal males, aged 20–38 years, were studied fasting and single (subject blind). Each subject was tested on two different days, the two treatments being given in random order at least 7 days apart. Starting at 08.30 h, an iv cannula was inserted in an antecubital vein for blood sampling and kept patent with 0.9% saline. A second iv cannula was placed in the opposite arm for the infusion of 0.9% saline or naloxone (Naloxone HCl, Endo Laboratories, Inc., New York, USA). Thirty min after cannulation, half hourly sampling was started for 420 min.
After 3 basal samples were obtained, 8 mg naloxone or 0.8 ml 0.9% saline was given over 1 min followed by 12 mg naloxone or 0.9% saline (each in a volume of 8 ml) over 3 h via a constant infusion pump.

Dynamic studies

Six healthy males, aged 21–28 years, were given, single (subject) blind and following an overnight fast, 100 µg LRH (Relisorm, Serono) iv and 200 µg TRH (Serono) iv. The TSH and Prl responses to TRH and the LH and FSH responses to LRH are the same, whether the releasing hormones are given together or independently (Mortimer et al. 1973). Blood specimens for hormone assay were drawn at −60, −30 and immediately before (time 0) TRH and LRH injection. Further samples were taken 20, 30, 60, 90 and 120 min after TRH and LRH administration.

Ten mg naloxone (or 1 ml 0.9% saline) was given iv as a bolus injection 30 min before TRH and LRH, immediately after the blood sample ‘−30’ was obtained. The two experiments were carried out in random order at least 7 days apart.

Each subject gave written informed consent.

Sera were immediately frozen until the assay was performed. The following hormones were measured by specific double-antibody radioimmunoassay: GH (Molnatti et al. 1969), Prl (McNeilly 1973), LH-FSH (Midgley 1966, 1967), TSH (Odell et al. 1967), using MRC standards 66/127 for GH, 71/222 for Prl, 68/38 for TSH.

Fig. 1.

Changes in LH and FSH from basal levels in 6 normal men given either naloxone or 0.9% saline. The mean basal levels for LH and FSH were 7.3 and 6.9 mIU/ml during naloxone, and 8.2 and 7.3 mIU/ml during saline experiment. Each point represents the mean ± SEM. LH and FSH values at time '0' are the mean of the three readings −60, −30 and 0 min.
68/40 for LH and 69/104 for FSH. 125I-labelled antigens and antisera were provided by Biodata, Milan. Serum cortisol was measured by competitive protein binding (Cortipac, Amersham). All specimens (control and experimental samples) were measured in duplicate in the same assay.

Student's two-tailed t-test was used for data evaluation. The secretion of each hormone during the control and test experiments was also determined by measuring the area under the curve.

Results

Naloxone infusion

Naloxone stimulated LH secretion when compared to the saline control (Fig. 1), with the response areas being significantly different ($P < 0.01$). The mean LH levels were significantly elevated 30 min after the start of the infusion and remained so throughout the experiment. Serum FSH levels after naloxone were higher than those after saline ($P < 0.05$ at 60, 90, 120, 180, 240 and 270 min; Fig. 1). The naloxone and saline response areas were also different ($P < 0.05$).

The individual pattern of LH levels during naloxone and saline infusion is shown in Fig. 2. In each of the subjects, naloxone increased the amplitude and the frequency of spontaneous LH secretion. When naloxone was replaced by saline, LH secretion remained significantly different from the control value till the end of the experiment.

Cortisol rose after naloxone infusion, peaked at 60 min and remained significantly above the control level throughout the period of naloxone in-

![Fig. 2](image_url)

The individual pattern of LH during and 3 h after the infusion of naloxone and in the control experiment.
fusion. When naloxone was replaced by saline, cortisol mean levels were comparable to those of the control study.

There was no effect of naloxone on serum Prl, GH and TSH levels (Fig. 3).

**Dynamic studies**

The effects of naloxone administration on LH, FSH, TSH and Prl response to TRH and LRH are shown in Fig. 4.

Naloxone did not alter the Prl and TSH response to TSH. The absolute mean peaks and the maximum increments were comparable with those observed in the control experiment.

LH and FSH absolute mean peaks in response to LRH were comparable in both experiments. The maximum increments in LH were slightly, but not significantly, lower in naloxone-treated subjects. After naloxone, mean LH levels at 0 min (i.e. before LRH administration) were significantly higher ($P < 0.05$) than those after saline.

No side effects were observed during naloxone administration in any of the subjects studied.

**Discussion**

Immunohistochemical studies have shown that the regional distribution of opioid peptides is similar to that of opioid receptor binding. In particular, beta-endorphin and enkephalin containing neu-
rons are present in the medial basal hypothalamus (Watson et al. 1978) suggesting that endogenous opioid peptides and their ligands may be involved in the neural regulation of pituitary hormone secretion. The relation of morphine and endogenous opioid peptides to neuroendocrine functions is well documented in experimental animals (Meites et al. 1979). The data presented here demonstrate that naloxone, an opiate antagonist with high affinity for endogenous opiate receptors, produces a rise in serum cortisol and gonadotrophins without altering TSH, Prl and GH secretion and support an important contribution of opioid receptors to the regulation of the hypothalamus-pituitary axis in man.

Morphine as well as endorphins and enkephalin acutely depress LH secretion in several species (Cicero et al. 1976; Bruni et al. 1977) and naloxone competitively inhibits the effect. Similar data were obtained in man with the administration of a long-acting met-enkephalin analogue (Stubbs et al. 1978). Morphine did not block the ability of synthetic LH-RH to release LH in vitro (Cicero et al. 1977), and there is no evidence that endogenous opioids can modulate gonadotrophin release at a pituitary level. Moreover, work done on rat hypothalamic fragments demonstrated that the release of LRH induced by dopamine is inhibited by met-enkephalin itself (Rotsztejn et al. 1978). Our additional data support the concept that endogenous opioids normally inhibit the hypothalamus-pituitary-gonadotrophin axis and suggest that the gonadotrophin rise may be secondary to the release to LRH from the hypothalamus rather than by an action of naloxone at the pituitary. The antagonist had no effect on the basal and LRH stimulated release of LH by the anterior pituitary in vitro (Cicero et al. 1979) and our observations show that naloxone does not increase the gonadotrophin response to LRH in vivo. Although the maximum LH increment observed 20 min after LRH administration was lower in naloxone-treated than in saline-treated subjects, basal LH levels after naloxone were higher than basal LH levels after saline.
and the absolute mean peak levels of gonadotrophins obtained were comparable after saline and naloxone pre-treatment. This suggests that the reduced maximum LH increment after LRH may result from a partial reduction (induced by naloxone through stimulation of endogenous LRH) of the stored gonadotrophin which is readily released after exogenous LRH injection.

Morphine has been shown to affect CRF-ACTH in experimental animals and to depress, when chronically administered, basal and stress-stimulated pituitary adrenal axis (Munson 1973). The rise in cortisol observed after naloxone is likely to be secondary to a rise in ACTH, since we could not find any cortisol rise after naloxone in ACTH-deficient patients (unpublished data). The ability of naloxone to stimulate cortisol is in agreement with the ACTH (and cortisol) fall observed after the administration of a met-enkephalin analogue in normal subjects (Stubbs et al. 1978) and in patients with Addison's disease or pituitary dependent Cushing's disease (Gaillard et al. 1980). The data, taken together, strongly suggest that endogenous brain opiates may have a physiological role in modulating basal activity of ACTH-cortisol axis.

Although basal TSH mean values tended to slightly decrease during the administration of the opiate antagonist, there was no significant change in basal as well as in the TRH stimulated TSH release after naloxone and saline infusion. There are few animal studies on the role of opioids in TSH secretion (Meites et al. 1979) and they suggest that opiate receptors suppress TSH release. In man, a single injection of morphine had no effect on TSH release (Tolis et al. 1975), whereas met-enkephalin caused a small TSH rise in normal subjects (Stubbs et al. 1978). More consistent data on the stimulatory role of morphine and related compounds on GH secretion are available in animal (Meites et al. 1979) and human studies (Von Graffenried et al. 1978; Stubbs et al. 1978). However, naloxone infusion failed to alter basal GH output. It is possible that opiate receptors do not play an important role in basal GH and, probably, TSH secretion in man.

The failure of naloxone to produce any effects on basal and TRH stimulated Prl secretion is difficult to explain, in view of the well documented action of morphine and endogenous opioids on Prl release in experimental animals (Bruni et al. 1977; Van Vugt et al. 1978) and in man (Tolis et al. 1975; Von Graffenried et al. 1978; Stubbs et al. 1978). Prl is under the inhibitory control of hypothalamic dopamine (DA) and opioids are known to decrease DA activity in the central nervous system and, possibly, at the pituitary level (Enjalbert et al. 1979), thus allowing Prl to be released. The failure of naloxone to alter basal Prl secretion suggests that endogenous opioid receptors do not play a major part in the basal (as well as TRH stimulated) Prl secretion, but a minor involvement in some aspects of Prl control cannot be excluded by our study.

During the preparation of this paper, Morley et al. (1980) reported that the injection of 10 mg naloxone stimulated cortisol, gonadotrophins and slightly enhanced the Prl response to TRH in man.

Although the dose of naloxone they and we used is 25–50 times higher than the usual adult dose of 0.4 mg used in treating narcotic overdose, the observed hormonal changes do not suggest any agonistic activity of the drug with respect to opiate receptors involved in neuroendocrine mechanisms.

In conclusion, the results show that naloxone can influence anterior pituitary hormone secretion suggesting an endogenous opioid modulation of some of these hormones.

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References


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