Effect of δ-opioid receptor agonist deltorphin on circulating concentrations of luteinizing hormone and follicle stimulating hormone in healthy fertile women

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There is evidence that endogenous opioid peptides exert an inhibitory effect on pituitary luteinizing hormone (LH) secretion both in animals and in humans, by interacting with µ-opioid receptors. However, a role for δ-opioid receptors in the regulation of gonadotrophin releasing hormone (GnRH) secretion has recently been suggested. In the present study, we evaluated the effect of the highly selective δ-opioid receptor agonist deltorphin on the LH and follicle stimulating hormone (FSH) responses to naloxone in six healthy fertile women during the luteal phase of the menstrual cycle. Deltorphin infusion alone (7 µg/kg/min for 60 min) did not significantly change the basal serum concentrations of LH in this group of women. The intravenous (i.v.) bolus administration of naloxone (15 mg) induced a significant (P < 0.001) increase in serum LH concentrations (from a mean basal value of 4.24 ± 1.10 IU/l to a peak of 13.27 ± 1.8 IU/l). The LH response to naloxone was significantly (P < 0.001) blunted by preinfusion of deltorphin (13.27 ± 1.80 IU/l versus 4.80 ± 1.18 IU/l). No significant changes in FSH concentrations were observed during deltorphin, naloxone or deltorphin plus naloxone administration. These data indicate that activation of δ-opioid receptors can reduce naloxone-induced LH release, suggesting a possible role of δ receptors in opioidergic modulation of LH secretion in women.

Key words: δ-opioid receptors/deltorphin/gonadotrophin/women

Introduction

It is well known that endogenous opioid peptides exert an important effect in the control of pituitary luteinizing hormone (LH) secretion, acting on gonadotrophin releasing hormone (GnRH) release at the hypothalamus (Genazzani and Petraglia, 1989; Kalra, 1993). In humans, the administration of morphine or other exogenous opiates induces a fall in circulating LH concentrations (Delitala et al., 1983). In contrast, endogenous opioid peptide receptor blockade by naloxone causes an increase in baseline LH concentrations (Quigley and Yen, 1980; Conte et al., 1996). The LH response to naloxone appears to be related to the concentrations of circulating gonadal steroids in healthy men (Conte et al., 1996) and in women during the late follicular and luteal phases of the menstrual cycle (Quigley and Yen, 1980). There is evidence indicating that the opioidergic inhibition of LH release is mainly mediated through µ-opioid receptors. Dermorphin, a highly selective µ-opioid agonist, decreases LH concentrations in fertile women and in postmenopausal women receiving oestrogen-progestogen replacement treatment (Petraglia et al., 1985). Pharmacological studies in animals indicate that µ-opioid receptor agonists inhibit GnRH secretion (Pfeiffer et al., 1983; Walsh and Clarke, 1996). In-vitro experiments demonstrate that pulsatile GnRH release from the human fetal mediobasal hypothalamus is suppressed by morphine, and this suppression is reversed by naloxone (Rasmussen et al., 1989). Moreover, a role of δ-opioid receptors in the regulation of GnRH secretion has recently been suggested (Weisner et al., 1985; Leadem and Yagenova, 1987).

Deltorphins are a group of opioid heptapeptides from amphibian skin that interact with high affinity and selectivity for δ-opioid receptors, and without any interaction at µ- and κ-opioid receptors (Kreil et al., 1989; Lazarus et al., 1989). In contrast to the enkephalin analogues, the deltorphins contain the common N-terminal tripeptide sequence H-Tyr1-D-Xaa2-Phe3, where D-Xaa2 is D-Met1 in deltorphin A, or D-Ala2 in deltorphins B and C ([Glu4] and [Asp5] in deltorphin II and I, respectively]. An L-isomer at the second residue virtually inactivates these peptides that display an affinity for δ-opioid receptors which is greater by one order of magnitude than that of DPDPE (D-Pen2-D-Pen5) enkephalin (Lazarus et al., 1989).

In the present study we investigated the effects of synthetic deltorphin A (H-Tyr-D-Met-Phe-His-Leu-Met-Asp-NH2) after i.v. administration on basal and naloxone-stimulated secretion of LH and FSH in healthy fertile women.

Materials and methods

Subject selection

Six healthy women, aged 20–35 years, with regular menstrual cycles (28–31 days in length), participated voluntarily in the study, which was previously approved by the local ethical committee. The women were studied during the midluteal phase (18–21 days) of the menstrual cycle; cycle length and ovulation were evaluated on the basis of flow charts, in which each subject recorded at rest morning vaginal temperature and menses occurrence. All subjects were within 10% of the ideal body weight and had not received any hormonal treatment for at least 6 months before the study. Smoking and use of alcohol,
tea and caffeine-containing foods were prohibited for 10 days before, as well as throughout, the study procedures.

**Drug investigations**

In each subject, after an overnight fast, indwelling intravenous (i.v.) cannulas were inserted in both forearms at 0730 h for separate blood sampling and drug administration. An equilibration period of 1 h was allowed before baseline blood samples were obtained. The women remained supine throughout all procedures; they were attended by a nurse and a physician and asked to report any side effects. All subjects underwent the following tests: (i) i.v. infusion of deltorphin (7 µg/kg/min diluted in 60 ml of saline, for 60 min, from –30 to 30 min of the study), plus an i.v. bolus of placebo (20 ml of normal saline) at time 0 of the study (deltorphin + placebo); (ii) i.v. infusion of normal saline (60 ml of saline from –30 to 30 min of the study), plus an i.v. bolus of placebo at time 0 of the study (saline + placebo); (iii) simultaneous i.v. infusion of normal saline (60 ml of normal saline, from –30 to 30 min of the study), plus an i.v. bolus of naloxone (Narcan, Crinos, Italy) 15 mg, at time 0 of the study (saline + naloxone); (iv) simultaneous i.v. infusion of deltorphin (7 µg/kg/min in 60 ml of saline for 60 min, from –30 to 30 min of the study), plus an i.v. bolus of naloxone, 15 mg, at time 0 of the study (deltorphin + naloxone). The women were investigated during two separate and successive menstrual cycles, receiving during each cycle two tests (deltorphin + placebo and saline + placebo, or saline + naloxone and deltorphin + naloxone) in random order with a three-day interval between treatments. Blood samples were taken at –60, –50, –40, –30 (basal samples), –20, –10, 0 (before bolus of placebo or naloxone), 10, 20, 30, 40, 50 and 60 min for measurements of LH and FSH serum concentrations. A blood sample was collected at time –60 of each procedure for oestradiol and progesterone measurements. Blood was centrifuged at 3000 g for 15 min immediately, and the serum removed and stored at –20°C until assayed. LH and FSH were measured by immunoradiometric assay (Serono Diagnostic, Milano, Italy) with monoclonal antibodies. The intra- and interassay coefficients of variation were 2.8% and 3.9% for LH, and 2.0 and 3.1% for FSH, respectively. The assay sensitivities were 0.15 IU/l for LH and 0.25 IU/l for FSH. Oestradiol and progesterone concentrations were determined by immunoradiometric assay (Diagnostic Products Corporation, Los Angeles, USA). The intra- and interassay coefficients of variation were 4.3 and 5.5% for oestradiol and 5.8 and 6.6% for progesterone, respectively. All samples were analysed in duplicate at the same time in each assay. Deltorphin (H-Tyr-D-Met-Phe-His-Leu) was synthetized by conventional methods in solutions, as previously described (Temussi et al., 1989), dissolved in sterile 0.15 M sodium chloride, and passed through a 0.45-µm Millipore filter (Millipore Corp., Bedford, MA). Deltorphin was available in lyophilized form and dissolved in 0.9% saline before i.v. administration.

**Statistical analysis**

The LH and FSH serum concentrations were expressed both as absolute values (IU/l) and as the area under the curve from 0 to 60 min (AUC 0–60 min, IU/l/min) calculated by the trapezoidal method. Results were expressed as mean ± SE. Statistical evaluation of the data was achieved using paired or unpaired Student’s t-test and analysis of variance, as applicable. In all tests, P < 0.05 was considered significant. The mean basal concentrations of LH and FSH were obtained from the mean (± SE) of the four values determined at times –60, –50, –40 and –30 min of the study for each test.

![Figure 1. The effect of deltorphin (DT) and naloxone (NX) administration on mean (± SE) serum concentrations of luteinizing hormone (LH) in six healthy fertile women. Arrows indicate the 60 min i.v. infusion of deltorphin (7 µg/kg/min) or saline (0.9% NaCl), and the administration of an i.v. bolus of naloxone (15 mg) or placebo. *P < 0.001 versus saline + placebo; *P < 0.01 and +P < 0.001 versus saline + naloxone.](image-url)

**Results**

The mean basal serum concentrations of LH, FSH, oestradiol and progesterone were within the normal range for the luteal phase of the cycle and did not show any significant differences between the four tests (Table I).

The infusion of deltorphin (deltorphin + placebo) did not affect LH concentrations significantly compared with either basal mean concentrations or after saline (saline + placebo) treatment (Figure 1). By contrast, a significant (P < 0.001) rise in LH concentration was observed in response to naloxone (saline + naloxone), increasing from 4.24 ± 1.10 IU/l at baseline to a peak of 13.27 ± 1.8 IU/l at 40 min and remaining significantly (P < 0.001) higher than both baseline and saline (saline + placebo) values from 20 to 60 min of the study (Figure 1). The LH response to naloxone (saline + naloxone) was significantly blunted by simultaneous infusion of deltorphin (deltorphin + naloxone) (12.07 ± 2.00 IU/l versus 5.28 ± 1.40 IU/l at 30 min, P < 0.01; 13.27 ± 1.80 IU/l versus 4.80 ± 1.18 IU/l at 40 min, P < 0.001) (Figure 1). In agreement with these results, the mean AUC for LH during deltorphin administration (deltorphin + placebo) did not differ significantly from that after saline + placebo treatment. However, the AUC for LH following naloxone (saline + naloxone) was significantly (P < 0.05) attenuated by deltorphin administration (deltorphin + naloxone) (9.27 ± 1.45 IU/l/min versus 5.10 ± 1.14 IU/l/min) (Table II).

Neither deltorphin infusion alone (deltorphin + placebo) nor naloxone alone (saline + naloxone) had any significant
Deltorphin

*The effect of deltorphin (DT) and naloxone (NX) administration on mean (± SE) serum concentrations of follicle stimulating hormone (FSH) in six healthy fertile women. Arrows indicate the 60 min i.v. infusion of deltorphin (DTLET) reduces the potassium-evoked luteinizing hormone-releasing hormone (LHRH) release in the median eminence and nucleus arcuate of the hypothalamus (Gerozissis et al., 1993). These data indicate that the δ-opioid receptors on Δ-opioid receptor agonist deltorphin blunts the naloxone-induced increase in LH concentrations, indicating a possible role of δ receptors in the opioidergic control of LH secretion by exerting an inhibitory action. Evidence has accumulated showing that opioidergic inhibition of LH release is mainly mediated through μ-opioid receptors (Pfeiffer et al., 1983; Walsh and Clarke, 1996). However, it has been shown in rats that intracerebroventricular administration of the selective δ-opioid receptor agonist DPDPE, inhibits LH secretion (Leadem and Yagenova, 1987), while the δ-opioid agonist (D-Thr²)-Leu-enkephalin-Thr⁶ (DTLET) reduces the potassium-evoked luteinizing hormone-releasing hormone (LHRH) release in the median eminence and nucleus arcuate of the hypothalamus (Gerozissis et al., 1993). These data indicate that the δ-opioid receptors may participate in the regulation of LHRH neuronal activity. Recently, Maggi et al. (1995a) have shown that DPDPE significantly inhibits binding of the non-selective opioid ligand diprenorphine in a neuronal LHRH-producing cell line (GT1-1), and thus suggested the presence of δ-opioid receptors on GT1-1 cells. Additionally, it has been shown that DPDPE

**Discussion**

The secretion of LH and FSH is controlled by the hypothalamic hormone GnRH (Yen, 1991) and various neurotransmitters are implicated in regulating the activity of GnRH neurons and consequently gonadotrophin secretion (Yen, 1991; Karla, 1993). Among the factors involved in the control of GnRH release, the endogenous opioid peptide may play an important role (Karla, 1993). It has been shown that, in women, opioid receptor agonists inhibit gonadotrophin release (Delitala et al., 1983; Petraglia et al., 1985), whereas the opioid antagonist, naloxone, raises circulating concentrations of gonadotrophin (Quigley and Yen 1980; Petraglia et al., 1986) and stimulates the frequency of spontaneous pulsatile LH secretion (Ropert et al., 1981).

In agreement with the literature, we have demonstrated that acute administration of naloxone results in a significant increase in circulating LH concentrations in fertile women during the luteal phase of the menstrual cycle. The i.v. administration of the highly selective δ-opioid agonist deltorphin fails to modify basal LH and FSH circulating concentrations suggesting that, under resting conditions, δ-opioid receptors may not be involved in the modulation of gonadotrophin release. However, pre-administration of the selective δ-opioid receptor agonist deltorphin blunts the naloxone-induced increase in LH concentrations, indicating a possible role of δ receptors in the opioidergic control of LH secretion by exerting an inhibitory action. Evidence has accumulated showing that opioidergic inhibition of LH release is mainly mediated through μ-opioid receptors (Pfeiffer et al., 1983; Walsh and Clarke, 1996). However, it has been shown in rats that intracerebroventricular administration of the selective δ-opioid receptor agonist DPDPE, inhibits LH secretion (Leadem and Yagenova, 1987), while the δ-opioid agonist (D-Thr²)-Leu-enkephalin-Thr⁶ (DTLET) reduces the potassium-evoked luteinizing hormone-releasing hormone (LHRH) release in the median eminence and nucleus arcuate of the hypothalamus (Gerozissis et al., 1993). These data indicate that the δ-opioid receptors may participate in the regulation of LHRH neuronal activity. Recently, Maggi et al. (1995a) have shown that DPDPE significantly inhibits binding of the non-selective opioid ligand diprenorphine in a neuronal LHRH-producing cell line (GT1-1), and thus suggested the presence of δ-opioid receptors on GT1-1 cells. Additionally, it has been shown that DPDPE

**Table I.** Mean (± SE) basal concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), oestradiol and progesterone in six healthy fertile women during the midluteal phase of the menstrual cycle

<table>
<thead>
<tr>
<th></th>
<th>Saline + placebo</th>
<th>Deltorphin + placebo</th>
<th>Saline + naloxone</th>
<th>Deltorphin + naloxone</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/l)</td>
<td>4.12 ± 0.78</td>
<td>4.05 ± 0.85</td>
<td>4.24 ± 1.10</td>
<td>3.78 ± 0.71</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>3.90 ± 0.80</td>
<td>3.45 ± 0.72</td>
<td>4.20 ± 0.51</td>
<td>4.30 ± 0.83</td>
</tr>
<tr>
<td>Oestradiol (pg/ml)</td>
<td>141.5 ± 18.5</td>
<td>162.1 ± 22.4</td>
<td>148.9 ± 19.6</td>
<td>155.6 ± 21.8</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>7.8 ± 1.9</td>
<td>8.6 ± 1.4</td>
<td>9.2 ± 2.1</td>
<td>8.1 ± 1.7</td>
</tr>
</tbody>
</table>

*P < 0.01 versus saline + placebo; **P < 0.05 versus saline + naloxone.

**Table II.** Effects of deltorphin administration on basal and naloxone-stimulated luteinizing hormone (LH) and follicle stimulating hormone (FSH) secretions (area under the curve, AUC) in six healthy fertile women

<table>
<thead>
<tr>
<th></th>
<th>Mean (± SE) AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LH (IU/l/min)</td>
</tr>
<tr>
<td>Saline + placebo</td>
<td>4.02 ± 0.78</td>
</tr>
<tr>
<td>Deltorphin + placebo</td>
<td>3.80 ± 0.82</td>
</tr>
<tr>
<td>Saline + naloxone</td>
<td>9.27 ± 1.45*</td>
</tr>
<tr>
<td>Deltorphin + naloxone</td>
<td>5.10 ± 1.14+</td>
</tr>
</tbody>
</table>

**Figure 2.** The effect of deltorphin (DT) and naloxone (NX) administration on mean (± SE) serum concentrations of follicle stimulating hormone (FSH) in six healthy fertile women. Arrows indicate the 60 min i.v. infusion of deltorphin (7 µg/kg/min) or saline (0.9% NaCl), and the administration of an i.v. bolus of naloxone (15 mg) or placebo.
induces inhibition of forskolin- or prostaglandin E$_2$-stimulated LH release in GT1-1 cells; moreover, this effect is reversed by the δ-opioid receptor antagonist naltrindole (Maggi et al., 1995b).

Our findings and the above-mentioned observations lead to the suggestion that δ-opioid receptors play a role in the opioidergic mechanisms involved in the control of LH release in women, possibly by acting on LH-RH-secreting neurons.

The administration of deltorphin, naloxone or deltorphin plus naloxone fails to affect FSH release. This is in accord with previous reports (Snowden et al., 1984; Petraglia et al., 1985) and confirms that opioids do not participate in the control of FSH secretion in women.

In conclusion, our results demonstrate that, in fertile women, the selective activation of δ-opioid receptors can reduce naloxone-induced LH release, thus suggesting a possible role of δ-opioid receptors in modulating opioid-mediated control of LH secretion. However, further investigation is required to clarify the site and mechanism of action in δ opioidergic modulation of LH release in women.

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References

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