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## Opioid Modulation of the Pre-ovulatory LH Surge in the Conscious Rat: Involvement of Indolamines

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**Abstract:** Endogenous opioid peptides play an important role in the generation of the pre-ovulatory LH surge. We investigated the interrelationship between the opioid and indolaminergic systems in the regulation of LH secretion, and the involvement of  $\mu$ - and  $\kappa$ -opioid subtypes in this process. Conscious female rats bearing cannula in the femoral artery were injected intraperitoneally with  $\mu$ -(diamorphine) and  $\kappa$ -(U-50488H) opioid agonists either alone or with their respective antagonists, naloxone and MR2266, on pro-oestrus. Blood samples were collected hourly between 15.00 and 19.00 and plasma LH levels were measured by RIA. 5-HT and 5-HIAA concentrations were determined by HPLC-ECD in the MPOA, SCN, ME and ARN. One-Way ANOVA was used to examine the results.

Both  $\mu$ - and  $\kappa$ - agonists inhibited the pre-ovulatory LH surge. Naloxone prevented

the suppressive effects of diamorphine on LH release, but MR2266 did not prevent the effects of U-50488H. Concentrations of 5-HT and 5-HIAA were selectively reduced by the  $\mu$ - and  $\kappa$ -agonists in the specific regions of the hypothalamus. Naloxone and MR2266 negated the inhibitory effects of diamorphine and U-50488H in an area-dependent manner. Only diamorphine lowered the ratio of 5-HIAA/5-HT in the MPOA, and these effects were reversed in the same hypothalamic region following its co-administration with naloxone.

The present results indicate that both  $\mu$ - and  $\kappa$ -opioid receptor subtypes may be involved in the inhibition of the LH surge. The physiological significance of an indolaminergic involvement in the opioid control of LH release appears to be minor.

**Key Words:** Indolamines, opioid receptors, LH surge and HPLC-ECD.

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### Introduction

Regulation of gonadotrophin-releasing hormone (GnRH) activity and hence luteinising hormone (LH) release involves a multiplicity of brain neurotransmitter systems including the monoamines and the opioids which modulate GnRH neurones within the hypothalamus (1, 2). The opioid system exerts a physiological tonic inhibitory effect on GnRH neurones as revealed by the enhancement of LH release after treatment with the opioid antagonist, naloxone (3, 4). Conversely, administration of opioid agonists, just before the critical period on the day of pro-oestrus, inhibits release of the pre-ovulatory LH surge and hence ovulation (5, 6). Allen and Kalra (7) have proposed that a reduction in the endogenous opioid tone before the onset of the pre-ovulatory LH surge may be the initial neural stimulus for the generation of the LH surge. The existence of

several different classes of opioid receptor subtypes has been shown in the hypothalamus (8, 9).

Although direct synaptic connections exist between opioid peptidergic nerve terminals and GnRH neurones in the medial preoptic area (MPOA) and median eminence (ME) (10) there is some evidence that the opioids can affect GnRH release directly (11). There is also a bulk of evidence indicating that the opioids act indirectly by influencing the brain monoaminergic systems (12-14).

In the present study, we further investigated the modulating effects of diamorphine ( $\mu$ -agonist) and U-50488H ( $\kappa$ -agonist), either alone or when co-administered with their receptor antagonists (naloxone and MR2266, respectively) on the release of LH and at the same time on the indolamine concentrations in specific regions of the rat hypothalamus. The

investigation was confined to the modulation of the pre-ovulatory LH surge in conscious female rats on the afternoon of pro-oestrus.

## Materials and Methods

Adult female Sprague-Dawley rats (Harlan UK Ltd., Oxon, England) weighing 220–300 g were maintained under controlled temperature ( $21 \pm 1$  °C) and light conditions (lights on from 07.00 to 19.00). Food and water were provided ad libitum. Vaginal smearing was performed each morning (09.00 to 10.00) and the morphology of the cells present used to identify the different stages of the oestrous cycle. Only those animals which had exhibited at least three consecutive four-day oestrous cycles were selected for experimentation.

On the late morning of pro-oestrus, a plastic cannula (Portex, o.d. 0.63mm) combined with winly tubing (i.d. 0.55 mm) was inserted into the right femoral artery under halothane anaesthesia (complete cessation of the hind limb flexor withdrawal reflex). With the aid of a stainless steel guide cannula, the vinyl tubing was fed under the dorsal skin to emerge at the back of the neck. The animals were allowed to recover and then in the early afternoon, just before the onset of the pre-ovulatory LH surge, intraperitoneally (IP) injected either with diamorphine (3 mg/kg,  $n=10$ ; Napp Laboratories Ltd, Cambridge UK), U-50488H (10 mg/kg,  $n=8$ ; Boehringer Ingelheim, Germany), diamorphine plus naloxone (15 mg/kg,  $n=13$ ; Sigma Chemicals Corporation, Poole, Dorset, UK) or U-50488H plus MR2266 (10 mg/kg,  $n=9$ ; Boehringer Ingelheim, Germany). The controls received saline alone (1 ml/kg;  $n=16$ ). Blood samples (200 $\mu$ l) were withdrawn through the heparinised cannula at hourly intervals from the freely-moving conscious animals throughout the afternoon of pro-oestrus, commencing at 15.00. Drug administrations were carried out under light halothane anaesthesia.

The blood samples collected were centrifuged at 4°C for 10 mins at 3000 rpm. The plasma was then transferred into fresh tubes and stored at -20°C until assayed for LH determination by radioimmunoassay (RIA). At 1900 the animals were decapitated, and the brains rapidly removed and frozen on dry ice. Five hundred micrometer coronal brain slices were cut. The MPOA, suprachiasmatic nucleus (SCN), ME and arcuate nucleus (ARN) were micropunched using modified stainless steel hypodermic needles (0.7 and 0.33mm internal diameter) under a dissection microscope, according to the stereotaxic atlas of the rat brain (15).

## Chromatography

The specific hypothalamic areas collected were kept at -80°C prior to the analysis. One hundred microliters of 0.1M HCl was added to each sample, along with 50 $\mu$ l of 3,4-dihydroxybenzylamine: 1ng) as an internal standard. The samples were homogenised and then centrifuged at 4°C for 10 mins at 3000 rpm. Ten microliter aliquots of supernatant were injected onto a reverse phase high performance liquid chromatographic (HPLC) column (S50DS2-250A, 5 $\mu$ m, 4.6mm i.d.x25cm, HICHRON) coupled to an electrochemical detector (ECD, Model 141, GILSON). The monoamine content of the hypothalamic regions was simultaneously detected. The method has been previously described (16).

## LH Assay

Plasma LH levels were measured by RIA. The standard used was NIADDK-rLH-RP-3 and the antibody NIADDK-anti-rLH S10. RIA reagents were obtained from the National Hormone and Pituitary Program (Baltimore, Maryland, USA). The inter- and intra-assay coefficients of variation were 8.0% and 9.5% respectively. The sensitivity of the assay was 10 pg/tube (1ng/ml).

## Protein Estimation

Protein estimations were made according to the modified method of Lowry et al. (17). The method is detailed in our previous report (16).

## Statistics

The data were statistically analysed by using One-Way Analysis of Variance (MINITAB for Windows, 10). The level of significance was set at  $P < 0.05$ .

## Results

### 1. Effect of $\mu$ - and $\kappa$ -agonists and antagonists on hypothalamic indolamine concentrations:

The indolamine results are presented in Figure 1 and Table 1. Although both diamorphine and U-50488H altered 5-hydroxytryptamine (5-HT) concentrations in all the hypothalamic regions examined, these changes were not significantly different from those values seen in the controls. MR2266 brought about significant increases in 5-HT levels in the SCN ( $P < 0.05$ ) and ARN ( $P < 0.05$ ), but decreases in the MPOA ( $P < 0.005$ ), compared with those which received U-50488H alone following its co-administration with the  $\kappa$ -opioid agonist. The 5-HT concentrations were also significantly elevated by naloxone in only the SCN compared with the values seen in the diamorphine-treated rats.

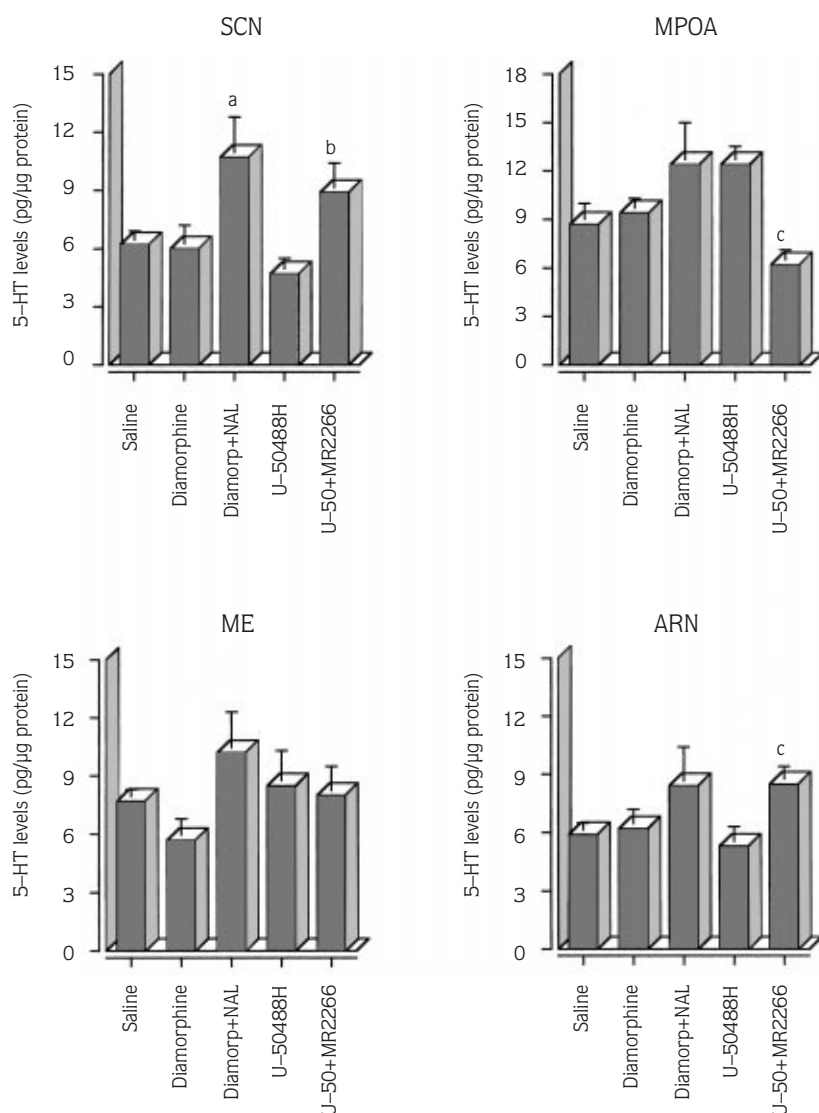


Figure 1. 5-HT concentrations (pg/μg protein±SEM) in the MPOA, SCN, ME and ARN of the rat hypothalamus at 19.00 on pro-oestrus following administration or co-administration of μ- and κ-opioid agonists and antagonists at 13.00 on the same day. a: P<0.05 compared with the diamorphine-treated animals. b: P<0.05. c: P<0.005 compared with the U-50488H-treated animals, using One-Way ANOVA.

Table 1. 5-HIAA concentrations (pg amine/μg protein±SEM) in the MPOA, SCN, ME and ARN of the rat hypothalamus at 19.00 on pro-oestrus after administration or co-administration of μ- and κ-opioid agonists and antagonists at 13.00 on the same day. a: P<0.05 compared with the saline-, b: P<0.01 compared with the diamorphine-, c: P<0.05 compared with the U-50488H-treated animals. ANOVA was used to examine the data.

Area	Saline (n=16)	Diamorphine (n=10)	Diamor+NAL (n=13)	U-50488H (n=8)	U-50+MR2266 (n=9)
MPOA	7.2±0.9	4.0±0.9 a	10.0±1.6 b	5.5±1.1	5.7±1.4
SCN	7.3±1.3	6.1±1.6	11.1±2.4	5.7±1.0	7.5±1.3
ME	7.8±1.1	4.5±1.1 a	14.5±3.0 b	4.1±0.6 a	5.6±1.2
ARN	6.7±1.1	6.0±1.5	13.8±5.0	4.1±0.9	7.4±1.2 c

Concentrations of the 5-HT metabolite (5-hydroxyindole acetic acid; 5-HIAA) were significantly decreased by the  $\mu$ -agonist in the MPOA ( $P<0.05$ ) and ME ( $P<0.05$ ). These inhibitory effects were prevented in the same hypothalamic areas following the co-administration of diamorphine with naloxone ( $P<0.01$ ). The  $\kappa$ -agonist reduced 5-HIAA levels in only the ME ( $P<0.05$ ), and its combination with MR2266 resulted in significant increases in the ARN compared with those which received U-50488H alone ( $P<0.05$ ).

The ratio of 5-HIAA/5-HT (Table 2) was significantly lowered by diamorphine in only the MPOA compared with that seen in the controls ( $P<0.01$ ). These suppressive effects were negated in the same hypothalamic area when the  $\mu$ -agonist was co-injected with naloxone ( $P<0.01$ ). Neither U-50488H nor its co-administration with the  $\kappa$ -opioid antagonist significantly altered the ratio of 5-HIAA/5-HT in any of the hypothalamic regions examined.

2. Effect of  $\mu$ - and  $\kappa$ -agonists and antagonists on LH release on the afternoon of pro-oestrus:

Plasma samples were obtained at hourly intervals between 15.00 and 19.00 on the afternoon of pro-oestrus and the LH concentrations were seen to rise significantly to a peak at 18.00 hours with a slight nonsignificant fall at 19.00 hours (LH levels ng/ml $\pm$ SEM: at 15.00 hours = 3.1 $\pm$ 1.3; 16.00 hours = 11.5 $\pm$ 4.1; 17.00 hours = 22.3 $\pm$ 7.9; 18.00 hours = 27.3 $\pm$ 9.4; 19.00 hours = 23.6 $\pm$ 6.3). These LH results are consistent with those reported by Dow et al. (18) using the same experimental technique. Administration of U-50488H at 13.00 completely suppressed plasma LH levels throughout the afternoon of pro-oestrus. The  $\kappa$ -antagonist, MR2266, failed to prevent the inhibitory effects of the  $\kappa$ -agonist on LH secretion in 8/9 rats with one animal showing a rise to peak LH levels of 19.5 ng/ml

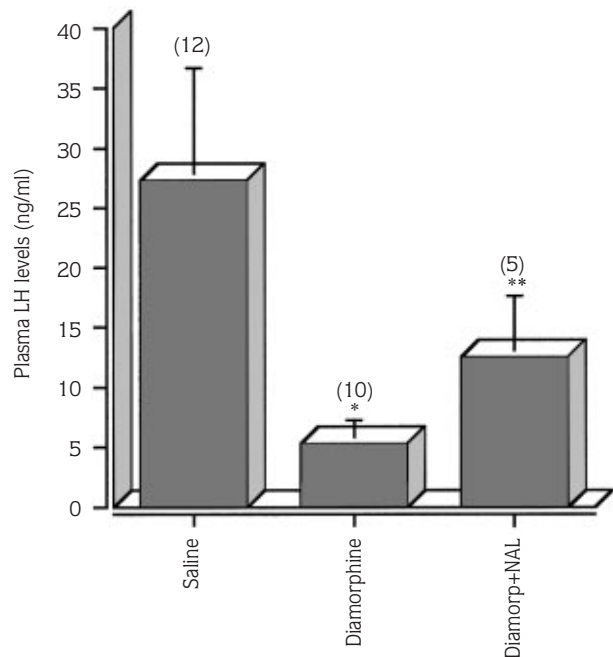


Figure 2. Peak concentrations of LH over the afternoon of pro-oestrus in conscious rats treated with saline, diamorphine alone or with naloxone. Numbers in each group are given in brackets. \*  $P<0.01$  compared with saline-treated animals; \*\*  $P<0.05$  compared with the diamorphine-treated animals using One-Way ANOVA.

at 19.00 hours. Administration of diamorphine suppressed plasma LH levels in 5/10 rats with five animals showing a rise to peak concentrations of 18.5, 12.8, 10.9, 10.7 and 5.5 ng/ml each at 18.00 hours and 19.00 hours sampling intervals. This inhibitory effect of diamorphine was reversed in 5/6 rats which were given 15 mg/kg naloxone concomitantly with the  $\mu$ -agonist. Peak concentrations of LH over the afternoon of pro-oestrus are shown in Figure 2.

Table 2. Ratio of 5-HT/5-HIAA (Mean $\pm$ SEM) in the MPOA, SCN, ME and ARN of the rat hypothalamus at 19.00 on pro-oestrus after administration or co-administration of  $\mu$ - and  $\kappa$ -opioid agonists and antagonists at 13.00 on the same day. a:  $P<0.01$  compared with the saline-, b:  $P<0.01$  compared with the diamorphine-treated animals. ANOVA was used to examine the data.

Area	Saline (n=16)	Diamorphine (n=10)	Diamor+NAL (n=13)	U-50488H (n=8)	U-50+MR2266 (n=9)
MPOA	0.962 $\pm$ 0.140	0.430 $\pm$ 0.106 a	1.048 $\pm$ 0.165 b	0.796 $\pm$ 0.324	0.783 $\pm$ 0.169
SCN	1.311 $\pm$ 0.300	1.365 $\pm$ 0.440	0.968 $\pm$ 0.156	0.997 $\pm$ 0.213	0.736 $\pm$ 0.116
ME	1.172 $\pm$ 0.234	1.153 $\pm$ 0.281	1.078 $\pm$ 0.112	0.624 $\pm$ 0.144	0.643 $\pm$ 0.149
ARN	6.7 $\pm$ 1.1	6.0 $\pm$ 1.5	13.8 $\pm$ 5.0	4.1 $\pm$ 0.9	7.4 $\pm$ 1.2 c

## Discussion

The hypothalamus is densely populated by opioid receptors while the anterior pituitary contains only a very low density of these (19). The opioids have been shown to have no direct action on LH release from the anterior pituitary (20). It is therefore suggested that the  $\mu$ - and  $\kappa$ -opioid agonists in this study exerted their inhibitory effects at the hypothalamic level. This is in line with the evidence that administration of morphine suppresses the release of GnRH (11) and naloxone infusion increases its secretion from the medial basal-preoptic area in vitro (21).

The ability of morphine (a  $\mu$ -agonist) to suppress LH secretion has been demonstrated in a variety of experimental conditions (see 6). The results of the present study support this hypothesis, as the  $\mu$ -opioid receptor agonist, diamorphine, also reduced the secretion of LH on the afternoon of pro-oestrus. When diamorphine was co-administered with naloxone, the opioid inhibition of the LH release was prevented. This suggests that increased LH release results from an antagonism at post-synaptic  $\mu$ -opioid receptors. Also, the inhibitory opioidergic tone, although decreased, is not totally eliminated during the critical period.

There are conflicting reports on the involvement of  $\kappa$ -opioid receptors in the regulation of LH secretion. Inhibition of LH release occurs after administration of specific  $\kappa$ -opioid agonists (22, 23). However, the specificity of the  $\kappa$ -opioid effect has been questioned (24). In the present study, the LH surge was completely abolished by a selective  $\kappa$ -agonist throughout the afternoon of pro-oestrus. The  $\kappa$ -opioid action on LH release is believed to be mediated at the level of the hypothalamus in view of the finding that  $\kappa$ -agonists inhibit GnRH release in vitro (25). The  $\kappa$ -receptor antagonist, MR2266, failed to reverse the suppressive effects of U-50488H on plasma LH levels. It has been reported that MR2266 has also  $\mu$ -agonist properties (26). Although the present results imply participation of  $\kappa$ -opioid receptors in the LH secretory systems, further studies using more selective antagonists may reveal additional information.

The studies on the involvement of the serotonergic system upon LH release have been controversial; both a stimulatory and an inhibitory role of 5-HT on LH secretion have been reported (27–29). 5-HT may mediate, at least in part, the effects of endogenous opioid peptides on the LH secretory systems. Morphine

stimulates LH release in the presence of exogenous 5-HT, but is inhibitory in its absence (30). Synaptic contacts between the serotonergic terminals and immunoreactive GnRH perikarya have been visualised in the rat hypothalamus (31). The  $\mu$ -opioid agonist, diamorphine, reduced the LH surge in association but had no effect on 5-HT levels in any of the four hypothalamic areas examined. However, it selectively decreased the concentrations of 5-HIAA in the MPOA and ME in a naloxone-reversible fashion. Moreover, the ratio of 5-HIAA/5-HT was also lowered in the MPOA, and the effect was negated by naloxone in this area. It has previously been suggested that naloxone enhancement of the pre-ovulatory LH surge is primarily mediated by 5-HT in the MPOA (4). These results would point to a modulatory action of the  $\mu$ -opioid subtype on the 5-HT metabolism (or turnover) in the MPOA and ME rather than its release from the nerve terminals. However, it was expected that the opioids would reduce not only the metabolism of this indoleamine but also its release as well.

Although U-50488H was very successful in blocking the LH surge, it did not significantly alter the concentrations of 5-HT or its metabolite in any of the hypothalamic areas examined except that 5-HIAA levels were lowered in the ME. Interestingly, 5-HT levels were elevated in the SCN and ARN, but reduced in the MPOA following the co-administration of U-50488H with MR2266. This  $\kappa$ -opioid antagonist also increased concentrations of 5-HIAA in only the ARN in comparison with those rats receiving U-50488H alone. Activation of  $\kappa$ -opioid receptors has been shown to pre-synaptically inhibit 5-HT release in the dorsal raphe nucleus (32). The results of the present study were not consistent and difficult to interpret. Previous attempts to reveal the putative nature of interaction between  $\kappa$ -opioid receptors and serotonergic neurotransmission have also been inconclusive (23).

In conclusion, activation of the  $\mu$ - and  $\kappa$ -opioid receptors may exert an inhibitory influence on LH release, since both diamorphine and U-50488H inhibited the pre-ovulatory LH surge. Although pharmacological studies have suggested a role for the serotonergic system in the central regulation of LH surge, the physiological significance of its involvement in this process appears to be minor. The present results suggest that  $\mu$ - and  $\kappa$ -opioid agonists (diamorphine and U-50488H) may have inhibitory effects on 5-HT release and/or turnover in discrete hypothalamic areas.

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