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Differential Regulation of Preovulatory Luteinizing Hormone and Follicle-Stimulating Hormone Release by Opioids in the Proestrous Rat

S. KUMRU, M. ŞIMŞEK, B. YILMAZ*, E. SAPMAZ, S. KUTLU*, S. SANDAL*, S. CANPOLAT*

*Fırat University Medical School, Departments of Obstetrics and Gynecology, and *Physiology, Elazığ, Turkey*

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Summary

We have investigated the role of μ- and κ-opioid receptors in the central control of preovulatory LH and FSH release in the proestrous rat. Animals were anesthetized with chloral hydrate at 14:00 h on proestrus day. Following femoral artery cannulation, they were mounted in a stereotaxic apparatus. Morphine and U-50488H (benzene-acetamide methane sulphonate) were infused intracerebroventricularly either alone or in combination with naloxone and MR1452, respectively. Controls received sterile saline alone. Blood samples were obtained at hourly intervals between 15:00 h and 17:00 h. Plasma LH and FSH levels were measured by radioimmunoassay. Morphine did not significantly change plasma LH levels at 15:00 h and 16:00 h sampling intervals. A significant increase was observed at 17:00 h compared to the controls ($p<0.05$). U-50488H significantly increased LH levels at 16:00 h and 17:00 h ($p<0.05$). The co-administration of naloxone and MR1452 with μ- and κ-agonist had no significant effect on LH levels at any sampling interval. In all groups, LH levels showed a linear rise over the sampling period between 15:00 h and 17:00 h. None of the treatments significantly altered plasma FSH levels which however, declined towards the end of the afternoon surge. In conclusion, we suggest that the secretion of LH and FSH is differentially regulated by μ- and κ-opioid receptors. It is thought that in all groups chloral hydrate interfered with the LH surge secretory systems.

Key words
LH • FSH • Morphine • Naloxone • U-50488H • MR1452.

Introduction

Endogenous opioid peptides have been reported to participate in the central regulation of luteinizing hormone (LH) release. It is believed that the effects of opioids on LH secretion are exerted at the hypothalamic level, i.e. that they modulate the release of the gonadotropin-releasing hormone (GnRH) by direct or indirect mechanisms (Mehmanesh *et al.* 1988, Kalra *et al.* 1997, Yilmaz and Gilmore 1999a). Opioid peptidergic neurones have been found to be in close proximity to GnRH neurons in various hypothalamic areas (Leranth *et al.* 1988). The existence of three major classes of opioid receptor subtypes (μ, κ and δ) has been shown in the

Fax+4202 24920590
E-mail physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

hypothalamus as well as in other brain areas (Mansour *et al.* 1988, Desjardins *et al.* 1990). Opioids have no direct action on LH release from the anterior pituitary (Bicknell 1985). Furthermore, the anterior pituitary is relatively poor in opioid receptors (Khachaturian *et al.* 1985).

There is a bulk of evidence indicating that opioids have an inhibitory influence on LH secretion. The administration of opioid agonists, just before the critical period on the day of proestrus, inhibits the preovulatory LH surge and hence ovulation (Kalra *et al.* 1989, Barraclough 1994). Conversely, the administration of an opioid antagonist, naloxone, overcomes the tonic inhibitory effect of endogenous opioid peptides on GnRH release and enhances the release of LH (Piva *et al.* 1985, Brown *et al.* 1994). Furthermore, it has been proposed that a reduction in endogenous opioid tone may be the trigger for initiating the LH surge in proestrous rats (Allen and Kalra 1986). However, this hypothesis has recently been challenged by Lieberman *et al.* (1998).

There is limited information regarding the involvement of opioids in the regulation of FSH secretion. It has been reported that butarphanol (a synthetic morphine derivative) reduced LH and FSH levels in ovariectomized rats (Fayez *et al.* 1991). In contrast, previous reports had shown that treatment of proestrous rats with morphine and naloxone only altered LH release (Ieri *et al.* 1980, Piva *et al.* 1985). Younglai and Byrne (1989) found that morphine reduces the frequency and amplitude of LH pulses, but does not affect FSH secretion in female rabbits. Secretion of FSH during the estrous cycle in rats consists of two different patterns – basal secretion and surge release. The surge pattern of FSH secretion occurs during the preovulatory period from the afternoon of proestrus to the morning of estrus, whereas the basal pattern can be seen at other stages of the estrous cycle (Noguchi *et al.* 1993).

The present study was designed to investigate the involvement of μ- and κ-opioid receptors in the central regulation of preovulatory LH and FSH release in the proestrous rat.

Material and Methods

Adult female Wistar rats weighing 220-250 g (Firat University Biomedical Unit, Elazig, Turkey) were used in this study. They were housed under controlled light (lights on from 07:00 h to 19:00 h) and temperature (21±1 ^{o}C) conditions. Food and water were provided *ad libitum.*

Vaginal smearing was performed each morning and the morphology of the cells present was used to identify the different stages of the estrous cycle. Only those rats (total of 42 animals) which had shown at least three consecutive four-day estrous cycles were included in the experiments. On the afternoon of proestrus, animals were anesthetized with chloral hydrate (400 mg/kg, i.p., Botafarma Laboratory, Ankara, Turkey) at 14:00 h. Surgical anesthesia was maintained by further periodic injections of the anaesthetic. After cannulation of the right femoral artery, the rats were mounted in a stereotaxic apparatus. They were intracerebroventricularly (icv) infused with either morphine (μ-agonist; 100 μg/kg/10 μl; n=8), U-50488H (benzeneacetamide methane sulphonate; μ-agonist; 40 μg/kg/10 μl; n=8), morphine plus naloxone (predominantly μ-antagonist; 4 μg/kg/10 μl; n=8) or U-50488H plus MR1452 (hydroxy 6,7-benzomorphan methanesulphonate; μ-antagonist; 80 μg/kg/10 μl; n=8) at 15:00 h on the afternoon of proestrus. The controls received sterile saline alone (10 μl; n=13). Blood samples (0.7 ml) were obtained at 15:00 h, 16:00 h and 17:00 h *via* the indwelling heparinized cannula and centrifuged at 3000 r.p.m. (4 ^{o}C for 10 min).

Morphine and naloxone were purchased from Galen Ilaç San. (Istanbul, Turkey) and Abbott Laboratories (North Chicago, USA), respectively.

Radioimmunoassay: Plasma levels of rLH and rFSH were determined by radioimmunoassay following the instructions given with the reagents generously provided by the National Hormone and Pituitary Program of NIDDK. The rLH reference preparation was rLH-RP-2 and the antiserum was anti-rat LH-RIA-11. The sensitivity (90 % B/Bo) of this assay using a 100 μl sample is about 0.16 ng/ml. The rFSH reference preparation was rFSH-RP-2 and the antiserum was anti-rat FSH-S-11. The sensitivity of this assay using a 200 μl sample is about 2 ng/ml. Both antigens were radiolabelled with ^{125}I (IMS 30 from Amersham Life Science Ltd, Bucks, UK). Bound and free radioiodinated antigens were separated using the double antibody generously provided by the Scottish Antibody Production Unit, Law Hospital, Carluke, Lanarkshire, Scotland. The mean intra-assay coefficient of variation for two quality control samples was <5 % in both assays.

The hormone results were statistically analyzed by one-way ANOVA (MINITAB, release 10 for Windows). Level of significance was set at $p<0.05$.

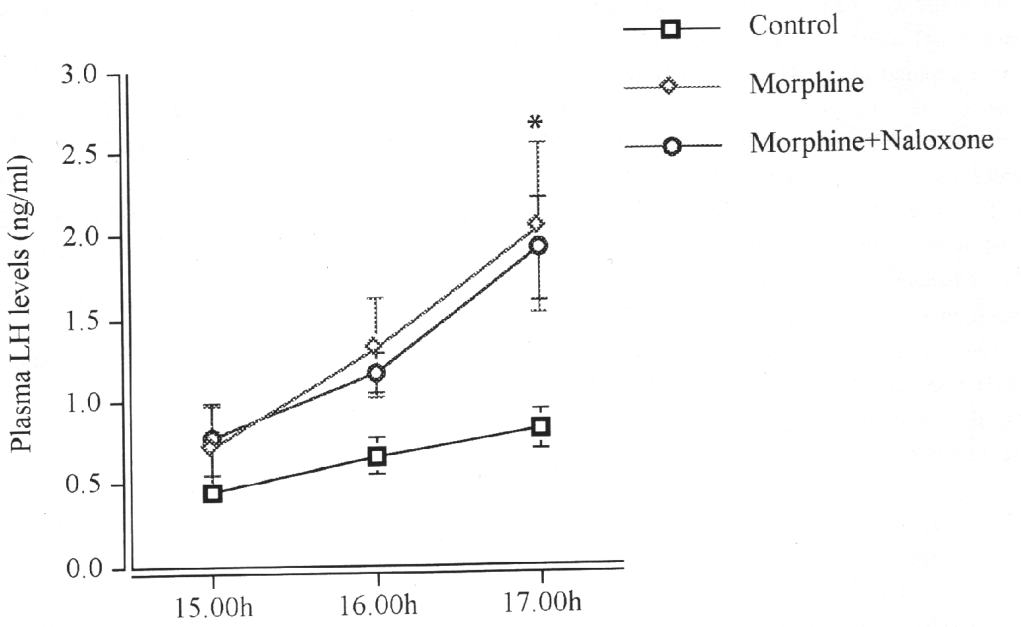

Fig. 1. *Plasma LH levels (ng/ml ± S.E.M.) at 15:00, 16:00 and 17:00 h sampling intervals on the afternoon of proestrus following administration of saline (n=10), morphine (n=8) or morphine plus naloxone (n=8) at 15:00 h on the same day * p<0.05 compared to the saline-treated animals, using one-way ANOVA.*

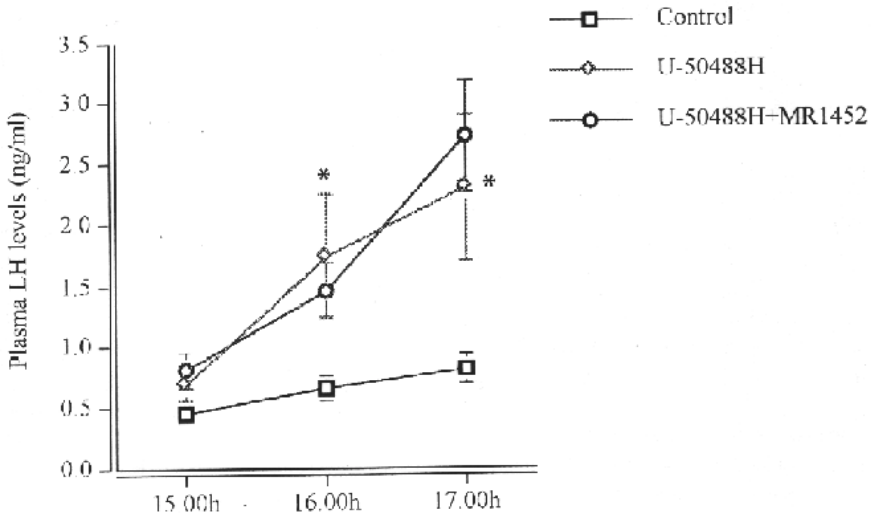

Fig. 2. *Plasma LH levels (ng/ml ± S.E.M.) at 15:00, 16:00 and 17:00 h sampling intervals on the afternoon of proestrus following administration of saline (n=10), U-50488H (n=8) or U-50488H plus MR1452 (n=8) at 15:00 h on the same day. * p<0.05 compared to the saline-treated animals, using one-way ANOVA.*

Results

The plasma LH results are shown in Figures 1 and 2. Morphine administration did not significantly change plasma LH levels at 15:00 h and 16:00 h sampling intervals. However, a significant increase in LH levels was observed at 17:00 h compared to the respective control group values ($p<0.05$). Co-administration of the

μ-opioid agonist with naloxone did not significantly alter the plasma LH concentrations compared to animals receiving morphine alone. However, LH levels in the morphine + naloxone group were significantly higher than the control group at 16:00 h and 17:00 h ($p<0.05$). Intracerebroventricular infusion of U-50488H increased plasma LH levels at 16:00 h and 17:00 h intervals compared to the control values at the same sampling time ($p<0.05$). Changes in LH concentrations following co-administration of the κ-agonist with MR1452 were not found to be significantly different from the rats receiving U-50488H alone. In all groups, LH levels showed a linear rise over the sampling period between 15:00-17:00 h on the late afternoon of proestrus. These increases were significant between the sampling intervals within each group ($p<0.05$). However, these LH patterns did not correspond to "preovulatory surge" features.

Table 1. Plasma FSH levels (ng/ml) at 15:00 h, 16:00 h and 17:00 h sampling intervals on the afternoon of proestrus following administration of saline, μ- and κ-opioid agonists and antagonists at 15 h on the same day.

	15:00 h	**16:00 h**	**17:00 h**
Control (n=10)	13.7±3.0	13.2±2.5	6.8±1.1*
Morphine (n=8)	16.5±3.4	13.8±2.6	10.3±2.8
Morphine + naloxone (n=8)	11.2±1.6	9.2±1.3	7.2±1.1*
U-50488H (n=8)	12.0±3.1	15.6±3.2	8.7±1.0*
U-50488H + MR1452 (n=8)	13.9±1.3	11.7±3.6	11.3±3.1

*Data are means ± S.E.M. *p<0.05 compared to 15:00 h or 16:00 h values within the same group, using one-way ANOVA..*

Plasma FSH levels are summarized in Table 1. Neither morphine nor U-50488H introduced any significant changes in plasma FSH levels at any sampling interval studied in comparison to the control group. The co-administration of μ- and κ-opioid agonists with naloxone and MR1452, respectively, did not significantly alter the FSH levels at 15:00, 16:00 and 17:00 h on the afternoon of the proestrus. However, it was found that FSH levels declined almost in all groups towards the end of the afternoon surge. The results on serum LH from our previous study are shown in Table 2. In a conscious rat model, the preovulatory LH surge occurred on the afternoon of proestrus.

Table 2. Plasma LH levels (ng/ml) in conscious rats at hourly sampling intervals between 15:00 and 19:00 h in the afternoon of proestrous following intraperitoneal admoinistration of saline, U-50488H (κ agonists) or U-50488H + MR2266 (κ antagonist) at 13:00 h on the same day.

Groups	**15:00 h**	**16:00 h**	**17:00 h**	**18:00 h**	**19:00 h**
Control	3.1±1.3	11.5±4.1	22.3±7.9		
	27.3±9.4		23.6±6.3		
U-50488H	Low	Low	Low	Low	Low
U-50488H + MR2266	Low	Low	Low	Low	Low

Data are means ± S.E.M. Low represents the concentration below the limit of detection (Yilmaz and Gilmore 1999a)

Discussion

The existence of a critical period of two or three hours on the afternoon of proestrus, beginning at approximately 14:00 h has been suggested. Opioid agonists such as morphine given after 14:00 h on the day of proestrus would not inhibit the preovulatory LH surge (Lieberman *et al.* 1998). It means that after this critical time, the LH surge becomes resistant to pharmacological blockade. It should be noted that in the present study, the lighting schedule started at 07:00 h, i.e. two hours earlier than the schedules reported by Lieberman *et al.* (1998). Therefore, the preovulatory LH surge was expected to occur late in the afternoon. In the present study, animals were anesthetized with chloral hydrate at 14:00 h and opioid agonists and antagonists were administered at 15:00 h. It has been reported that general anesthetics may have unexpected effects in neuroendocrine studies (Hartman *et al.* 1989, Yilmaz and Gilmore 1999b). We have previously reported that urethane, saffan (althesin) and ketamine all suppressed LH levels in proestrous or ovariectomized and steroid-primed rats (Yilmaz *et al.* 1996, Yilmaz and Gilmore 1999b). Preovulatory LH levels (ng/ml) following saline administration to rats under different anesthetics were as follows: conscious group: 27.3±9.4; urethane group: 0.5±0.05; ketamine group: 5.2±0.8 and saffan group: 2.5±0.5 (Yilmaz 1998). Since anesthesia was required for serial collection of

blood samples on the afternoon of proestrus, chloral hydrate was chosen as an alternative anesthetic agent in the present study. We found that plasma LH levels were low in all groups, probably due to a general depression by the anesthetic agent. However, the mechanisms by which chloral hydrate interferes with the LH secretory systems is not known.

It has been widely reported that administration of morphine inhibits the surge release of LH on the day of proestrus (Pfeiffer *et al.* 1987, Lieberman *et al.* 1998). However, several studies have shown that activation of μ-opioid receptors stimulates LH release (Pang *et al.* 1977, Brown *et al.* 1994). In these reports, it appears that low doses of morphine and duromorph have stimulatory effects on LH release, whereas high doses of these compounds are required for blocking of the LH surge. In the present study, application of a single dose of morphine had no significant effect at 15:00 and 16:00 h of sampling, but significantly increased plasma LH levels at 17:00 h on the afternoon of proestrus. Although it is difficult to reconcile the literature with the present findings, the stimulatory action of low doses of morphine might account for this discrepancy.

There are conflicting reports on the involvement of κ-opioid receptors in the regulation of LH secretion. Inhibition of LH release occurs after administration of specific κ-opioid agonists (Leadem and Yagenova 1987, Gopalan *et al.* 1989). However, the specificity of the κ-opioid effect has been questioned since the inhibition induced by tifluadom, a κ receptor agonist, was reversed by naloxone (Pfeiffer *et al.* 1987). In our previous study, the LH surge was completely abolished by a selective κ-agonist throughout the afternoon of proestrus in a conscious rat model, but the κ-opioid antagonist (MR2266) failed to exert this effect (Yilmaz and Gilmore 1999a). In the present experiments, U-50488H increased plasma LH levels at 16:00 h and 17:00 h on proestrus day. The co-administration of this κ-agonist with MR1452 had no significant effect on LH release. A recent study has shown that U-50488H stimulated the *in vitro* LH release from entire rat pituitary in a dose-dependent manner (Dragatsis *et al.* 1995). This finding appears to be contradictory since κ-opioid action on LH release is believed to be mediated at the hypothalamic level in view of the finding that κ-agonists inhibit GnRH release *in vitro* (Muraki *et al.* 1979).

It is still controversial whether the release of both LH and FSH is diminished by an opioidergic influence. Some authors have found that opioid agonists and antagonists concomitantly alter FSH and LH release (Fayez *et al.* 1991, Leposavic *et al.* 1991), while others have reported that these agents modify LH, without affecting FSH secretion (Piva *et al.* 1985). In the present study, μ- and κ-opioid agonists and antagonists had no significant effect on plasma FSH levels at any sampling interval on the afternoon of proestrus. These results suggest that μ- and κ-opioid receptors are not involved in the central control of preovulatory FSH secretion.

Although it is generally accepted that the release of both FSH and LH is stimulated by the hypothalamic decapeptide, GnRH (Wise *et al.* 1979), the existence of a separate hypothalamic FSH-releasing factor (FSHRF) has been suggested by McCann *et al.* (1998). Indeed, the administration of an antiserum against GnRH abolished LH release, but had no effect upon FSH secretion (Kovacs *et al.* 1993). In addition, their hormone profiles throughout the estrous cycle are known to be dissociated. Although the proposed FSHRF has been purified from rat hypothalamus, it has not yet been identified (Yu *et al.* 1997). In our study, μ- and κ-opioid agonists and antagonists did not significantly modify FSH release at any sampling interval studied, whereas they caused concomitant changes in plasma LH levels on the afternoon of proestrus. Thus, these results provide further evidence for the existence of FSHRF to selectively regulate FSH secretion.

In conclusion, it is postulated that μ- and κ-opioid receptors may be involved in the central regulation of the LH surge. The effect of opioid action on LH release may depend on the dose applied. No relationship was found between μ- and κ-receptor types and FSH secretion. The overall suppression of the preovulatory LH surge may be attributed to the general anesthetic, chloral hydrate used in this study.

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Reprint requests

Dr. Bayram Yılmaz, Fırat University, Tıp Fakültesi (Medical School), Department of Physiology, 23119 Elazığ, Turkey. Fax: + 90 424 237 91 38. E-mail: b.yilmaz@excite.com