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The Role of Endogenous Opioid Peptides in the Effects of Constant Illumination on Reproductive Function in the Rat

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HULSE, G. K., G. J. COLEMAN, D. L. COPOLOV AND V. W. K. LEE. *The role of endogenous opioid peptides in the effects of constant illumination on reproductive function in the rat.* PHARMACOL BIOCHEM BEHAV **23**(4) 535–539, 1985.—The present experiments assessed the involvement of endogenous opioids in the inhibition of FSH and LH release, ovulation and continuous sexual receptivity following exposure to constant illumination. In the first experiment, exposure to constant illumination resulted in persistent vaginal oestrus in all rats. The injection of naloxone resulted in marked elevations in serum FSH and LH, induced ovulation and increased the frequency of lordosis behaviour. It was concluded that endogenous opioid(s) participate in these effects. In Experiment 2, levels of β-endorphin were found to be elevated in anterior pituitary and neurointermediate lobe tissue extracts from rats exposed to constant illumination, compared to levels in pro-oestrus rats. Naloxone injection into those rats exposed to constant illumination significantly increased hypothalamic levels of β-endorphin compared to saline injected controls. This suggests that the blockade of opiate receptors increases β-endorphin production, uptake and/or decreases its release from the hypothalamus. These results, and the known inhibitory action of β-endorphin on LH release suggest that it may be this opioid, perhaps in conjunction with pineal products, which is responsible for the observed anti-reproductive effects of constant illumination.

β-Endorphin Constant illumination Lordosis Naloxone

EXPOSURE of cyclic female rats to constant illumination (LL) produces abnormal behavioural, physiological and endocrinological events, including an absence of the pre-ovulatory follicle stimulating hormone (FSH) and luteinizing hormone (LH) surge [17] and an associated absence of oestrous cyclicity and ovulation [14].

Endogenous opioids have been shown to play a role in regulating LH release in the rat. Endogenous opioid peptides depress serum LH concentrations, while administration of naloxone increases LH levels [1]. Naloxone administration also antagonizes the anti-LH and anti-ovulatory actions of immobilization [10] and unpredictable electric shock [11].

The similarities of the effects of LL and stress on reproductive function raises the question of whether endogenous opioids are involved in the effects of LL on reproductive function in a manner similar to their involvement in the effects of stress on reproduction. If endogenous opioids are involved, the administration of naloxone might result in FSH/LH release and ovulation.

The anti-FSH/LH action of opioids is initiated at the level of the hypothalamus, by inhibiting luteinizing hormone releasing hormone (LH-RH) release [4]. Recently, it has been demonstrated that β-endorphin (β-EP), but not met-enkephalin antibodies injected into the arcuate nucleus of the mediobasal hypothalamus increase plasma levels of LH [25], suggesting that β-EP rather than met-enkephalin plays a role in regulating LH release.

Constant secretion of oestrogen following exposure to LL has been reported to result in almost continuous sexual receptivity as indicated by lordosis behaviour in the female rat [14,17]. However, lordosis behaviour is facilitated by the administration of LH-RH in the presence of high levels of oestrogen [19]. Following exposure to LL, LH-RH release is inhibited [26]. Reinitiation of LH-RH release by naloxone should lead to an increase in the frequency of lordosis behaviour.

The aim of this study was to examine whether endogenous opioids are involved in the effects of LL on FSH/LH release, ovulation and lordosis behaviour. In Experiment 1, we have examined whether a single naloxone injection into rats exposed chronically to LL results in FSH/LH release, ovulation and increased lordosis behaviour and, in Experiment 2, whether rats chronically exposed to LL show higher levels of immunoreactive (Ir-) β-EP in hypothalamic, anterior pituitary gland and neurointermediate lobe tissue extracts and plasma compared to control rats at pro-oestrus.

EXPERIMENT 1

Method

Forty-six naive female Wistar rats (175–210 g) were housed under a controlled 12:12 light/dark (LD) regimen (lights on 12:00 hr) in individual cages (550 × 330 × 450 mm) with air temperature at 21 ± 2°C and rat food (GR 2 +,

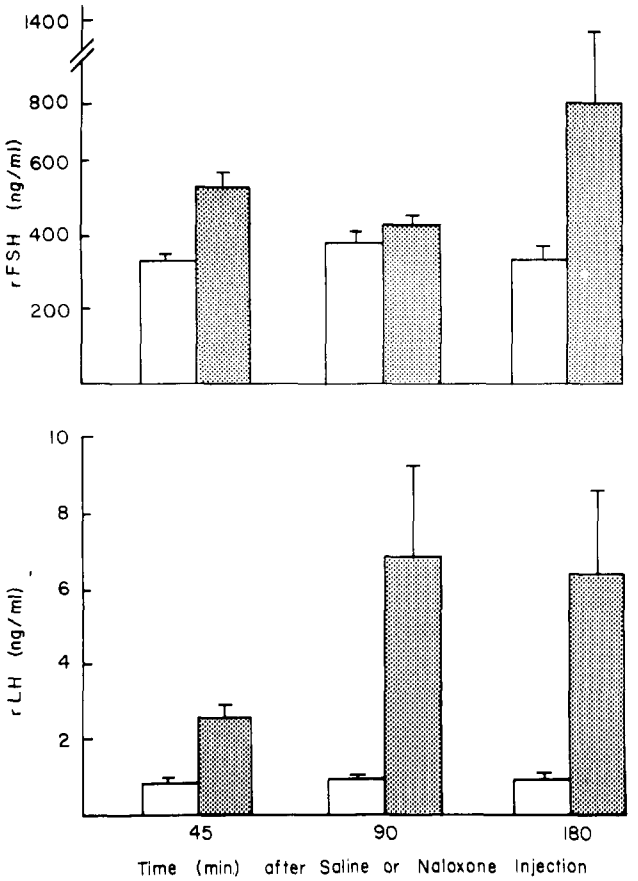

FIG. 1. Effect of naloxone (shaded areas) or saline (unshaded areas) on serum FSH and LH levels in rats exposed to LL. For all time periods in both conditions N = 5.

Clark King, Melbourne, Australia) and tap water available ad lib.

Daily vaginal smearing and classification commenced from day 80. Only rats exhibiting at least three normal 4-day oestrous cycles before experimentation were included in the study.

At 99 days of age, rats were placed in LL. On the afternoon (17:00 hr) of day 134 all rats were randomly assigned to one of two conditions. In condition 1, rats were given both an interperitoneal (IP) and subcutaneous (SC) injection of 4 mg/kg naloxone hydrochloride dissolved in 0.2 ml saline (0.154 $_M$-NaCl). Rats in condition 2 received an IP and an SC injection of 0.2 ml saline (0.154$_M$-NaCl).

Fifteen rats from each condition were then killed by decapitation, five at 17:45, 18:30 and 20:00 hr respectively and trunk blood collected into tubes. Blood was allowed to clot, centrifuged and serum separated and frozen by placing it in liquid nitrogen. Serum was stored at $-20°$ C until assayed for FSH and LH.

Serum levels of LH and FSH were measured by double antibody systems. Radioimmunoassay used reagents provided by NIAMDD; the characteristics of the rat FSH [6] and LH [15] assays have been described previously.

At 08:00 hr on day 135, the remaining eight rats in each condition were removed to an adjoining room illuminated by red lights and tested for lordotic behaviour [21]. All rats

TABLE 1

EFFECT OF NALOXONE OR SALINE ON LORDISIS AND OVULATION IN RATS EXPOSED TO LL

	No. of rats displaying lordosis behaviour	Lordotic quotient	No. of rats ovulating	Mean No. of oocytes per rat
LL naloxone injected (N = 8)	8	66.3	6	8.6
LL saline injected (N = 8)	8	28.8	0	0

were placed separately in boxes (500 × 330 × 450 mm) and manually stimulated for 30 sec per trial for 10 trials each spaced 10 min apart. The number of lordotic responses was recorded. The ratio of lordosis/times stimulated × 100 (lordosis quotient = LQ) was used as a measure of sexual receptivity. Scoring of lordotic behaviour was carried out under a single blind design.

At 12:00 hr on the morning of day 135 these remaining rats were anaesthetized and their ovaries and oviducts removed and placed in physiological saline. Each ovary and oviduct was then inspected separately under a dissecting microscope and oocyte counts made using a well-established technique [23].

Results

Exposure of rats to LL for 35 days resulted in constant vaginal oestrus. The effect of a single injection of saline or naloxone on serum FSH and LH levels in rats exposed to LL can be seen in Fig. 1.

Two separate analyses of variance with Planned Contrasts (Hays, 1970) were carried out. Significant elevations in serum FSH, $F(1,24) = 4.83$, $p<0.05$, and LH, $F(1,24) = 15.92$, $p<0.01$, levels occurred following naloxone injection compared to those following saline injection. While LH levels in naloxone treated rats at 90 and 180 min were similar, these levels were significantly greater than those found at 45 min, $F(1,24) = 6.05$, $p<0.025$. A similar result was obtained for FSH, $F(1,24) = 5.55$, $p<0.05$.

Although all rats injected with naloxone or saline displayed lordosis, saline injected rats had a much lower lordotic quotient (mean LQ = 28.8) compared to those injected with naloxone (mean LQ = 66.3), $t(14) = 4.4$, $p<0.01$. While 6 out of 8 rats injected with naloxone ovulated (mean number of oocytes/rat = 8.6), none of the saline injected rats ovulated (Table 1).

EXPERIMENT 2

Method

Twenty-one rats, similar to those described in Experiment 1, were initially housed under a 12:12 LD regime. At 99 days of age, they were randomly assigned to one of three conditions. Conditions 1 and 2 were housed under LL conditions and condition 3 remained under the controlled 12:12

LD regime. Daily vaginal smearing and classification was carried out as described in Experiment 1.

On the afternoon (17:00 hr) of day 134, all rats in conditions 1 and 2 were injected with saline and naloxone respectively as described in Experiment 1. At 18:30 hr all rats in conditions 1 and 2 were killed by decapitation and trunk blood collected into chilled heparinized tubes for centrifugation at 4°C; plasma was then removed and frozen. Hypothalami were dissected using an established method [7]. The anterior pituitary and neurointermediate lobe of killed rats were also removed and placed into 1 ml 0.1 M-HCl and quickly frozen by immersing in liquid nitrogen.

When rats in condition 3 first reached pro-oestrus after day 134, they were killed at 17:00 hr by decapitation and plasma, the hypothalamus, anterior pituitary and neurointermediate lobe were removed and processed as described above. Tissues and plasma were stored at −20°C for up to 6 weeks for radioimmunoassay of β-EP [12].

Results

The Ir-β-EP content of neural tissue extracts and plasma in rats exposed to LL and the pro-oestrus control condition can be seen in Fig. 2.

Analysis of variance revealed that levels of Ir-β-EP for hypothalamic, $F(2,17) = 4.69$, $p<0.01$, anterior pituitary, $F(2,17) = 18.6$, $p<0.01$, and neurointermediate lobe, $F(2,18) = 6.1$, $p<0.01$, tissue extracts, but not plasma were significantly different across the three groups.

Duncan's Multiple Range test [13] revealed that anterior pituitary Ir-β-EP levels for naloxone, $q(17) = 4.52$, and saline, $q(17) = 5.83$, injected LL rats were significantly higher than were Ir-β-EP pro-oestrus control values. Similarly, neurointermediate lobe Ir-β-EP levels of saline, $q(18) = 8.13$, and naloxone, $q(18) = 11.49$, injected rats were significantly higher than those for pro-oestrus controls. Hypothalamic Ir-β-EP levels of naloxone injected rats were significantly higher than those observed in saline, $q(17) = 8.68$, and pro-oestrus, $q(17) = 10.67$, controls. Hypothalamic Ir-β-EP levels of pro-oestrus control and saline injected rats were not significantly different.

While analysis failed to show significant differences between plasma Ir-β-EP levels, differences in the range, mean and s.e.m. for pro-oestrus controls and rats exposed to LL were observed. Whereas pro-oestrus controls showed plasma Ir-β-EP values ranging from 0.14 to 0.34 μg/l with a mean and s.e.m. of 0.23 ± 0.03, saline and naloxone injected rats housed under LL showed plasma Ir-β-EP values ranging from 0.03 to 0.81 μg/l and 0.13 to 0.84 μg/l with mean and s.e.m. values of 0.34 ± 0.17 and 0.40 ± 0.1 μg/l respectively.

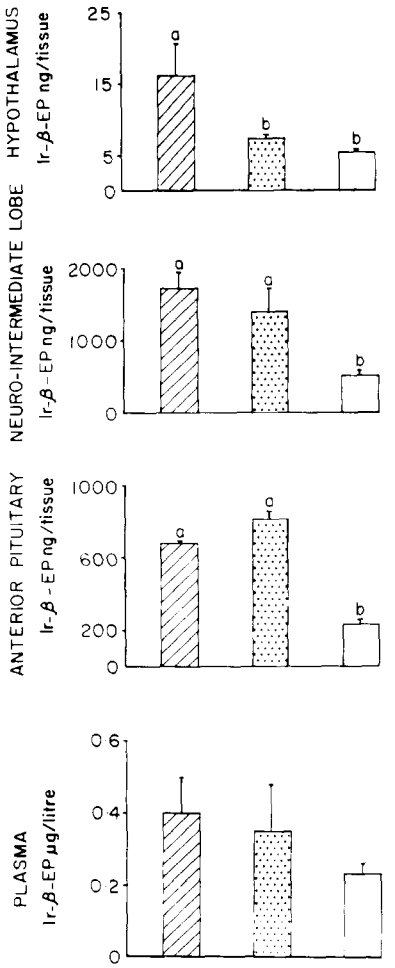

FIG. 2. Changes in immunoreactive β-endorphin (Ir-β-EP) content (mean ± s.e.m.) of hypothalamus, anterior pituitary gland, neurointermediate lobe and plasma in rats injected with naloxone hydrochloride (hatched bars) or saline (stippled bars) following exposure to constant illumination and pre-ovulatory pro-oestrus controls (open bars). Mean ± s.e.m. values with different superscripts are significantly different ($p<0.05$). In all conditions N = 7.

DISCUSSION

Despite previous findings that naloxone inhibits [27] or has no effect on [22] FSH release, those from Experiment 1, similarly to those from others [18,29] support a facilitatory role of naloxone. Of these studies, one finding [27] may be interpreted as a depletion effect, rather than an inhibitory one, but no explanation can be offered for the discrepancy of these results from others [22].

Elevated levels of serum FSH/LH account for the presence of ova retrieved from the oviducts of rats, since FSH/LH treatment is known to result in the rupture of mature ovarian follicles [24]. That LH release and ovulation is induced by a single naloxone treatment in female rats exposed to LL is consistent with the finding that naloxone administration effectively antagonizes the anti-LH and anti-ovulatory action of immobilization [10] and unpredictable electric shocks [11], suggesting a common mechanism by which stress and LL disrupt reproductive function.

Administration of melatonin to rats following exposure to LL results in LH/FSH release and ovulation [16]. LL exposure for a time sufficient to block ovulation and vaginal cyclicity results in a decrease in rat midbrain serotonin concentration, while treatment of LL exposed rats with melatonin raises midbrain serotonin to levels found in cyclic rats at ovulation, suggesting that melatonin acts via the midbrain serotoninergic system in inducing ovulation [28].

Collectively, these findings suggest that opioids, as well as pineal-related substances are involved in the anti-reproductive effects of LL. Some research suggests that there is an interaction between opioids and the pineal [3], and opioids and the serotoninergic system [5].

Experiment 2 demonstrated that the induction of persistent vaginal oestrus by exposure to LL results in anterior pituitary and neurointermediate lobe tissue levels of Ir-β-EP that are

higher than those observed in preovulatory rats. The large range of plasma Ir-β-EP levels observed in Experiment 2 in rats exposed to LL but not in those at pro-oestrus, suggests that LL might cause episodic Ir-β-EP release with only some rats being observed at the time of release. Naloxone administration elevates hypothalamic tissue levels of Ir-β-EP in rats exposed to LL compared to saline injected LL rats. This suggests first, that naloxone administration either increases production, uptake and/or decreases secretion of hypothalamic Ir-β-EP and secondly, that a feedback system to regulate hypothalamic opioid levels involving endogenous opiate receptors exists.

The anti-LH actions of opioids are initiated at the level of the hypothalamus by inhibiting LH-RH release [4]. It is possible, therefore, that naloxone facilitates LH release and ovulation by antagonizing the anti-LH-RH activity of opioids. This hypothesis is consistent with the finding that LH-RH administered to rats exposed to LL induces pituitary LH release [26].

If hypothalamic β-EP is involved in the inhibition of LH-RH release, then elevated levels of hypothalamic β-EP might be expected following exposure to LL. Hypothalamic Ir-β-EP levels, however, were not elevated. There are a number of possible explanations for this observation. First, an endogenous opioid other than β-EP may be involved in the blockage of LH release. This is not supported by the finding that β-EP, but not met-enkephalin antibodies placed into the hypothalamus of normal cyclic rats facilitate LH release [25]. Second, the radioimmunoassay technique employed cross-reacts with β-LPH, an inactive opioid, and it is possible that β-EP changes in hypothalamic tissue extracts might be masked by changes in β-LPH. The third and most likely explanation is that only a small amount of extracellular hypothalamic β-EP is necessary to inhibit LH-RH release. Ir-β-EP values may predominantly reflect intracellular stores unavailable to receptors and mask small changes in extra-cellular levels. The stimulation of hypothalamic LH-RH secreting cells may also result in the stimulation of neurones activating lordosis behaviour. Additional evidence to support the involvement of LH-RH in lordosis behaviour has been shown by induction of lordosis in female rats after injection of LH-RH [19,20].

The present study using naloxone hydrochloride provides clear evidence that opioids are involved in reproductive disruptions observed following exposure to LL. Research utilizing refined assay techniques and specific opioid antibodies are now necessary to evaluate the function of specific opioid peptides in the observed LL disruptions, particularly in the context of the role of the pineal gland and its related hormones.

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