Pharmacology Biochemistry & Behavior, Vol. 19, pp. 795–799, 1983.

The Role of Endogenous Opioids in the Blockade of Reproductive Function in the Rat Following Exposure to Acute Stress

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Received 7 February 1983

HULSE, G. K. AND G. J. COLEMAN. *The role of endogenous opioids in the blockade of reproductive function in the rat following exposure to acute stress.* PHARMACOL BIOCHEM BEHAV **19**(5) 795–799, 1983.—The present experiments investigated the involvement of endogenous opioids in the preovulatory disruption of luteinizing hormone release and ovulation following exposure to an acute stressor. The effect of exposure to an acute stressor and/or treatment with naloxone hydrochloride on the proestrus/estrus display of lordosis behavior is also reported. Electric shocks (2 mA) delivered at 10-min intervals for three hours during the period of the preovulatory luteinizing hormone surge in adult cyclic female rats resulted in the blockage of luteinizing hormone release, decreased lordosis behavior, and inhibition of ovulation. The anti-luteinizing hormone and anti-ovulatory actions were antagonized by the administration of naloxone hydrochloride shortly prior to the application of the stressor, which suggests that the observed blockage of ovulation is mediated by endogenous opioids known to be elevated in response to a stressor. It is argued that the most likely mechanism by which endogenous opioids inhibit luteinizing hormone release and ovulation is by inhibiting luteinizing hormone releasing hormone release, and/or decreasing its production in the hypothalamus. In contrast, naloxone only partially antagonizes the anti-lordotic actions of electric shock. It is therefore further argued that the inhibition of luteinizing hormone releasing hormone release alone is not sufficient to explain the dramatic decrease in lordosis behavior observed following exposure to an acute stressor. Adrenal progesterone released in response to adrenocorticotropic hormone release following exposure to an acute stressor, in association with inhibited luteinizing hormone releasing hormone release is hypothesized to be responsible for the observed decrease in lordotic behavior, and the inhibition of luteinizing hormone release.

Stress Endogenous opioids Naloxone Luteinizing hormone

THE exposure of adult cyclic female rats to stressors such as immobilization or unpredictable electric shock (UES) on the afternoon of proestrus prevents ovulation by inhibiting luteinizing hormone (LH) release [12]. Exposure of immature female rats primed with pregnant mare serum gonadotrophin (PMSG) to stress of immobilization just prior to the LH surge also results in the blockage of ovulation [9]. This anti-ovulatory action of stress is effectively antagonized by the administration of naloxone hydrochloride (naloxone), shortly prior to the application of the stress regime [9].

Since naloxone is a known opiate antagonist [3], the observed blockage of ovulation may be mediated by the endogenous opioids, which are reported to be elevated by acute stressors [7]. Reproductive disruptions observed in adult rats following exposure to stressors might, similarly to those observed in immature PMSG-primed rats, be due to increments in endogenous opioids.

The stress induced opioid disruption to the preovulatory LH surge could occur at the level of the hypothalamus, either where the opioids inhibit the neurogenic stimulation necessary to trigger the release of luteinizing hormone releasing hormone (LHRH) or reduce LHRH production [4]. Naloxone would block the actions of hypothalamic opioids and allow LHRH production or release. This hypothesis is supported by the identification of opioid receptors on hypothalamic neural tissue known to be associated with LHRH production and release [17].

In the cyclic rat, pre-ovulatory LHRH [13], progesterone and an elevated level of estrogen [19] are necessary for the initiation and intensity of lordosis behavior. Both progesterone and estrogen levels are governed by the release of hypothalamic LHRH [6]. If the increase in endogenous opioids, resulting from exposure to a stressor, disrupts LHRH release, then lordosis behavior, as well as ovulation, will be inhibited. Recent evidence suggests, however, that the effect of an acute stressor (ether exposure) on lordosis behavior may not be so unequivocal. Adrenocorticotrophic hormone (ACTH) from the anterior pituitary, released following exposure to an acute stressor, has been found to result in progesterone release from the adrenals [8] which in turn increases the frequency of lordosis behavior in estrogen-primed, ovariectomized rats [5].

Accordingly, the resulting influence on lordosis behavior of an acute stressor presented during the afternoon of proestrus may depend on the relative importance of first, the opioid blockage of hypothalamic LHRH which has been shown to decrease lordosis behavior and second, increments in adrenal progesterone which have been shown to increase lordosis behavior.

This study was designed to investigate, first, whether

UES applied on the afternoon of proestrus interferes with LH release, lordosis behavior and ovulation in adult cyclic rats, and, second, whether prior naloxone administration antagonizes any anti-reproductive actions of UES.

METHOD

Naive female Wistar 90-day-old rats (175–210 g) were housed under conditions of 12:12 light/dark (LD) regimen (lights on 0800 hr). All rats were housed in individual cages (550×330×160 mm) with air temperature 21±2°C and rat food (GR2+, Clark King) and tap water available ad lib.

Stress apparatus comprised four modified Skinner boxes constructed from clear Plexiglas of internal dimensions 34×23×33 cm high with a floor of 19 stainless steel 0.65 cm diameter rods evenly spaced at 1.9 cm intervals. UES was delivered through the floor grid (2 mA DC, 50 pulses per sec, square wave).

At 90 days of age, 36 rats underwent jugular catheterization using an established technique [16]. Blood samples and injection of reconstituted red blood cells, naloxone and/or 0.154 M NaCl were made via this route. Following surgery, rats were allowed to recover for ten days. On every alternate day following surgery, catheters were flushed with 0.5 ml of heparinized saline. During flushings, blood was also drawn into a syringe to ensure that catheters were not blocked. Five rats who developed a blocked catheter were discarded from the experiment.

Daily vaginal smearing and classification commenced from day 100. Only rats exhibiting at least three normal 4-day estrous cycles before experimentation were included in the study. Twenty-eight of the 31 rats met this classification criterion.

On Days 120–123 (depending on the age at which rats reached proestrus) rats were randomly assigned to one of the four conditions: (1) UES + 0.5 ml 0.154 M NaCl; (2) UES + 5 mg/kg naloxone in 0.5 ml 0.154 M NaCl; (3) 5 mg/kg naloxone in 0.5 ml 0.154 M NaCl; (4) 0.5 ml 0.154 M NaCl.

On the afternoon of proestrus at 1555 hr, conditions 2 and 3 were injected with 5 mg/kg naloxone hydrochloride in 0.5 ml 0.154 M NaCl and placed in the stress apparatus with up to 4 rats per Skinner box. Conditions 1 and 4 were similarly injected with 0.5 ml 0.154 M NaCl and placed in identical shock boxes. At 1600 hr conditions 1 and 2 were subjected to 18 electric foot shocks every 10 min.

During the experiment, 1 ml blood samples were withdrawn 5 min before injection (1550 hr), 90 min after injection (1730 hr), and 180 min after injection (1900 hr) respectively and collected into tubes. Following each blood collection, catheters were flushed with 0.2 ml heparinized saline. Blood samples were stored for no longer than 15 min, then centrifuged at 4°C and serum separated. Serum was then frozen by placing it in liquid nitrogen and stored at −40°C until assayed for LH. Radioimmunoassay for rat LH was carried out by a double antibody system using standard rat LH-I-6 and anti rat-LH-S-6 reagents supplied by the National Institute of Arthritis, Metabolism and Digestive Diseases. The characteristics of the rat LH RIA system have been described previously [11]. Following blood collection and plasma separation, remaining red blood cells were reconstituted with 0.5 ml 0.154 M NaCl and injected back into their respective donors.

At 0800 hr on day 135, seven rats in each condition were removed to an adjoining room illuminated by red lights and tested for lordotic behavior [18]. All rats were placed separately in boxes (500×330×450 mm) and manually stimulated for 20 sec per trial for 10 trials each spaced 10 min apart. The number of lordotic responses elicited by the stimulation was recorded. The ratio of lordosis/times stimulated × 100 (lordosis quotient=LQ) was used as a measure of sexual receptivity. Scoring of lordotic behavior was carried out under a single blind design.

On the morning (0800 hr) of day 136, all experimental rats were vaginal smeared, then sacrificed and ovaries and oviducts were removed. Each ovary and oviduct was inspected separately under a dissecting microscope. Tubal oocyte counts were then made [23].

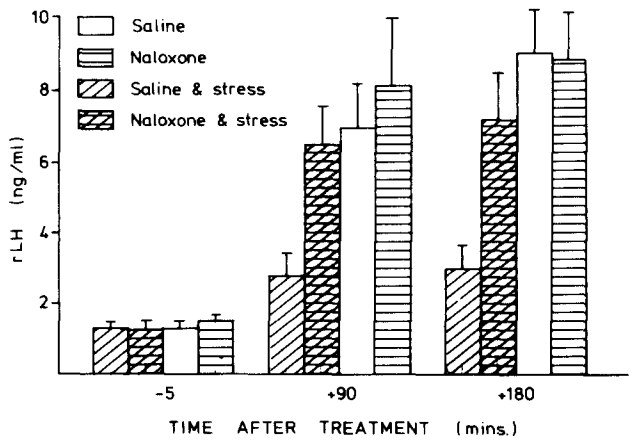

FIG. 1. Effect of naloxone or saline on serum LH levels in the proestrus female rat exposed to UES.

RESULTS

The effect of 180 min shock stress and/or treatment with naloxone or saline on LH levels can be seen in Fig. 1.

Serum LH values were analyzed using a two-way analysis of variance with one repeated measure and significant effects were investigated using Newman-Keuls post hoc analysis [10] with $\alpha=0.05$. A significant difference in serum LH between the four experimental treatments was observed, $F(3,24)=5.1$, $p<0.01$. Subsequent post hoc testing showed no significant overall differences between pairs of groups. Serum LH varied according to the time serum samples were taken, $F(2,48)=54.8$, $p<0.01$, with post hoc analysis showing that samples at 1550 hr had lower serum LH levels than at the other two times, which were not significantly different from each other. Interaction between these factors, $F(6,48)=3.5$, $p<0.01$, was observed.

Post hoc tests showed that saline injected controls displayed normal preovulatory serum LH values with nadir levels at 1550 hr (1.2 ng/ml) which continued to increase significantly at 1730 hr (7.2 ng/ml) to reach peak values (9.2 ng/ml) at 1900 hr. Naloxone injected rats showed significant LH increments at 1730 (8.2 ng/ml) and 1900 hr (9.0 ng/ml) compared to pre-injection values (1.4 ng/ml) that were not dissimilar from saline injected controls. Saline injection plus exposure to UES resulted in significantly lower mean levels of serum LH at 1750 (2.6 ng/ml) and 1900 hr (2.8 ng/ml) compared to saline injected controls, although serum LH values at 1900 hr were significantly greater than pre-injection values (1.2 ng/ml).

TABLE 1

EFFECT OF NALOXONE OR SALINE ON LORDOSIS BEHAVIOUR AND OVULATION IN THE PROESTRUS FEMALE RAT EXPOSED TO UES

	No. of rats displaying lordosis behaviour	Mean Lordotic quotient (N=7)	No. of rats ovulating	Mean ± SEM of oocytes per rat
Saline + UES (N=7)	4	17	2	3.6 ± 2.2
Naloxone + UES (N=7)	5	34	6	13 ± 2.1
Naloxone (N=7)	7	73	7	15.7 ± 2.0
Saline (N=7)	7	61	7	15.6 ± 0.5

Prior administration of naloxone significantly attenuated the effect of exposure to UES on serum LH levels at 1750 (6.5 ng/ml) and 1900 hr (7.3 ng/ml), although peak serum LH levels at 1900 hr were still significantly lower than those of saline injected controls.

The number of rats displaying lordosis, mean LQ, mean number of rats ovulating and mean rate of ovulation (number of oocytes/rat) following stress and/or treatment with naloxone or saline can be seen in Table 1.

Exposure to UES reduced the number of rats displaying lordosis behavior (4 in 7) compared to naloxone (7 in 7) and saline (7 in 7) injected controls. Naloxone administration prior to UES did not substantially increase the number of rats displaying lordosis (5 in 7) compared to saline injected rats exposed to UES.

LQ results were analyzed using a 2×2 (Stress/No-Stress; Saline/Naloxone) factorial design analysis of variance. Stressed groups were significantly different from non-stressed groups, $F(1,24)=27.88, p<0.01$. There was no effect due to naloxone treatment and no interaction. Inspection of Table 1 suggests that LQ for the saline and UES rats was lower than that for the naloxone and UES rats although naloxone does not reinstate lordosis behavior fully in UES treated rats.

The number of oocytes ovulated/rat were analyzed using a one-way analysis of variance [10]. Significant differences in oocytes counted between the four experimental treatments, $F(3,24)=8.38, p<0.01$, were observed.

Post hoc analysis showed that rats treated with saline plus UES had significantly decreased ovulation rates compared to rats exposed to naloxone plus UES, naloxone alone or saline alone. Ovulatory values of naloxone injected rats exposed to UES were not significantly different from saline or naloxone treated rats. While all 7 naloxone or saline treated and 6 of 7 UES-naloxone treated rats ovulated, only 2 of 7 stress-saline pretreated rats ovulated.

DISCUSSION

Exposure to acute UES inhibited preovulatory LH release, decreased lordosis behavior and inhibited ovulation. The anti-ovulatory actions of UES were effectively antagonized by the administration of naloxone shortly prior to UES. Naloxone also effectively antagonized the effect of UES on serum LH 90 min following UES, although the peak serum LH levels at 180 min following UES were significantly lower than those of saline injected controls. The action of UES on lordosis behavior was only partially antagonized by pre-treatment with naloxone.

Since LH release, lordosis and ovulation were all normal in the saline treated controls, there is good reason to believe that mere placement of rats in the Skinner boxes did not induce significant levels of novelty stress.

The effects on LH and ovulation of UES are consistent with those in other studies where an acute stressor applied prior to the LH surge blocks the LH surge and ovulation [12]. The ability of naloxone to antagonize these effects is consistent with previous research showing a similar effect on ovulation in PMSG-primed immature rats previously exposed to immobilization stress [9].

That naloxone antagonized the effects of UES on serum LH at 90 min but not 180 min may be explained by its half-life of 40 min [1]. This short half-life makes it likely that a single injection of naloxone at the dose employed in this study would be insufficient to maintain blockage of opioid receptors for the duration of UES. Utilizing longer acting opioid antagonists or increasing the dose of naloxone could yield very different results. Increased serum LH in UES-exposed rats pre-treated with naloxone is analogous to similar increments in LH with amenorrheaic women suffering from stress following injection with naloxone [20].

It is probable that elevated serum LH levels observed in this study are responsible for the occurrence of ovulation since the presence of LH is known to readily result in ovulation [24]. Therefore, the previous finding that naloxone antagonizes the anti-ovulatory action of immobilization in PMSG primed rats [9] can perhaps be explained by increments in serum LH.

The effects of UES on lordosis behavior reported here do not support the findings that acute exposure of female rats to a stressor (ether) increases lordosis behavior [8]. This discrepancy may be due to a number of factors: first, different types of stressors were employed in the two studies; second, different strains of rats were used; and, third, the previously reported study [8] used estrogen primed ovariectomized rats, representing a different hormonal environment and phase of the estrous cycle, compared to the high estrogen/progesterone proestrus/estrus rats used in the present experiment. These arguments are supported by recent evidence showing

that different stressors [2], genetic differences between rats [9], and different stages of the estrous cycle [14] result in differing responses to a stressor.

The role of naloxone in antagonizing the antireproductive effects of a stressor reported here and elsewhere [9,20] is taken to imply that endogenous opioids were instrumental in producing these stress effects. The value of naloxone as an experimental tool lies in its ability to bind to endogenous opiate receptors, thereby preventing the action of opioids upon those receptors. Naloxone binding, unlike opioid binding, does not result in synaptic transmission [26].

The effects of UES on plasma LH levels and on ovulation can be explained by findings which demonstrate that stressors elevate peripheral levels of endogenous opioids [7]. Endogenous opioids in turn have been implicated in the control of hypothalamic LHRH and pituitary LH release. A number of endogenous opioid peptides administered exogenously have been shown to depress serum LH concentrations [3] while suppression of their action either by using naloxone [3] or an opioid antibody (anti-ir-β-endorphin) facilitates LH release [25].

No direct evidence showing the role of endogenous opioids on ovulation is available, but the fact that LH release is essential for ovulation implies that endogenous opioids can block ovulation.

Additional, indirect evidence implicating opioids in the blockage of LH and ovulation comes from those studies using morphine which show that acute administration of morphine inhibits the LH surge and ovulation while prior administration of naloxone reverses these effects [15].

The mechanism by which opioids inhibit LH release may be by the blockage of LHRH release from the median eminence (ME) of the hypothalamus [4]. Electrical stimulation of the ME reverses morphine blockage of ovulation in adult rats [24] while exogenous administration of LHRH reverses morphine blockage of the LH surge [15]. It is hypothesized here that the actions of endogenous opioids on the ME are similar to morphine. Support for this proposition comes from a study showing that injection of naloxone into a superfusion system containing medial basal hypothalami from female rats caused a significant increase in LHRH [22]. Further evidence suggests that β-endorphin is one of the opioids controlling LHRH release [25].

Two issues arise from the finding that UES decreased lordosis behavior and that naloxone only partially reinitiated it. The first issue relates to the mechanism by which stress inhibited lordosis and the second to why naloxone did not cause a reversal.

Lordosis is facilitated in estrogen primed rats by the administration of LHRH [13] or progesterone [19]. Evidence has already been presented here to show that a stressor inhibits LHRH release. Since LHRH is one of the hormones underlying lordosis, its blockage by UES would be expected to reduce lordosis.

The relationship between UES, progesterone levels and lordosis behavior is not so clear-cut. Stress has been found to produce increments in peripheral progesterone levels [27]. Further, progesterone is known to exert a biphasic influence on lordosis behavior, facilitating it at low doses and inhibiting it at higher doses [19]. Thus, an additional reason for the decrease in lordosis behavior observed following UES may be the elevation of circulating progesterone.

The fact that naloxone only partially reinstated lordosis behavior suggests that if inhibition of lordosis is caused by stress induced production of adrenal progesterone, then it is not mediated by a naloxone sensitive system.

ACTH is elevated following exposure to a stressor [7] and is known to result in progesterone release [21]. There is no evidence to suggest that naloxone reverses this effect, therefore, the fact that lordosis behavior was only partially reinstated by naloxone may be explained by an ACTH-induced progesterone release.

The findings of this study clearly implicate endogenous opioids in the disruption of the preovulatory LH surge and inhibition of ovulation observed following the exposure of cyclic female rats to acute UES. Little support was found for the implication of endogenous opioids in the suppressive effect of UES on lordosis behavior. The involvement of a non-naloxone sensitive process is implied.

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