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BRIEF COMMUNICATION

Dual Effect of Morphiceptin on Lordosis Behavior: Possible Mediation by Different Opioid Receptor Subtypes[1]

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PFAUS, J. G., N. PENDLETON AND B. B. GORZALKA. *Dual effect of morphiceptin on lordosis behavior: Possible mediation by different opioid receptor subtypes.* PHARMACOL BIOCHEM BEHAV **24**(5) 1461–1464, 1986.—Intracerebroventricular (ICV) infusions of the selective μ receptor agonist morphiceptin produce a dual effect on lordosis behavior in ovariectomized, steroid-primed rats. Low doses of morphiceptin (20 ng) inhibit lordosis whereas higher doses (2000 ng) facilitate this behavior. The present experiment tested whether naloxone, an antagonist of both high- and low-affinity μ receptors, or the long-acting high-affinity μ receptor antagonist naloxazone could block the dual effect of morphiceptin on lordosis. Ovariectomized rats primed with estrogen and progesterone received naloxone, naloxazone, or a control solution prior to ICV infusions of either 0, 20, or 2000 ng of morphiceptin. Naloxone reversed both the inhibition and facilitation of lordosis produced by morphiceptin, but had no effect on lordosis when administered before control infusions. In contrast, naloxazone reversed the inhibition but not the facilitation of lordosis. These results indicate that the inhibitory effect of morphiceptin on lordosis reflects the activation of high-affinity μ receptors whereas the facilitatory effect reflects either the activation of low-affinity μ receptors or other opioid receptor subtypes. The failure of naloxone or naloxazone to affect lordosis in rats receiving control infusions of saline further suggests that endogenous opioid systems do not exert a tonic inhibitory or facilitatory action on lordosis behavior.

Morphiceptin Lordosis behavior Naloxone Naloxazone Opioid receptors

OPIOID agonists such as heroin, morphine, methadone, Met-enkephalinamide, and β-endorphin have been shown to inhibit sexual behavior in a variety of mammalian species [1, 5, 6, 8–11, 16, 18, 21]. Although an extensive literature exists on the neuroendocrinology of opioid administration [1], little is known concerning the opioid receptor mechanisms that may underlie the inhibition of sexual behavior. This lack of information is due, in part, to a lack of receptor specificity in the drugs tested. Moreover, these drugs possess very different relative affinities for individual opioid receptor subtypes. Heroin and morphine, for example, are 50 times more potent at μ receptors than at δ receptors [15]. β-endorphin, however displays approximately equal affinity for μ and δ receptors [15]. In light of this, the opioid receptor mechanisms that may be involved in the inhibition of sexual behavior by opioid agonists, especially β-endorphin, cannot be determined. A more appropriate analysis of opioid receptor mechanisms would require the use of highly selective agonists and antagonists.

Morphiceptin (NH_2-Tyr-Pro-Phe-Pro-$CONH_2$), an opioid tetrapeptide derived from the milk protein β-casein, has an affinity 1000 times greater for μ than δ or κ opioid receptors [3,4] and produces a long-term, naloxone-reversible analgesia in mice following central administrations [3, 12, 23]. Recently, we reported that infusions of morphiceptin to the lateral ventricles had a dual effect on lordosis behavior in ovariectomized rats primed with estrogen and progesterone [18]. A low dose of morphiceptin (20 ng) significantly inhibited lordosis up to 2 hr following infusion. Higher doses (200, 2000 ng), however, significantly facilitated lordosis over the same time course. Both of these effects were reversed with naloxone (10 mg/kg) injected 30 min before testing. Because

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morphiceptin has been shown to interact with both high- and low-affinity μ receptors [3, 20, 23], our results suggested that the inhibition of lordosis produced by the low dose of morphiceptin may be due to the activation of high-affinity (μ_1) sites, whereas the facilitation of lordosis produced by the higher doses may be due to the activation of low-affinity (μ_2) sites or other opioid receptor subtypes.

The long-acting μ_1 antagonist naloxazone [13,15] abolishes the competitive inhibition of opioid binding produced by nanomolar but not micromolar concentrations of morphiceptin *in vitro*, and blocks the analgesic activity of morphiceptin in mice following intracerebroventricular infusions [23]. In order to examine whether the dual effect of morphiceptin on lordosis is a function of the differential activation of μ receptor subtypes, we tested the ability of either naloxone or naloxazone to antagonize the inhibition or facilitation of lordosis produced by different doses of morphiceptin.

METHOD

Animals and Surgery

Female Sprague-Dawley rats were obtained from Charles River Canada Inc., Montreal, at 60 days of age. At approximately 100 days of age, these females were bilaterally ovariectomized and implanted with guide cannulae aimed at the right lateral ventricles under sodium pentobarbital anesthesia (Somnital, 40 mg/kg). Guide cannula placements were made according to the atlas of Pellegrino, Pellegrino, and Cushman [17]. Surgical procedures and guide cannula construction have been fully described elsewhere [7].

Following surgery, females were housed individually in standard wire-mesh cages in a room maintained on a reversed 12 hr light/12 hr dark cycle at 21±1°C. Females were allowed free access to food and water. The placement of cannulae was tested 1 week before the experiment by infusing 2 μg of angiotensin II into the right lateral ventricle of each rat. Only those females that displayed vigorous drinking within 5 min of infusion were used as subjects.

Drug Procedures

Estradiol benzoate (EB) and progesterone (P) (Steraloids) were dissolved in peanut oil and injected subcutaneously in 0.1 ml of the vehicle. Angiotensin II (Bachem) was dissolved in physiological saline at a concentration of 1 μg/μl. Morphiceptin (Bachem) was dissolved in physiological saline to obtain concentrations of 10 ng or 1000 ng/μl of solvent. Both doses of morphiceptin were infused in 2 μl of solvent. Control rats received an equivolume infusion of physiological saline. All central infusions were made with an electrically-driven infusion pump (Sage Instruments Model 3H1A) at a rate of 5 μl/min. Naloxone hydrochloride (du-Pont) was dissolved in physiological saline and glacial acetic acid (0.0007 M) at a concentration of 10 mg/ml. Naloxazone was synthesized according to the method of Pasternak and Hahn [14] and suspended in physiological saline with glacial acetic acid at a concentration of 10 mg/ml. Both naloxone hydrochloride and naloxazone were administered subcutaneously at a dose of 10 mg/kg.

Lordosis Testing

Lordosis testing involved the presentation of each experimental female to a sexually vigorous male rat in a 29×45 cm Plexiglas testing chamber filled with 4 cm of San-i-cel bedding. Each female was placed with a male until 20 mounts with pelvic thrusting had occurred. Lordosis quotients were calculated as the percentage of mounts with pelvic thrusting that resulted in a lordosis posture. A moderate degree of sexual receptivity was induced in each female by subcutaneous injections of 10 μg EB 48 hr and 250 μg P 4 hr before each test.

Experimental females were randomly assigned to 1 of 3 morphiceptin dose groups (n=8/group), each receiving either 0, 20, or 2000 ng of morphiceptin 1 hr before each test. Females in each dose group received injections of naloxone, naloxazone, or the control solution of weak acetic acid in saline in a latinized fashion. Injections of naloxone or the control solution were made 30 min before morphiceptin infusions. Naloxazone was injected 12 hr before morphiceptin infusions in order to decrease possible nonspecific effects by permitting the elimination of unbound antagonist [12]. Lordosis testing occurred at weekly intervals during the middle third of the dark cycle in a room dimly lit by red lights.

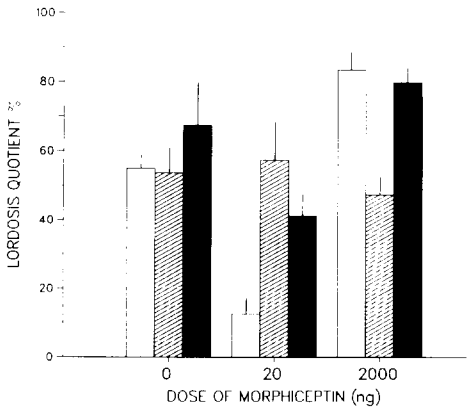

FIG. 1. Effect of naloxone, naloxazone, or a control solution (weak acetic acid in saline) on lordosis behavior in steroid-primed female rats given infusions of morphiceptin to the lateral ventricles. Bars represent means±standard errors. Open bars: saline; hatched bars: naloxone (10 mg/kg); solid bars: naloxazone (10 mg/kg).

RESULTS

Following histological verification of cannula placements, data were analyzed using a 3×3 analysis of variance for dependent measures. Results are shown in Fig. 1.

A significant main effect was detected for dose of morphiceptin, $F(2,21)=14.94$, $p<0.0001$. There were no main effects of antagonist administration or testing order. However, a significant interaction was found between dose of morphiceptin and antagonist administration, $F(4,21)=7.38$, $p<0.0002$. Subsequent use of the Newman-Keuls method of post-hoc comparisons revealed that the low dose of morphiceptin (20 ng) significantly inhibited lordosis ($p<0.01$) whereas the higher dose (2000 ng) significantly facilitated this behavior ($p<0.01$). Naloxone significantly reversed both the inhibition and facilitation of lordosis produced by morphiceptin ($p<0.01$), but had no effect on lordosis when injected before control infusions of saline. In contrast, naloxazone significantly reversed the inhibition ($p<0.05$) but not the facilitation of lordosis. Although naloxone appeared

more effective than naloxazone in preventing the inhibition produced by 20 ng morphiceptin, this difference was not statistically significant. Likewise, although naloxazone appeared to facilitate lordosis behavior in the absence of morphiceptin, this effect did not reach statistical significance.

DISCUSSION

Consistent with our previous findings [18], infusions of morphiceptin to the lateral ventricles produced a dual effect on lordosis behavior. The low dose of morphiceptin (20 ng) inhibited whereas the higher dose (2000 ng) facilitated lordosis. The ability of naloxazone to reverse the inhibitory but not the facilitatory effect of morphiceptin indicates that the inhibition of lordosis produced by morphiceptin is due to the activation of μ_1 receptors. Although the present experiment did not conclusively identify the opioid receptor subtype involved in the facilitation of lordosis by higher doses of morphiceptin, the reversal of this effect with naloxone suggests that it may be due to the activation of μ_2 sites, as naloxone is approximately 10 times as potent at both μ_1 and μ_2 receptors than δ receptors [15]. In addition, morphiceptin possesses a 250-fold greater potency for μ_2 binding compared to δ binding [23]. However, we have previously shown that the relatively selective activation of δ receptors with δ-receptor peptide produces a dose-dependent and naloxone-reversible facilitation of lordosis in rats given an identical steroid priming regimen [18]. Because morphiceptin has a lower affinity for δ receptors relative to μ_1 and μ_2 receptors, we cannot rule out the possibility that higher doses of morphiceptin facilitate lordosis by an action on δ receptors.

Recent evidence suggests that agonist activity at μ receptors reduces the number of δ receptors in rat brain [19]. This is presumed to occur as a function of allosteric inhibition, in which agonist activity at μ receptors inhibits the conformational expression of the δ receptor. Likewise, the high- and low-affinity conformations of the μ receptor also appear to be allosterically coupled, although the relationship between these receptor subtypes has not been determined. The activation of μ_2 or δ sites with highly selective agonists, however, produces a number of similar effects, such as respiratory depression and increased dopamine turnover in the striatum [13,22]. This latter effect is of interest because higher affinity binding of morphine to μ receptors in the striatum decreases dopamine turnover [22]. If the same opioid receptor interactions apply to the central regulation of lordosis behavior, then agonist activity at μ_2 or δ receptors may serve a similar facilitatory role and may suppress the inhibition of lordosis produced by agonist activity at μ_1 receptors through an allosteric mechanism.

Finally, it has been suggested that endogenous opioid systems exert a tonic inhibitory influence on lordosis behavior in female rats [2]. In that study, subcutaneous administrations of the long-acting opioid antagonist naltrexone significantly increased the lordosis quotients of ovariectomized, estrogen-primed rats 3–4 hr after injection. Adrenalectomy did not reverse this effect, nor did the administration of the protein synthesis inhibitor anisomycin 15 min before naltrexone. This led the authors to suggest that the facilitation of lordosis by naltrexone was not dependent upon the presence of progesterone, although naltrexone was not tested in rats receiving both estrogen and progesterone. In the present experiment, however, the failure of naloxone or naloxazone to affect the lordosis behavior of rats receiving control infusions of saline indicates that endogenous opioid systems do not exert a tonic action on lordosis in ovariectomized rats primed with estrogen and progesterone. These results are consistent with previous reports indicating that peripherally-administered naloxone has no effect on lordosis behavior [21].

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