Pergamon

Pharmacology Biochemistry and Behavior, Vol. 47, No. 4, pp. 771-778, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)E0032-D

Enkephalinase Inhibition Facilitates Sexual Behavior in the Male Rat but Does Not Produce Conditioned Place Preference

ANDERS ÅGMO,[1] MONICA GOMEZ AND YANIRA IRAZABAL

Department of Psychology, Universidad Anahuac, Mexico City, Mexico

Received 7 December 1992

ÅGMO, A., M. GOMEZ AND Y. IRAZABAL. *Enkephalinase inhibition facilitates sexual behavior in the male rat but does not produce conditioned place preference.* PHARMACOL BIOCHEM BEHAV 47(4) 771-778, 1994.—The effects of two enkephalinase inhibitors, SCH 34826 and phospho-leu-phe, on male rat sexual behavior and conditioned place preference were evaluated. SCH 34826, administered intraperitoneally, reduced the ejaculation latency to both the first and second ejaculation at a dose of 30 mg/kg. This dose also reduced the first postejaculatory interval. No other effect was obtained with this drug. Phospho-leu-phe, administered intracerebroventricularly, increased mount and intromission latency at doses of 50 and 100 μg. A dose of 25 μg reduced the latency to the first ejaculation as well as the number of preejaculatory intromissions. The postejaculatory interval was also reduced at this dose. SCH 34826, 100 and 30 mg/kg, and phospho-leu-phe, 25 μg, had no effect in the conditioned place preference procedure. These observations seem to suggest that there is no functionally relevant tonic release of enkephalins. Therefore, the effects obtained on sexual behavior may indicate that enkephalins are released before and during the course of sexual activity. The function of such a release could be to facilitate ejaculatory mechanisms in the way found in the present studies. Previous work has shown that ejaculation-induced reward is opioid dependent, further supporting the hypothesis of opioid release during sexual activity. Taken together, these data suggest an important role for opioids, probably enkephalins, in the physiological control of sexual behavior.

Sexual behavior Enkephalins Place preference

SEVERAL studies have shown that systemic administration of morphine inhibits sexual behavior in the male rat (4,28,34). Administration of β-endorphin or d-ala^2-met^5-enkephalinamide (DALA) into a lateral ventricle also inhibits male sexual behavior (29,37). However, it has been shown that ejaculation is facilitated in the small proportion of rats that copulate after systemic morphine (4). This facilitation is shown as reduced ejaculation latency and number of preejaculatory intromissions. Further, an injection of DALA into the lateral ventricle immediatey after the first intromission has similar effects (4). Because the second ejaculation, in the rat, is achieved after fewer intromissions and with a shorter latency than the first [reviewed in (24)], and because morphine and DALA produce exactly these effects, it was proposed that endogenous opioids were released during the course of sexual activity (4). Such a release could have two effects. First, facilitate subsequent sexual behavior, and second, afford the rewarding properties to ejaculation.

Ejaculation-induced reward is blocked by naloxone (1), suggesting that release of endogenous opioid peptides, indeed, is important for that reward. Moreover, ejaculation produces hypoalgesia (42) that is reversible by naloxone (18). There is, thus, substantial indirect evidence suggesting that opioid peptides are released during sexual activity. It is not known, however, where such a release may be located. Plasma concentration of β-endorphin appears to be elevated by sexual activity (35), and it has been reported that prolonged copulation reduces midbrain endorphin concentration (42). It is unlikely that enhanced plasma concentration of opioids leads to facilitated sexual behavior. A recent study showed that the inhibi-

[1] Requests for reprints should be addressed to Anders Ågmo, Laboratoire de Psychophysiologie, Faculté des Sciences, Parc de Grandmont, 37200 Tours, France.

tory effects of opiates on this behavior are caused by activation of peripheral opioid receptors (5). When these receptors were blocked by methylnaloxone, systemic morphine facilitated sexual behavior. The location of the facilitatory effects within the brain is not known, but possible sites include the medial preoptic area (see below), the nucleus accumbens (10), and the ventral tegmental area (32).

At the moment, there are no data allowing for a hypothesis as to which opioid peptide is released during sexual behavior. Both morphine, dynorphin(1-13) (10), and Met-enkephalin (40) facilitate ejaculation when infused into the medial preoptic area in low doses. However, the specific μ receptor agonist morphiceptin inhibits sexual behavior (27) as does β-endorphin (22). It is, therefore, unlikely that endogenous μ receptor ligands participate in facilitation of sexual behavior. On the other hand, opioid kappa agonists do not have rewarding effects (33), and it is, therefore, not probable that the κ agonist dynorphin is involved in sexual reward. Available data suggest that Met-enkephalin is the most likely candidate for having these two effects. Besides its facilitatory action on ejaculatory mechanisms, infusion of this peptide into the preoptic area produces reward, as manifested in the conditioned place preference paradigm (2).

If Met-enkephalin is released during sexual activity, then an enkephalinase inhibitor should reinforce the effect of such release. Consequently, ejaculation should be facilitated in rats given an enkephalinase inhibitor. One purpose of the present experiments was to evaluate this hypothesis. To that end, two enkephalinase inhibitors were administered to male rats. SCH 34826 is an enkephalinase inhibitor active after systemic administration. Its enkephalinase inhibiting properties appear to be due to its metabolite, SCH 32615. This latter compound is about one order of magnitude less potent than thiorphan. It has been reported that SCH 34826 does not inhibit aminopeptidase, diaminopeptidase, or angiotensin converting enzyme (13). The effects of the drug, in a variety of assays, are blocked by naloxone, suggesting that its actions are specific to opioid systems (8,12,13). Phosphoryl-leu-phe is another enkephalinase inhibitor, about one order of magnitude more potent than thiorphan (9). This compound has been shown to potentiate the analgesic actions of DALA after intracerebroventricular administration (7).

Even if the enkephalinase inhibitors would facilitate sexual behavior, this would not constitute evidence for enkephalin release during sexual activity unless it is demonstrated that these agents are ineffective in the absence of such activity. Enkephalins may be tonically released, and administration of enkephalinase inhibitors could, therefore, enhance enkephalin concentrations and, hence, produce a pharmacological facilitation of sexual behavior. To control for this possibility, an additional experiment was performed.

Opioids, including Met-enkephalin, readily produce conditioned place preference [(43) and references therein]. Thus, if enkephalins were tonically released in functionally relevant amounts, enkephalinase inhibition should produce conditioned place preference. SCH 34826 and phospho-leu-phe were, therefore, administered to rats subjected to place preference conditioning in the absence of sexual activity.

METHOD

Subjects

Male Wistar rats (350–450 g) from a local colony were used in all experiments. They were housed, two per cage, in a room with a controlled 12 L : 12 D (lights off 0900) and given commercial rat pellets and tap water ad lib. Females (Wistar, 200–300 g) used in tests for sexual behavior were ovariectomized under Brevital (40 mg/kg) anesthesia at least 2 weeks before experiments. They were subcutaneously injected with estradiol benzoate (Sigma), 25 μg/rat, 48–56 h before use, and with progesterone (Sigma), 1 mg/rat, 4–8 h before. Both steroids were dissolved in corn oil and the volume of injection was 0.2 ml/rat. Only males that had ejaculated at each of three pretests performed as described below were included in the experiments. After the pretests, they were castrated under Brevital anesthesia and subcutaneously implanted with a 20 mm long testosterone (Sigma)-filled Silastic capsule (o.d. 0.125 in.; i.d. 0.062 in.; Dow-Corning). This implant maintains relatively constant and physiological plasma testosterone concentrations and sexual behavior for several months (15,21).

Enhanced opioidergic neurotransmission inhibits pituitary LH release and thereby causes reduced plasma testosterone concentrations [reviewed in (30)]. Because even rapid and short-lasting changes in this concentration may modify sexual behavior (26), it was considered important to supply the subjects with a constant release androgen source. In that way, possible behavioral effects of enkephalinase inhibition cannot be attributed to actions on androgen secretion.

Those males that were to be treated with phospho-leu-phe were implanted with a stainless steel guide cannula (21 gauge) in the left cerebral ventricle using standard stereotaxic techniques (coordinates: 0.1 mm anterior to bregma, 1.5 mm lateral to the midline, and 4.0 mm below the dura matter. The head was inclined so that lambda was 1.0 mm lower than bregma). Brevital anesthesia (40 mg/kg) was used. The subjects were allowed to recover for 1 week, and drug treatment was then initiated. After the end of the experiments, 5 μl methylene blue was injected through the cannula, the subject killed by an overdose of anesthesia and the brain removed. It was immediately cut and examined under a dissection microscope to verify correct cannula placement. Only animals with a wide distribution of blue within the ventricles were included in the statistical analysis.

Behavioral Testing Procedures

Sexual behavior was recorded as described previously (4). The following parameters were registered: Mount and intromission latency (time from introduction of the male into the mating test cage until the first mount and intromission, respectively), ejaculation latency (time from the first intromission in a series until the following ejaculation), postejaculatory interval (time from ejaculation until the next intromission), number of preejaculatory mounts and intromissions. Tests were ended at the end of the second postejaculatory interval or 30 min after introduction of the male without intromission or 30 min after the first intromission in a series without ejaculation or when a postejaculatory interval was longer than 30 min.

The place preference conditioning procedure has also been described in detail previously (1). Briefly, three-compartment cages were used. The lateral compartments offered distinct stimuli (odor, color, and floor texture). After a pretest where the time spent in each lateral compartment was recorded, animals were reinforced in the nonpreferred compartment. Three reinforced and three nonreinforced sessions were given to each subject. Daily sessions were performed Monday to Friday. Twenty-four hours after the last conditioning session, the test was performed. The pretest and the test lasted 10 min, and the conditioning sessions lasted 30 min. Two basic variables were used to quantify place preference: The time spent in the

reinforced compartment and the preference score [time spent in the reinforced compartment/(time spent in the reinforced compartment + time spent in the nonreinforced compartment)]. To consider a place preference, both variables should show a significant change between pretest and test. These variables are the most commonly used in place preference studies (41). In addition, the time spent in the nonreinforced and neutral compartments was also analyzed. This would illustrate how the subjects distributed their time between the three compartments, and provide further information as to the effects of the drugs.

All behavioral tests were performed between the 5th and the 8th h of the dark phase of the light/dark cycle under dim white light.

Drugs

Morphine HCl (Ministry of Health, Mexico) was dissolved in physiological saline and injected intraperitoneally (IP) at a dose of 1 mg/kg in a volume of 1 ml/kg b.wt. 1 min before the experimental session. SCH 34826 [(S)-N-N-1-(2,2-dimethyl-1,3-dioxolan-4yl) methoxy carbonyl-2-phenylethyl-l-phenylalanine-β-alanine) (a gift from Dr. A. Barnett, Schering-Plough Corporation, Bloomfield, NJ) was suspended in physiological saline to which two drops of Tween 80 had been added. This drug was injected IP in a volume of 2 ml/kg b.wt. 30 min before behavioral observation. Doses of 10, 30, and 100 mg/kg were used. Control treatment consisted of an equivalent volume of saline with Tween 80.

Phospo-leu-phe (phosphoryl-l-leucyl-l-phenylalanine KCl) was generously provided by Dr. S. Blumberg, Department of Biophysics, The Weizmann Institute, Rehovot, Israel. The dipeptide was dissolved in physiological saline and infused into the lateral ventricle in a volume of 5 μl over 2 min. The infusion cannula (27 gauge) protruded 0.5 mm beyond the tip of the guide cannula. After the end of infusion, the cannula was left in place for 1 min and then replaced by a flush dummy cannula. During infusion, the animal was freely moving in its home cage. Immediately after the end of the infusion procedure, the experiment was begun. Doses of 25, 50, and 100 μg were used. Control treatment consisted of an identical infusion of physiological saline.

Design

In experiments on sexual behavior, both drugs were administered according to a Latin square design in such a way that all subjects received all doses plus vehicle. At each session, an equal number of subjects received each dose. The interval between drug treatments was 48 h for animals given phospho-leu-phe and 1 week for those given SCH 34826. Twelve animals were used in the experiment with SCH 34826 and eight animals in that with phospho-leu-phe.

Two place preference experiments were performed. In the first one, seven animals were treated with SCH 34826 at a dose of 100 mg/kg before each reinforced session and vehicle before the nonreinforced sessions. For camparison, another group of 10 animals received morphine, 1 mg/kg, before each reinforced session.

At a later date, three more groups were subjected to place preference conditioning. One group of eight animals was injected with saline before both reinforced and nonreinforced sessions (control), the second and third groups were treated with SCH 34826, 30 mg/kg (n = 8), or phospho-leu-phe, 25 μg (n = 7), before the reinforced sessions. These doses were chosen because they affected sexual behavior.

Statistical Analysis

Only animals that ejaculated twice after all treatments were included in the analyses of the parameters of sexual behavior. Mount and intromission latencies were analyzed with a one-factor ANOVA for repeated measures. All other parameters were evaluated with a two-factor ANOVA for repeated measures on both factors. The factors were drug dose and ejaculation (first vs. second). Simple main effects were always calculated for each dose in order to avoid confounding interactions. In case of significant effect of dose, the Neuman-Keul's procedure was used for a posteriori comparisons between doses.

Each place preference experiment was evaluated with two-factor ANOVAs for repeated measures on one factor. The factors were pretest-test and treatment.

RESULTS

Sexual Behavior

SCH 34826. Of the 12 animals treated with the drug, 9 achieved ejaculations after all treatments. Two animals failed to initiate sexual behavior after a dose of 50 mg/kg and three failed after 100 mg/kg.

The drug had no effect on mount or intromission latencies, $F(3, 24) = 1.95$, NS; $F(3, 24) = 2.40$, NS, respectively. There was a significant effect of dose on ejaculation latency, $F(3, 24) = 4.31$, $p < 0.05$, and postejaculatory interval, $F(3, 24) = 4.02$, $p < 0.05$, but not on preejaculatory intromissions, $F(3, 24) = 2.18$, NS, in the first series. In the second series, SCH 34826 reduced the ejaculation latency, $F(3, 24) = 6.47$, $p < 0.01$, without affecting other parameters (all $p > 0.1$). A posteriori comparisons showed that a dose of 30 mg/kg reduced the ejaculation latency to both the first and second ejaculation, while the postejaculatory interval was reduced after the first ejaculation only. The other doses had no effects. Data are shown in Fig. 1.

When the copulatory series were compared, it was found that the latency to the second ejaculation was reduced after all treatments except 100 mg/kg. The number of preejaculatory intromissions was reduced and the postejaculatory interval was prolonged in the second series after all treatments.

Phospho-leu-phe. Of the eight animals with correct cannula placement, only six achieved two ejaculations after all treatments. Two subjects failed to initiate sexual behavior after a dose of 100 μg.

There was a significant effect on mount and intromission latencies, $F(3, 15) = 4.62$, $p < 0.05$; $F(3, 15) = 4.35$, $p < 0.05$, respectively. A posteriori comparisons showed that the mount latency was longer after 100 μg than after vehicle, while the intromission latency was prolonged both after 50 and 100 μg.

There was a significant effect of dose on ejaculation latency, $F(3, 15) = 6.69$, $p < 0.01$, number of preejaculatory intromissions, $F(3, 15) = 8.70$, $p < 0.01$, and postejaculatory interval, $F(3, 15) = 4.38$, $p < 0.05$, in the first copulatory series. A posteriori comparisons showed that 25 μg reduced the ejaculation latency, the number of preejaculatory intromissions, and the postejaculatory interval. Higher doses had no effect on these parameters. In the second copulatory series, only the postejaculatory interval was reduced by the enkephalinase inhibitor, $F(3, 15) = 4.48$, $p < 0.05$.

When the second ejaculation was compared with the first, it was found that animals treated with saline ejaculated with a shorter latency, $F(1, 5) = 16.19$, $p < 0.05$, made fewer preejaculatory intromissions, $F(1, 5) = 35.59$, $p < 0.005$, and

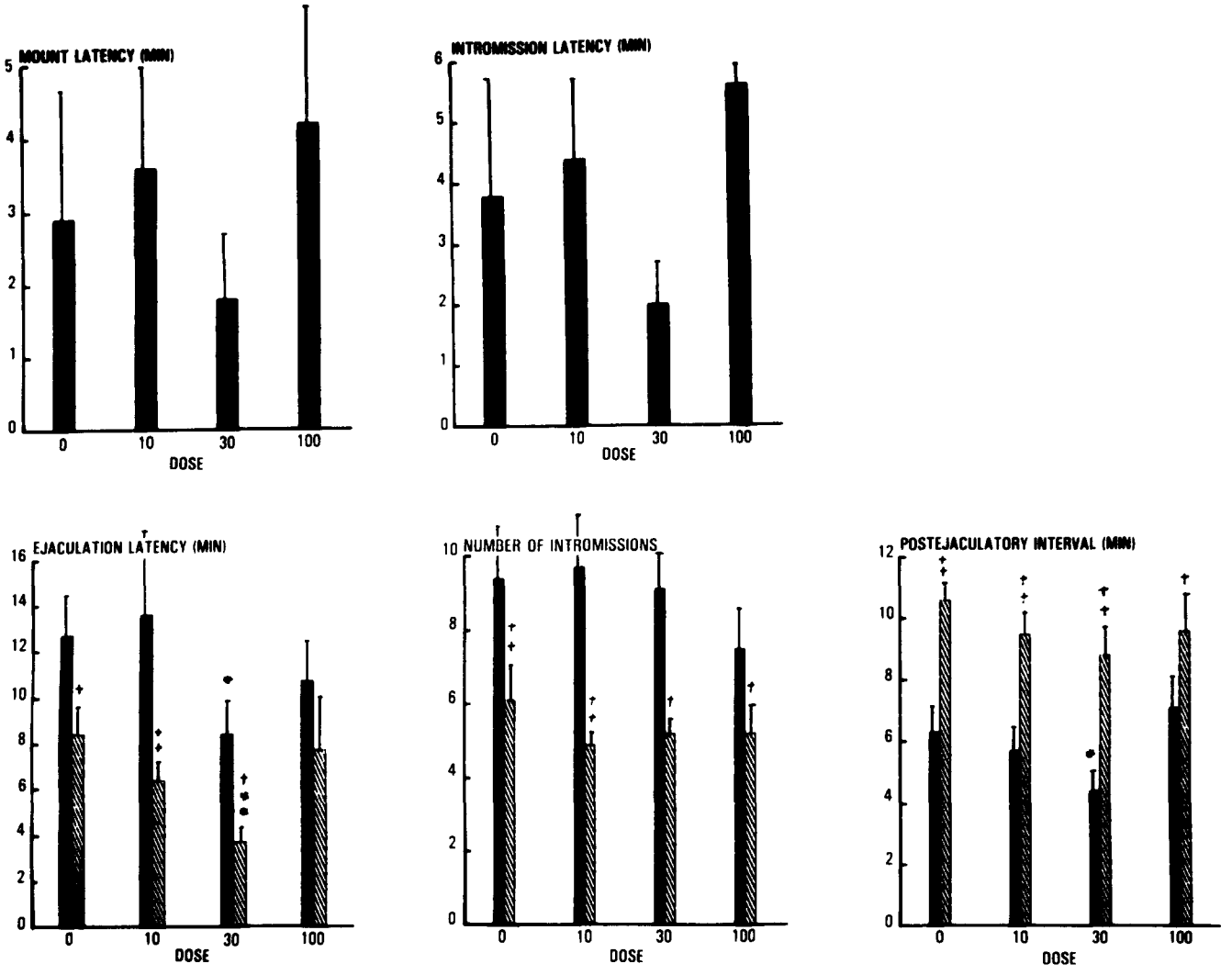

FIG. 1. Parameters of sexual behavior in male rats treated with varying doses of the enkephalinase inhibitor SCH 34826. Data are mean + SE. Doses in mg/kg (n = 9). Black bars, first copulatory series; striped bars, second copulatory series. *Different from saline in the same series, $p < 0.05$; **$p < 0.01$. $^{+}$Different from the same treatment in the first series, $p < 0.05$; $^{++}p < 0.01$.

had longer postejaculatory intervals, $F(1, 5) = 9.13$, $p < 0.05$, in the second series than in the first. There were no differences between copulatory series for animals trated with 25 μg with regard to ejaculation latency and preejaculatory intromissions ($p > 0.1$), while the postejaculatory interval was increased, $F(1, 5) = 7.35$, $p < 0.05$. Animals treated with 50 or 100 μg did not show reduced ejaculation latency to the second ejaculation in comparison to the first ($p > 0.1$), but the number of preejaculatory intromissions was reduced, $F(1, 5) = 14.02$, $p < 0.05$, and $F(1, 5) = 24.41$, $p < 0.01$, respectively. The postejaculatory interval increased between series in animals treated with 100 μg, $F(1, 5) = 6.98$, $p < 0.05$, but not in those treated with 50 μg, $F(1, 5) = 4.32$, NS. Data are shown in Fig. 2.

Conditioned Place Preference

Data are shown in Table 1. Upon analysis of the time spent in the reinforced compartment in the experiment where the effect of SCH 34826, 100 mg/kg, was compared to that of morphine, 1 mg/kg, a significant difference between groups was obtained, $F(1, 14) = 7.12$, $p < 0.05$. There was also a significant difference between pretest-test, $F(1, 14) = 9.40$, $p < 0.01$, while the group × pretest-test interaction was non-significant, $F(1, 14) = 3.82$, NS. Tests for simple main effects showed that there was no difference between the groups at the pretest, $F(1, 14) = 3.50$, NS. At the test, however, a group difference was obtained, $F(1, 14) = 9.18$, $p < 0.01$. Furthermore, there was a significant difference between pretest and test in the group treated with morphine, $F(1, 14) = 14.40$, $p < 0.01$. No difference was found in the group treated with SCH 34826, $F(1, 14) = 0.55$, NS.

When analyzing the preference score, significant effects of group, $F(1, 14) = 7.05$, $p < 0.05$, pretest-test, $F(1, 14) = 17.88$, $p < 0.001$, and an interaction group × pretest-test, $F(1, 14) = 7.38$, $p < 0.05$, were found. Tests for simple main effects showed that the groups were not different at the pretest, $F(1, 14) = 4.10$, NS, while the preference score was sig-

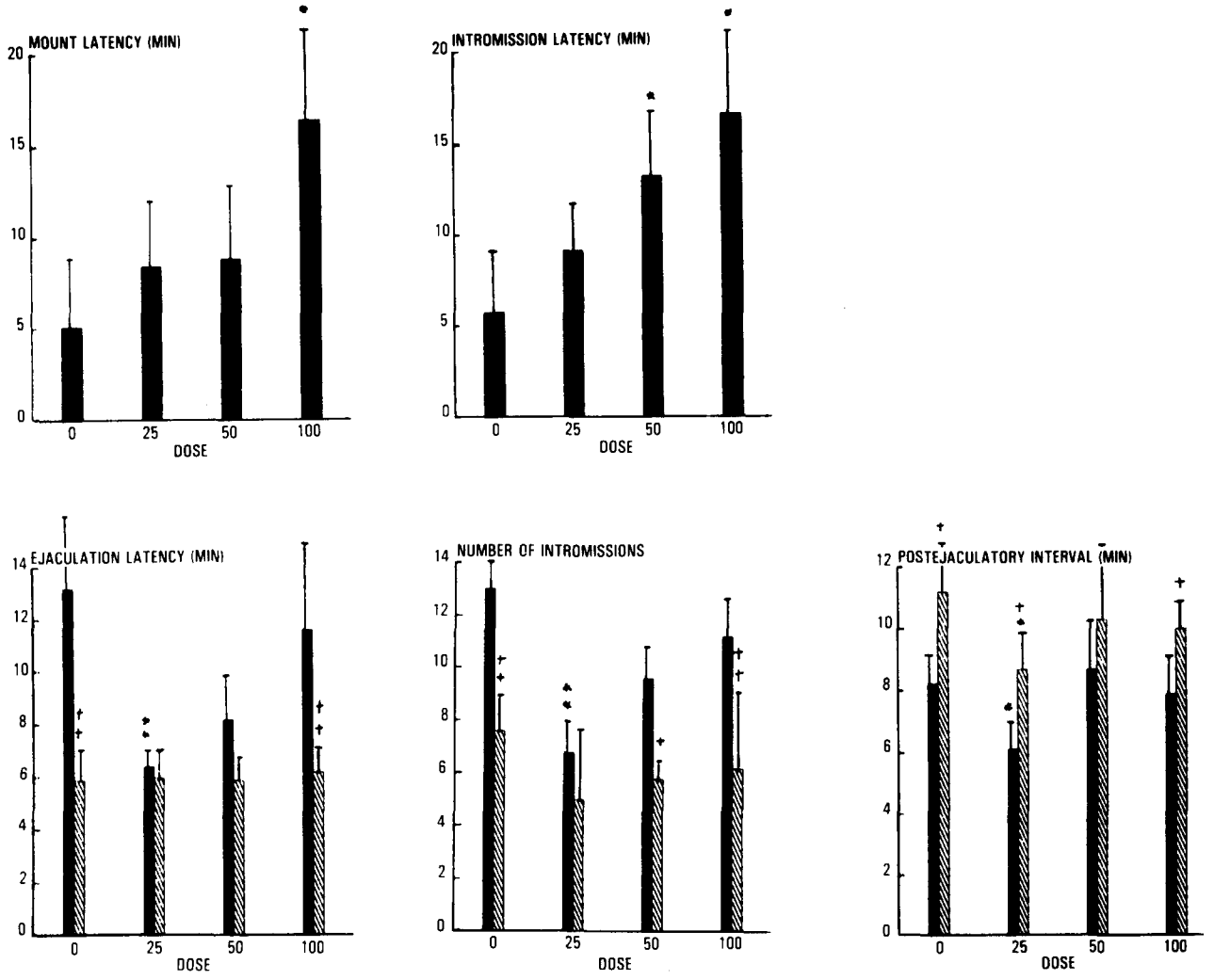

FIG. 2. Effects of the enkephalinase inhibitor phospho-leu-phe on sexual behavior in male rats. Data are mean + SE. Doses in μg (n = 6). Black bars, first copulatory series; striped bars, second copulatory series. *Different from saline in the same series, $p < 0.05$; **$p <$ 0.01.

TABLE 1

PLACE PREFERENCE DATA FROM ONE EXPERIMENT WHERE THE EFFECTS OF MORPHINE WERE COMPARED TO THOSE OF SCH 34826 AND FROM ANOTHER EXPERIMENT WHERE THE EFFECTS OF SALINE, SCH 34826, AND PHOSPHO-LEU-PHE, WERE EVALUATED

	Time in Reinforced Compartment		Time in Nonreinforced Compartment		Time in Neutral Compartment		Preference Score	
Treatment	Pretest	Test	Pretest	Test	Pretest	Test	Pretest	Test
Morphine 1 mg/kg	100 ± 29	158 ± 26*	336 ± 50	209 ± 36†	164 ± 32	232 ± 16*	0.25 ± 0.06	0.45 ± 0.08†
SCH 34826 100 mg/kg	42 ± 15	55 ± 19	457 ± 46	424 ± 56	100 ± 31	120 ± 38	0.10 ± 0.04	0.14 ± 0.05
Saline	93 ± 16	82 ± 16	315 ± 40	349 ± 40	192 ± 30	168 ± 30	0.24 ± 0.05	0.21 ± 0.25
SCH 34826 30 mg/kg	93 ± 12	103 ± 23	330 ± 40	301 ± 51	177 ± 30	196 ± 32	0.24 ± 0.04	0.24 ± 0.08
Phospho-leu-phe 25 μg	118 ± 20	125 ± 20	286 ± 35	286 ± 45	195 ± 22	188 ± 34	0.30 ± 0.05	0.33 ± 0.06

Data (in s) are mean ± SE n = 9 (morphine 1 mg/kg); 7 (SCH 34826, 100 mg/kg); 8 (saline and SCH 34826, 30 mg/kg); 7 (phospho-leu-phe). *†Different from pretest, *$p < 0.01$; †$p < 0.001$.

nificantly different at the test, $F(1, 14) = 8.73$, $p < 0.01$. When the pretest was compared with the test in the morphine-treated animals, a significant effect was found, $F(1, 14) = 27.56$, $p < 0.001$. No effect was found in the group treated with SCH 34826, $F(1, 14) = 1.02$, NS.

ANOVA of the time spent in the nonreinforced compartment showed a significant effect of group, $F(1, 14) = 6.82$, $p < 0.05$, pretest-test, $F(1, 14) = 19.87$, $p < 0.001$, and of the interaction group × pretest-test, $F(1, 14) = 6.86$, $p < 0.05$. Test for simple main effects of groups at the pretest revealed no difference, $F(1, 14) = 3.02$, NS. At the test, however, the groups differed, $F(1, 14) = 11.39$, $p < 0.01$. There was a significant reduction in the time spent in the nonreinforced compartment between pretest and test in the group treated with morphine, $F(1, 14) = 28.61$, $p < 0.001$, but not in the group treated with SCH 34826, $F(1, 14) = 1.50$, NS. There was also a significant difference between groups with regard to the time spent in the neutral compartment, $F(1, 14) = 5.32$, $p < 0.05$. A difference was also obtained between preetest and test, $F(1, 14) = 7.38$, $p < 0.05$, while the interaction group × pretest-test was nonsignificant, $F(1, 14) = 2.21$, NS.Tests for simple main effects revealed that the groups did not differ at the pretest, $F(1, 14) = 1.95$, NS. A significant difference was obtained at the test, $F(1, 14) = 9.25$, $p < 0.01$. Furthermore, the group treated with morphine increased the time spent in the neutral compartment between pretest and test, $F(1, 14) = 10.09$, $p < 0.01$. No effect was found in the group given SCH 34826, 100 mg/kg, $F(1, 14) = 1.38$, NS. When the reinforcing properties of SCH 34826, 30 mg/kg, and phospho-leuphe, 25 μg, were evaluated, no effect was obtained neither on time spent in the reinforced compartment nor on preference score (all $p > 0.3$). Similarly, the enkephalinase inhibitors did not have effect on the time spent in the nonreinforced or neutral compartments (all $p > 0.4$).

DISCUSSION

Enkephalinase inhibition had several effects upon male sexual behavior. Phospho-leu-phe not only facilitated the first ejaculation at a low dose, but also reduced the postejaculatory interval after both ejaculations at this same dose. Higher doses delayed the initiation of sexual behavior without affecting ejaculatory mechanisms. On the other hand, SCH 34826, at a dose of 30 mg/kg, reduced the ejaculation latency to both the first and second ejaculation as well as the postejaculatory interval after the first. However, this drug did not affect the number of preejaculatory intromissions, nor did a high dose delay the initiation of sexual behavior.

To explain some of the observed effects it seems necessary to suppose either that enkephalins are tonically released, independently of sexual activity, or that they are released before the onset of sexual behavior as a consequence of stimuli related to it. The fact that mount latency was prolonged makes it obligatory to consider a precopulatory release. The results of the place preference experiments do not support a tonic release at brain sites related to opioid effects on reward mechanisms. Some of the structures where opioid reward is reliably produced are the ventral tegmental area and the nucleus accumbens [reviewed in (11,43)]. Recently it has been shown that Met-enkephalin infused into the medial preoptic area also produces conditioned place preference (2), suggesting that this site may be involved in opiate reward. The nucleus accumbens, ventral tegmental area, and the medial preoptic area are brain structures that have been shown to mediate opioid effects on male sexual behavior (10,22,32,40). Because there is at least a partial overlap between sites mediating opioid reward and opioid effects on male sex behavior, a tonic release that could have modified sexual behavior should also have produced conditioned place preference. In the absence of this latter effect it is, therefore, unlikely that there exists a tonic enkephalin release at relevant brain sites.

It could be maintained that the lack of effect of enkephalinase inhibition on place preference was due to inadequate doses. However, it appears that place preference can be produced by lower doses of morphine and DALA than those required to modify sexual behavior [see (2,4) and present data with morphine]. Further, doses of opioids sufficiently large to drastically inhibit sexual behavior continues to cause place preference (5). It seems, then, that place preference can be obtained within a larger range of doses than that affecting sexual behavior. Because of that, it is rather unlikely that doses of the enkephalinase inhibitors higher or lower than those used here would produce place preference.

In this context, it is important to note that enkephalinase does not appear to act on intraneuronal peptide storage sites (44). Rather, the enzyme seems to be located on synaptic membranes (16) and directed into the synaptic cleft (17,25). In consequence, only synaptically released enkephalin is subject to the action of enkephalinase. Thus, in the absence of synaptic release, enkephalinase inhibition would be ineffective (8). One study has reported that infusions of thiorphan into the ventral tegmental area produces place preference (19). The dose employed was high (60 μg), and the vehicle (5% sodium bicarbonate) could have tissue effects by itself. Curiously, the controls were given physiological saline. Moreover, thiorphan is not specific for enkephalinase (14). It is, therefore, not certain that the reinforcement observed was due to enhanced enkephalinergic acitivity. Finally, in a review of the literature it was concluded that the evidence for functionally relevant tonic release of enkephalins is slight (6).

If the observed effects on sexual behavior are not a consequence of tonically released enkephalins, then their release must be activated by events preceding sexual activity. There is indirect evidence that this may be the case. Dopamine release in the nucleus accumbens is enhanced by exposure to a receptive female in a situation where sexual interaction is not possible (39), and exposure to bedding material from cages housing receptive females enhances dopamine release (31). This latter effect is blocked by administration of naloxone, suggesting that opioid release activates dopamine neurons innervating the nucleus accumbens. It has previously been shown that dopamine release induced by foot shock can be attenuated by infusion of methylnaloxone into the ventral tegmental area (23). There is, thus, evidence that activation of opioid systems precedes or is causative of dopamine release. Because dopamine is released before the initiation of sexual activity, but as a response to sexually relevant stimuli, it is possible that opioids are released at the same time. It is tentatively suggested that the effects of enkephalinase inhibition on mount and intromission latencies are a consequence of such release. Indeed, the effects observed on these latencies with phospho-leu-phe are quite similar to those obtained after an infusion of the μ agonist morphiceptin into the preoptic area (27). Although enkephalins preferentially bind to the δ receptor, they have considerable affinity for μ receptors (20). Prolonged mount and intromission latencies were only obtained with doses higher than those required to facilitate ejaculatory mechanisms, and were not observed after treatment with SCH 34826. This makes it feasable to propose

that the primary effect of enkephalinase inhibitors is to facilitate ejaculation. Perhaps that the delayed onset of sexual behavior is due to an unphysiological accumulation of opioid peptides.

It may be noted that no consistent dose-effect relationship could be obtained, except for the effects of phospho-leu-phe on mount and intromission latencies. This is in agreement with previous studies where it was found that morphine, enkephalin, and dynorphin(1-13) facilitated ejaculatory mechanisms at some doses but were inhibitory or lacked effect at higher doses (10,40). There is, at the moment, no unambiguous explanation for these phenomena.

The fact that phospho-leu-phe had several effects that SCH 34826 did not have is not readily explained. However, it is possible that the former compound produced a larger inhibition of enkephalinase because of the route of administration and of its higher potency. Nevertheless, two effects were in common for the two drugs, reduced ejaculation latency and postejaculatory interval. These effects have previously been obtained with intraventricular infusion of DALA (4). Taken together, these data clearly suggest that release of endogenous opioid peptides, most likely enkephalins, facilitates ejaculatory mechanisms and enhances postejaculatory sexual motivation. Because sexual reward seems to be opioid dependent (1,3), enkephalinase inhibition could enhance the reward value of ejaculation. Enhanced reward may increase motivation to copulate, thereby reducing the postejaculatory interval exactly as observed in the present studies. There is one problem with the hypothesis that opioid release facilitates ejaculatory mechanisms. Naloxone has no reliable effect on the first copulatory series [(4) and references therein], while this drug has been reported to increase ejaculation latencies in subsequent series (38). One possibility to explain the unreliable effect of naloxone is that preejaculatory opioid release, in the absence of enkephalinase inhibition, is insufficient to modify ejaculatory mechanisms. It is also possible that opposing actions of opioids at different receptors (see the Introduction section), all blocked by naloxone, may obscure the effects of the antagonist.

There are some additional data suggesting that opioid release is associated with sexual behavior. Male rats subjected to a kindling procedure showed a prolonged postictal behavioral depression when stimulated shortly after ejaculation. The increase was blocked by naloxone and was specific to electrodes located within the medial preoptic area (36). These observations suggest that sexual activity is associated with a localized opioid release. Furthermore, sexual reward, as evaluated by the conditioned place preference procedure, can be blocked by infusion of methylnaloxone into the medial preoptic area but not in the nucleus accumbens (3). These data provide additional evidence for opioid release in the medial preoptic area during sexual behavior.

Much additional work is needed to determine the exact functions of endogenous opioids in the control of male sexual behavior. Present data at least suggest that they may not only be pharmacologically interesting but also physiologically relevant, making further studies particularly worthwile.

ACKNOWLEDGEMENTS

Financial support was received from the Universidad Anahuac. The technical assistance of Mr. Mario Diaz is gratefully acknowledged.

REFERENCES

1. Ågmo, A.; Berenfeld, R. Reinforcing properties of ejaculation in the male rat: Role of opioids and dopamine. Behav. Neurosci. 104:177-182; 1990.
2. Ågmo, A.; Gómez, M. Conditioned place preference produced by infusion of met-enkephalin into the medial preoptic area. Brain Res. 550:343-346; 1991.
3. Ågmo, A.; Gómez, M. Sexual reinforcement is blocked by infusion of naloxone into the medial preoptic area. Behav. Neurosci. 107:812-818; 1993.
4. Ågmo, A.; Paredes, R. Opioids and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 30: 1021-1034; 1988.
5. Ågmo, A.; Rojas, J.; Vázquez, P. Inhibitory effect of opiates on male rat sexual behavior may be mediated by opiate receptors outside the central nervous system. Psychopharmacology (Berlin) 107:89-96; 1992.
6. Akil, H.; Watson, S. J.; Young, E.; Lewis, M. E.; Khachaturian, H.; Walker, J. M. Endogenous opioids: Biology and function. Annu. Rev. Neurosci. 7:223-255; 1984.
7. Algeri, S.; Altstein, M.; de Simone, G.; Guardabasso, V. In vivo potentiation of (d-ala^2) met-enkephalin amide central effects after administration of an enkephalinase inhibitor. Eur. J. Pharmacol. 71:261-262; 1981.
8. Al-Rodhan, N.; Chipkin, R.; Yaksh, T. L. The antinociceptive effects of SCH-32615, a neutral endopeptidase (enkephalinase) inhibitor, microinjected into the periaqueductal, ventral medulla and amygdala. Brain Res. 520:123-130; 1990.
9. Altstein, M.; Blumberg, S.; Vogel, Z. Phosphoryl-leu-phe: A potent inhibitor of the degradation of enkephalin by enkephalinase. Eur. J. Pharmacol. 76:299-300; 1982.
10. Band, L. C.; Hull, E. M. Morphine and dynorphin(1-13) microinjected into the medial preoptic area and nucleus accumbens: Effects on sexual behavior in male rats. Brain Res. 524:77-84; 1990.
11. Bozarth, M. A. Ventral tegmental reward system. In: Engel, J.; Oreland, L., eds. Brain reward systems and abuse. New York: Raven; 1987:1-17.
12. Chipkin, R. E.; Coffin, V. L. Analgesic and acute central nervous system side effects of the intravenously administered enkephalinase inhibitor SCH 32615. Pharmacol. Biochem. Behav. 38:21-27; 1991.
13. Chipkin, R. E.; Berger, J. G.; Billard, W.; Iorio, L. C.; Chapman, R.; Barnett, A. Pharmacology of SCH 34826, an orally active enkephalinase inhibitor analgesic. J. Pharmacol. Exp. Ther. 245:829-838; 1988.
14. Chipkin, R. E.; Billard, W.; Ahn, H. S.; Sybertz, E. J.; Iorio, L. C. In vitro and in vivo activities of enkephalinase and angiotensin converting enzyme inhibitors: Relationship of enzyme inhibition to analgesic and cardiovascular effects. In: Ehrenpreis, S.; Sicutiri, F., eds. Degradation of endogenous opioids: Its relevance in human pathology and therapy. New York: Raven: 1983; 91-106.
15. Damassa, D. A.; Smith, E. R.; Tennent, B.; Davidson, J. M. The relationship between circulating testosterone levels and male sexual behavior in rats. Horm. Behav. 8:275-286; 1977.
16. De la Baume, S.; Patey, G.; Schwartz, J. C. Subcellular distribution of enkephalindipeptidyl carboxypeptidase (enkephalinase) in the rat brain. Neuroscience 6:315-321; 1981.
17. Devault, A.; Nault, C.; Zollinger, M.; Fournie-Zaluski, M. C.; Roques, B. P.; Crine, P.; Boileau, G. Expression of neutral endopeptidase (enkephalinase) in heterelogous COS-1 cells. J. Biol. Chem. 263:4033-4040; 1988.
18. Forsberg, G.; Wiesenfeld-Hallin, Z.; Eneroth, P.; Sodersten, P. Sexual behavior induces naloxone-reversible hypoalgesia in male rats. Neurosci. Lett. 81:151-154; 1987.
19. Glimcher, P. W.; Giovino, A. A.; Margolin, D. H.; Hoebel, B. G. Endogenous opioid reward induced by an enkephalinase

inhibitor, thiorphan, injected into the ventral midbrain. Behav. Neurosci. 98:262–268; 1984.
20. Goldstein, A. Binding selectivity profiles for ligands of multiple receptor types: Focus on opioid receptors. Trends Pharmacol. Sci. 8:456–460; 1987.
21. Greene, W. A.; Foote, R. H. Release rate of testosterone and estrogen from polydimethylsiloxane implants for extended periods in vivo compared with loss in vitro. Int. J. Fertil. 23:128–132; 1978.
22. Hughes, A. M.; Everitt, B. J.; Herbert, J. Selective effects of β-endorphin infused into the hypothalamus, preoptic area and bed nucleus of stria terminalis on the sexual and ingestive behaviour of male rats. Neuroscience 23:1063–1073; 1987.
23. Kalivas, P. W.; Abhold, R. Enkephalin release into the ventral tegmental area in response to stress: modulation of mesocorticolimbic dopamine. Brain Res. 414:339–348; 1987.
24. Larsson, K. Features of the neuroendocrine regulation of masculine sexual behavior. In: Beyer, C., ed. Endocrine control of sexual behavior. New York: Raven; 1979:77–163.
25. Malfroy, B.; Schofield, P. R.; Kuang, W. J.; Seeburg, P. H.; Mason, A. J.; Henzel, W. J. Molecular cloning and amino acid sequence of rat enkephalinase. Biochem. Biophys. Res. Commun. 144:59–66; 1987.
26. Malmnas, C. O. Short-latency effects of testosterone on copulatory behavior in sexually experienced male rats. J. Reprod. Fertil. 51:351–354; 1977.
27. Matuszewich, L.; Dornan, W. A. Bilateral injections of a selective mu-receptor agonist (morphiceptin) into the medial preoptic nucleus produces a marked delay in the initiation of sexual behavior in the male rat. Psychopharmacology (Berlin) 106:391–396; 1992.
28. McIntosh, T. K.; Vallano, M. L.; Barfield, R. J. Effects of morphine, β-endorphin and naloxone on catecholamine levels and sexual behavior in the male rat. Pharmacol. Biochem. Behav. 13: 435–441; 1980.
29. Meyerson, B. J.; Terenius, L. β-Endorphin and male sexual behaviour. Eur. J. Pharmacol. 42:191–192; 1977.
30. Millan, M. J.; Herz, A. The endocrinology of the opioids. Int. Rev. Neurobiol. 26:1–84; 1985.
31. Mitchell, J. B.; Gratton, A. Opioid modulation and sensitization of dopamine release elicited by sexually relevant stimuli: A high speed chronoamperometric study in freely behaving rats. Brain Res. 551:20–27; 1991.
32. Mitchell, J. B.; Stewart, J. Facilitation of sexual behavior in the male rat associated with intra-VTA injections of opiates. Pharmacol. Biochem. Behav. 35:643–650; 1990.
33. Mucha, R. F.; Herz, A. Motivational properties of kappa and mu opioid receptor agonists studied with place and taste preference conditioning. Psychopharmacology (Berlin) 86:274–280; 1985.
34. Mumford, L.; Kumar, R. Sexual behavior of morphine-dependent and abstinent male rats. Psychopharmacology (Berlin) 65:179–185; 1979.
35. Murphy, M., Bowie, D.; Pert, C. Copulation elevates plasma β-endorphin in the hamster. Soc. Neurosci. Abstr. 5:470; 1979.
36. Paredes, R.; Manero, M. C.; Haller, A. E.; Alvarado, R.; Ågmo, A. Sexual behavior increases postictal behavioral depression in medial preoptic area kindled rats: Evidence for opioid involvement. Behav. Brain Res. 52:175–182; 1992.
37. Pellegrini Quarantotti, B.; Paglietti, E.; Bonnani, E.; Petta, M.; Gessa, G. L. Inhibition of copulatory behavior by d-ala^2-met-enkephalinamide. Life Sci. 23:673–678; 1978.
38. Pfaus, J. G.; Gorzalka, B. B. Opioids and sexual behavior. Neurosci. Biobehav. Rev. 11:1–34; 1987.
39. Pfaus, J. G.; Damsma, G.; Nomikos, G. G.; Wenkstein, D. G.; Blaha, C. D.; Phillips, A. G.; Fibiger, H. C. Sexual behavior enhances central dopamine transmission in the male rat. Brain Res. 530:345–348; 1990.
40. Ruiz, E.; Ågmo, A. Enkephalin infusion into the medial preoptic area and sexual behavior in the male rat. 20th Ann. Conf. Reprod. Behav. Omaha, Nebraska, June 11–14; 1988:83.
41. Schechter, M. D.; Calcagnetti, D. J. Trends in place preference conditioning with a cross-indexed bibliography; 1957–1991. Neurosci. Biobehav. Rev. 17:21–41; 1993.
42. Szechtman, H.; Hershkowitz, M.; Simantov, R. Sexual behavior decreases pain sensitivity and stimulates endogenous opioids in male rats. Eur. J. Pharmacol. 70:279–285; 1981.
43. Wise, R. A. Opiate reward: Sites and substrates. Neurosci. Biobehav. Rev. 13:129–133; 1989.
44. Yaksh, T. L.; Chipkin, R. E. Studies on the effects of SCH-34826 and thiorphan on (Met^5) enkephalin levels and release in rat spinal cord. Eur. J. Pharmacol. 167:367–373; 1989.