Pharmacology Biochemistry and Behavior, Vol. 46, pp. 391–395, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Behavioral Effects of the μ-Opioid Peptide Agonists DAMGO, DALDA, and PL017 on Locomotor Activities

MERLE E. MEYER[1] AND MELISSA E. MEYER

Department of Psychology, University of Florida, Gainesville, FL 32611

Received 8 September 1992

MEYER, M. E. AND M. E. MEYER. *Behavioral effects of the μ-opioid peptide agonists DAMGO, DALDA, and PL017 on locomotor activities.* PHARMACOL BIOCHEM BEHAV **46**(2) 391–395, 1993.—The relative role of central μ-opioid receptor agonists Tyr-D-Ala-Gly-*N*-Methyl-Phe-Gly-*ol* (DAMGO), Tyr-D-Arg-Phe-Lys (DALDA), and Tyr-Pro-MePhe-D-Pro (PL017) (0.00, 0.01, 0.1, or 1.0 μg, ICV) on behavior was investigated in rats for 60 min in activity monitors. DAMGO (0.1 and 1.0 μg) and PL017 (1.0 μg) resulted in biphasic effects, inhibition followed by hyperactivity for linear locomotor, whereas the 0.01-μg dosage was associated with hyperactivity. On the other hand, DALDA (0.1 and 1.0 μg) suppressed locomotor activity over the 60-min session.

μ-Opioid peptide agonists DAMGO DALDA PL017 Locomotor activity

THERE is an abundance of evidence to show that there are at least three major pharmacologically different opioid receptor subtypes, μ-, δ-, and κ-receptors (4,7,16,19,27,28). However, subclassifications of the μ-receptor has been suggested, as well as for the κ-receptor. With the development of selective ligands and their availability in [^{3}H]-labeled form, the μ binding sites have been labeled (8,15).

The effects of various opioid peptides and opiate agonists on locomotor activity has been described in rodents. Morphine, β-endorphin, and some metabolically stable enkephalin analogs result in biphasic effects on locomotor activity. In general, low dosages induce stimulation of activity, whereas large dosages result an initial suppression followed by hyperactivity (2,10–12,23,25). Recent studies with mice using Tyr-D-Ala-Gly-*N*-Methyl-Phe-Gly-*ol* (DAMGO), a specific μ-opioid peptide, injected ICV, suggests that DAMGO induced an increase in horizontal activity and a decrease in rearing without the typical biphasic effect (18). On the other hand, ICV-injected DAMGO, in rats, resulted in generalized biphasic effects across linear locomotor, rearing, and stereotypy behaviors and an inhibition of thigmotaxis (17).

While recognizing the possibility of other subclasses and subtypes of opioid receptors, this research study focused on the μ-receptor subtype. The present study expands the generality of DAMGO, Tyr-D-Arg-Phe-Lys (DALDA), and Tyr-Pro-MePhe-D-Pro (PL017) opioid peptide effects on locomotor activities in rats. We report here our findings of various ICV-injected μ-opioid peptide agonists on linear locomotor activity in cm during six consecutive 10-min time blocks.

METHOD

Animals

Male Long-Evans rats weighing 200–225 g were obtained from Charles River. Rats were individually housed in stainless steel cages, had food and water ad lib, and were maintained on a 12 L : 12 D (light 0700–1900 h) cycle. Animals were tested in the light phase between 1000–1600 h. The room in which animals were maintained was at a constant temperature (21 ± 2°C). This study was carried out in compliance with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals.

Surgery

Animals, while under Equithesin anesthesia, were cannulated unilaterally with the use of a stereotaxic instrument. Guide cannulae, 7 mm long, fabricated from 21-ga hypodermic needles were permanently fixed to the skull with microscrews and dental cement. The guide cannulae were implanted following the coordinates from Paxinos and Watson (20), 0.8 mm posterior to bregma and 1.5 mm lateral to midline on the right side to allow injections into the lateral cerebral ventricle (ICV). The vertical depth of the injection cannula was 4 mm

[1] To whom requests for reprints should be addressed.

below the surface of the skull. Animals were allowed 2 weeks recovery before behavioral testing. During recovery, animals were not handled or transported except for routine cleaning.

Drugs and Drug Administration

Drugs used were the μ-receptor agonists DAMGO (mol. wt. 513.7; Bachem, Torrance, CA), DALDA (mol. wt. 611.1; Bachem), and PL017 (mol. wt. 535.3; Peninsula Laboratories, Belmont, CA). All peptides were dissolved in distilled water. Distilled water was also given for vehicle control injections (0.00 μg). As the molecular weights of these peptides were similar, the drug solutions were made up daily to the appropriate concentrations of 0.01, 0.1, and 1.0 μg. The 0.5 μl of solution was microinjected over a period of 60 s and the cannulae remained in place for another 30 s. Immediately after the injection procedure, animals were placed directly into activity chambers. Injection sites were verified by the perfusion of a methylene blue dye solution into the lateral ventricles prior to autopsy. In two animals, verification was not possible and they were replaced.

Apparatus

Immediately following ICV injections, each rat was placed in an Omnitech Digiscan Animal Activity Monitor (Columbus, OH) for 1 h. In the three experiments, data were collected every 10 min. The acrylic cage within the monitor measured approximately 42 × 42 × 30.5 cm. The monitor was equipped with 16 beams 2.54 cm apart from front to back and from side to side. The Digiscan analyzer converted the patterns of the beams broken into different measures of locomotor activity. The measure analyzed in this study was the distance the animal moved in cm during six consecutive 10-min time blocks. This measure was most comparable to other methods of response measurements as described in the experimental literature.

Statistics

Each independent treatment group consisted of 12 animals chosen at random. Animals were treated only once.

A two-factor mixed-design analysis of variance (ANOVA) was used to analyze the within measures (six consecutive 10-min time blocks), between the treatment conditions (four dose levels), and the time × dose interaction effect. Significant interactions for the dose × time interval were followed up within time blocks by Dunnett's multiple comparison tests between the control group and the treatment groups. p values equal to or less than 0.05 were judged statistically significant. The vehicle control group data were used in the independent analyses for DAMGO, DALDA, and PL017.

RESULTS

Locomotor Effects of DAMGO

Figure 1A illustrates a biphasic dose-response curve over the 60-min time course of locomotor activity of rats, as measured in cm, treated with one of four dose levels of DAMGO (vehicle, 0.01, 0.1, and 1.0 μg/rat, ICV).

The four treatment groups × six 10-min time blocks interaction was highly significant $F(15, 220) = 16.00$, $p < 0.001$. The subsequent analyses between the 1.0-μg group and the vehicle control group revealed a biphasic effect for DAMGO. At the 10-, 20-, and 30-min time blocks, there were significant suppression or hypoactivity ($p < 0.05$) and at the 50- and 60-min time blocks there were significant potentiation or hyperactivity ($p < 0.05$). The subsequent analyses between

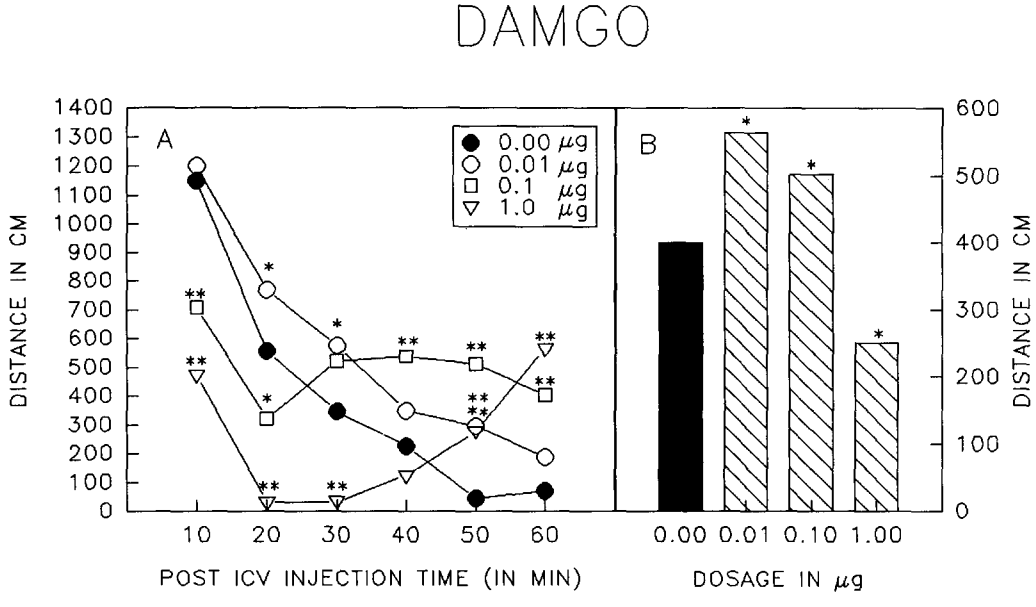

FIG. 1. (A). Significant interaction effects of ICV-injected 0.00-, 0.01-, 0.1-, and 1.0-μg dosages of the μ-opioid peptide Tyr-D-Ala-Gly-*N*-Methyl-Phe-Gly-*ol* (DAMGO) on locomotor activity in cm over six 10-min intervals. (B). Significant dose effects of DAMGO averaged across the six consecutive 10-min intervals. Significant differences from the vehicle control group (0.00 μg) at each time point: $^*p < 0.05$; $^{**}p < 0.01$.

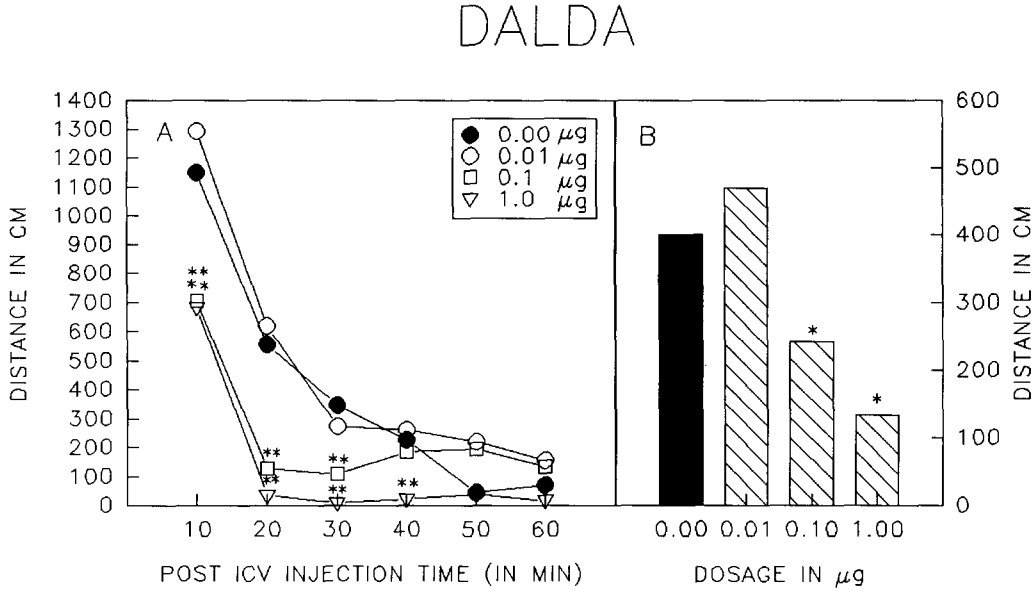

FIG. 2. Significant interaction effects of ICV-injected 0.00-, 0.01-, 0.1-, and 1.0-μg dosages of Tyr-D-Arg-Phe-Lys (DALDA) on locomotor activity in cm over six 10-min intervals. (B). Significant dose effects of DALDA averaged across the six consecutive 10-min intervals. Significant differences from the vehicle control group (0.00 μg) at each time point: $*p < 0.05$; $**p < 0.01$.

the 0.1-μg group and the vehicle control group also revealed a biphasic effect for DAMGO. At the 10- and 20-min time blocks, there was significant hypoactivity ($p < 0.05$) and at time blocks of 40, 50, and 60 min significant hyperactivity. The subsequent analyses for the 0.01-μg group revealed significant hyperactivity at time blocks of 20, 30, and 50 min.

Figure 1B shows the significant differences between the three dose levels and the vehicle control group, $F(3, 44) = 7.11, p < 0.001$. The subsequent analyses revealed that across the six time blocks the 0.01- and 0.1-μg groups were significantly hyperactive ($p < 0.05$) whereas the 1.0-μg group was significantly hypoactive ($p < 0.01$).

Locomotor Effects of DALDA

Figure 2A illustrates a single-slope dose-response curve of the locomotor activity of rats, as measured in cm, treated with one of the four dose levels of DALDA (vehicle, 0.01, 0.1, and 1.0 μg/rat, ICV) over the 60-min time course.

The four treatment groups × six 10-min time blocks interaction was significant, $F(15, 220) = 7.42, p < 0.001$. The subsequent analyses between the 1.0-μg group and the vehicle control group resulted in hypoactivity at the 10-min interval through the 40-min time block ($p < 0.01$ and 0.05). Similarly, the 0.1-μg group showed significant suppression from the 10-min time block through the 30-min block ($p < 0.01$ and 0.05).

Figure 2B shows the significant differences between the three dose levels and the vehicle controls, $F(3, 44) = 7.42, p < 0.001$. Both the 0.1- and 1.0-μg groups were significantly suppressed when compared to their vehicle controls ($p < 0.01$).

Locomotor Effects of PL017

Figure 3A illustrates a biphasic dose-response curve of the linear locomotor activity, as measured in seconds, of rats treated with one of four dose levels of PL017 (vehicle, 0.01, 0.1, and 1.0 μg/side, ICV) over the 60-min time course.

The dose × time block interaction was highly significant, $F(15, 220) = 11.90, p < 0.001$. The subsequent analyses between the 1.0-μg group and the vehicle control group revealed a biphasic effect for PL017. At time blocks 10 and 20 min, there was significant suppression of locomotor activity ($p < 0.01$), and at time blocks 40–60 min there was significant hyperactivity ($p < 0.01$). The subsequent analyses between the 0.1-μg group and the vehicle controls revealed an initial suppression at the 10-min time block ($p < 0.05$) and hyperactivity at the 30- through 60-min time blocks ($p < 0.01$ and 0.01). The 0.01-μg group when compared to the vehicle controls showed hyperlocomotion between the time blocks of 20–60 min ($p < 0.01$ and 0.05).

Figure 3B shows the significant effects of the three dose levels in comparison to the vehicle controls, $F(3, 44) = 4.18$, $p = 0.01$. Both the 0.01- and 0.1-μg groups were hyperactive ($p < 0.01$).

DISCUSSION

The results presented in this article provide information on the role of the μ-receptor in mediating changes in locomotor behavior following ICV administration of three μ-receptor peptide agonists—DAMGO, DALDA, and PL017.

Morphine has been considered the μ-receptor prototype agonist as this nonpeptide agonist has a high affinity for the μ site (21,22). Low dosages of morphine typically elicit hyperactivity. As the dosages are increased, morphine results in a biphasic effect on locomotor activity in rats, where there was an initial suppression of activity followed by excitation.

The biphasic dose-response curve of the μ-receptor peptide agonist, DAMGO, in this present study on locomotor activity was similar to the effects of morphine in rats (23) expect for

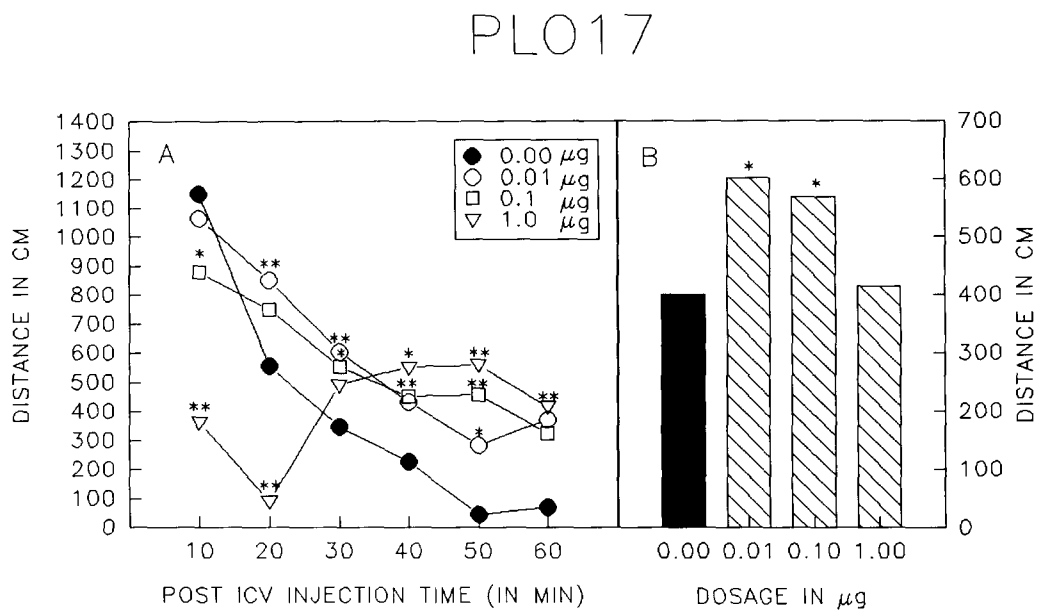

FIG. 3. Significant interaction effects of ICV-injected 0.00-, 0.01-, 0.1-, and 1.0-μg dosages of Tyr-Pro-MePhe-D-Pro (PL017) on locomotor activity in cm over six 10-min intervals. (B). Significant dose effects of PL017 averaged across the six 10-min intervals. Significant differences from the vehicle control group (0.00 μg) at each time point: $*p < 0.05$; $**p < 0.01$.

the duration of the effects (1,10). The 1.0-μg dose (ICV) exerted a significant U-shaped biphasic effect with an initial suppression and an intermediate marked hypoactivity, followed by significant hyperactivity. The 0.1-μg dose initially suppressed locomotion, followed with hyperactivity. On the other hand, the 0.01-μg dose effectively enhanced locomotor activity without the initial suppression. These biphasic effects were different from mice, where only a single-slope dose-hyperactivity response was observed (18). This species difference may be a function of the opioid to which mice show especially significant excitation (10,25).

The behavioral profile of PL017 was in part comparable to DAMGO. The 1.0-μg dose (ICV) also resulted in a significant U-shaped curve with an initial suppression followed by activation of locomotor activity. However, the suppression effect was of a shorter duration and the overall inhibitory effects did not differ from the vehicle controls. Overall, the 0.01- and 0.1-μg groups of PL017, as with DAMGO, resulted in a single-slope hyperactivity.

DALDA, on the other hand, at dose levels of 0.1 and 1.0 μg was associated with a single-slope dose-response hypoactivity across the session and without the later activation phase as seen with DAMGO and PL017. Overall, these two dose levels significantly suppressed locomotor activity. The behavioral profile of DALDA differs from morphine, DAMGO, and PL017, where the primary effect elicited by DALDA was suppression. The DALDA-induced suppression may have a significantly longer duration than was observed with DAMGO and PL017. Because of the limited duration of the postinjection, the activation phase may not have been measured. The explanation of these behavioral effects warrants further investigation.

To account for the biphasic effect of the μ-receptor opiates and opioid peptides, it has been proposed that they activate two different processes, resulting in opposite changes in locomotor activity (23). It has been suggested that locomotor suppression is mediated by μ-receptors and the activation is a function of the δ-receptor subtype (9). However, with morphine and the ligands used in the present study, all with a high affinity at the μ-receptor, it is highly unlikely that the two-opioid process theory could explain the data.

On the other hand, it has been established that opiates and opioid peptides induce monophasic hypoactivity if injected into the periaqueductal gray and unidirectional excitation via the striatum (6,13). Further, opioid peptides modulate locomotor activity within both the nigrostriatal and mesolimbic dopaminergic systems and opioid-elicited locomotor activity can be disrupted by dopaminergic antagonists (3,14,21,26). The biphasic effects of DAMGO and PL017 are similar to morphine and may be explained by a two-process theory. Conversely, this biphasic effect may be due to an initial peptide-induced akinesia and local diffusion of the peptide to various brain regions. For example, DAMGO microinjected into the ventral tegmental area elicits hyperactivity (13,26).

Perhaps the most important area for future research will be studies that focus upon the anatomic substrates that underlie various behavioral effects elicited by selective opioid receptors agonists and antagonists and are also functionally related to the dopaminergic systems (5,6,24).

ACKNOWLEDGEMENTS

This research was supported by a grant to Merle E. Meyer from the Whitehall Foundation. The authors thank Bonnie McLaurin for excellent technical assistance and Jeanene Griffin for excellent secretarial assistance.

REFERENCES

1. Bhargava, H. N. Effects of methionine-enkephalin and morphine on spontaneous locomotor activity: antagonism by naloxone. Pharmacol. Biochem. Behav. 9:167–171; 1978.
2. Brady, L. S.; Holtzman, S. G. Effects of intraventricular morphine and enkephalins on locomotor activity in nondependent, morphine-dependent and postdependent rats. J. Pharmacol. Exp. Ther. 218:613–620; 1981.
3. Bunny, W. C.; Massari, V. J.; Pert, A. Chronic morphine-induced hyperactivity in rats is altered by nucleus accumbens and ventral tegmental lesions. Psychopharmacology (Berl.) 82:318–321; 1984.
4. Corbett, A. D.; Paterson, S. J.; Kosterlitz, H. W. Selectivity of ligands for opioid receptors. In: Herz, A.; Akil, H.; Simon, J., eds. Handbook of experimental pharmacology. Heidelberg: Springer-Verlag; 1993:645–679.
5. Costall, B.; Fortune, D. H.; Naylor, R. J. The induction of catalepsy and hyperactivity by morphine administered directly into the nucleus accumbens of rats. Eur. J. Pharmacol. 49:49–64; 1978.
6. Dauge, V.; Rossignol, P.; Roques, B. P. Comparison of the behavioral effects induced by administration in rat nucleus accumbens or nucleus caudatus of selective μ and δ opioid peptides or kelatorphan, an inhibitor of enkephalin-degrading enzymes. Psychopharmacology (Berl.) 96:343–352; 1988.
7. Goldstein, A.; James, I. F. Multiple opioid receptors. Criteria for identification and classification. Trends Pharmacol. Sci. 5: 503–505; 1984.
8. Handa, B. K.; Lane, A. C.; Lord, J. A. H.; Morgan, B. A.; Rance, M. J.; Smith, C. F. C. Analogues of β-LPH61-64 possessing selective agonist activity at the μ-opiate receptors. Eur. J. Pharmacol. 70:531–540; 1981.
9. Havemann, U.; Kuschinsky, K. Locomotor activity of rats after injection of various opioids into the nucleus accumbens and septum mediale. Naunyn Schmiedeberg Arch. Pharmacol. 331:175–180; 1985.
10. Hecht, A.; Schiorring, E. Behaviour effects of low and high acute doses of morphine in solitary mice. Psychopharmacology (Berl.) 64:73–81;1979.
11. Iwamoto, E. T. Locomotor activity and antinociception after putative mu, kappa, and sigma opioid receptor agonists in the rat: Influence of dopaminergic agonists and antagonists. J. Pharmacol. Exp. Ther. 217:451–460; 1981.
12. Iwamoto, E. T. An assessment of the spontaneous activity of rats administered morphine, phencyclidine, or nicotine using automated and observational methods. Psychopharmacology (Berl.) 84:374–378; 1984.
13. Jenck, F.; Bozarth, M.; Wise, R. A. Contraversive circling induced by ventral tegmental microinjections of moderate doses of morphine and [D-Pen^2, D-Pen^5] enkephalin. Brain Res. 450:382–386; 1988.
14. Joyce, E. M.; Iverson, S. D. The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. Neurosci. Lett. 14:207–212;1979.
15. Kosterlitz, H. W.; Paterson, S. J. Tyr-D-Ala-Gly MePhe-NH-$(CH_2)_2$ OH is a selective ligand for the μ opiate binding site. Br. J. Pharmacol. 73:299; 1981.
16. Martin, W. R. Pharmacology of opioids. Pharmacol. Rev. 35: 283–323; 1984.
17. Meyer, M. E.; Meyer, M. E. Behavioral effects of μ, δ, and κ opioid peptide agonists DAMGO, DPDPE, and DAKLI on locomotor activities. Pharmacol. Biochem. Behav. 45:315–320; 1993.
18. Michael-Titus, A.; Dourmap, N.; Costentin, J. Mu and delta opioid receptors control differently the horizontal and vertical components of locomotor activity in mice. Neuropeptides 13:235–242; 1989.
19. Paterson, S. J.; Robson, I. E.; Kosterlitz, H. W. Classification of opioid receptors. Br. Med. Bull. 39:31–36; 1983.
20. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. New York: Academic Press; 1986.
21. Pert, A.; Sivit, C. Neuroanatomical focus for morphine and enkephalin-induced hyperactivity. Nature 265:645–647; 1977.
22. Rees, D. C.; Hunter, J. C. Opioid receptors. In: Hansch, C.; Sammes, P. G.; Taylor, J. B., eds. Comprehensive medicinal chemistry. New York: Pergamon Press; 1990:805–846.
23. Schnur, P.; Hang, D. Naloxone reversal of morphine elicited hyperactivity. Life Sci. 40:329–333; 1987.
24. Szekely, J. I. Analysis of behavioral actions of opioid peptides. In: Szelely, J. I., ed. Opioid peptides. Boca Raton, FL: CRC Press; 1990:235–250.
25. Szekely, J. I.; Miglecz, E.; Ronai, A. Z. Biphasic effects of a potent enkephalin analogue (D-Met^2, Pro^5)-enkephalinamide and morphine on locomotor activity in mice. Psychopharmacology (Berl.) 72:299–301; 1980.
26. Vezina, P.; Kalivas, P. W.; Stewart, J. Sensitization occurs to the locomotor effects of morphine and the specific opioid receptor agonist, DAGO, administered repeatedly to the ventral tegmental area but to the nucleus accumbens. Brain Res. 417:51–58; 1987.
27. Wood, R. L. Multiple opiate receptors support for unique mu, delta, and kappa sites. Neuropharmacology 21:487–497; 1982.
28. Zukin, R. S.; Zukin, S. R. The case for multiple opiate receptors. Trends Neurosci. 7:160–164; 1984.