Pharmacology Biochemistry and Behavior, Vol. 46, pp. 587–591, 1993

0091-3057/93 $6.00 + .00

Receptor-Selective Opioid Peptides Fail to Affect Behavioral Responses Induced by a Low Dose of Apomorphine in the Mouse

MAKOTO UKAI,[1] TOHRU TOYOSHI AND TSUTOMU KAMEYAMA

Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan

Received 13 October 1992

UKAI, M., T. TOYOSHI AND T. KAMEYAMA. *Receptor-selective opioid peptides fail to affect behavioral responses induced by a low dose of apomorphine in the mouse.* PHARMACOL BIOCHEM BEHAV **46**(3) 587–591, 1993.—The effects of intracerebroventricular injections of the mu-selective opioid agonist DAMGO ([D-Ala^2,NMePhe^4,Gly-ol]enkephalin), the kappa-selective opioid agonist dynorphin A-(1–13), and the delta-selective opioid agonist DPLPE ([D-Pen^2,L-Pen^5]enkephalin) on the decrease in different behavioral responses induced by a low dose of apomorphine (0.03 mg/kg) were investigated in the mouse. A low dose (0.03 mg/kg) of apomorphine produced a marked decrease in behavioral responses such as circling and rearing. Although the dopamine D_1 antagonist SCH 23390 (0.01 and 0.03 mg/kg) did not influence behavior induced by apomorphine (0.03 mg/kg), the dopamine D_2 antagonist sulpiride (3.0 mg/kg) reversed the decrease in circling and rearing behavior induced by apomorphine, suggesting that the effects of apomorphine on circling and rearing are mediated through dopamine D_2 autoreceptors. DAMGO (0.003 or 0.01 μg), dynorphin A-(1–13) (3.0 or 10.0 μg), or DPLPE (0.3 and 1.0 μg) had no significant effects on the apomorphine-induced decrease in circling and rearing behavior. These in vivo results suggest that opioid peptides selective for receptor types fail to influence drug effects mediated by dopamine D_2 autoreceptors.

[D-Ala^2,NMePhe^4,Gly-ol]Enkephalin Dynorphin A-(1–13) [D-Pen^2,L-Pen^5]Enkephalin Apomorphine Locomotor activity Mouse

OPIOIDS are well known to influence a variety of behavioral responses in mammals (4,7), possibly by interacting with brain dopamine systems. For example, U-50,488H, a kappa opioid agonist, diminishes the K^+-evoked release of dopamine in rat brain slices (19). Intracerebroventricular injection of the dynorphin analog E-2078 inhibits dopamine release in the nucleus accumbens (8). Dynorphin A-(1–13) inhibits the marked increase in rearing behavior induced by apomorphine (16) and RU 24213 (17), suggesting that kappa opioid receptors play an inhibitory role in dopamine neurotransmissions. In contrast, the effects of mu and delta opioid agonists on dopamine neurons appear to be inconsistent. It has been shown that morphine acts presynaptically to inhibit dopamine release (2), whereas Kalivas and Duffy (5) have reported that higher doses of DAMGO injected into the A10 region elicit a marked increase in dopamine release in the nucleus accumbens, leading to hypermotility. Furthermore, the delta opioid agonist DPLPE ([D-Pen^2,L-Pen^5]enkephalin) has no significant effects on behavior induced by apomorphine (15) or RU 24213 (11), whereas DPDPE ([D-Pen^2,D-Pen^5]enkephalin), another delta opioid agonist, produces a marked increase in dopamine release (8). However, the involvement of the autoreceptors of dopamine neurons in the behavioral effects of opioid peptides still remains uncertain.

In the present study, the effects of DAMGO, dynorphin A-(1–13), and DPLPE on behavioral responses induced by a low dose of the direct dopamine agonist apomorphine were examined by using multidimensional behavioral analyses, which can classify various types of behavior.

METHOD

Animals

Male ddY mice (Nihon SLC, Inc., Hamamatsu, Japan), weighing between 20–30 g, were employed in the experiments. The animals were randomly assigned to groups consisting of 14 mice per group. Before the experiments, the mice were given free access to food and water, and individual mice were housed in a cage in a constantly illuminated room at a temperature of 23 ± 1°C and a relative humidity of 55 ± 2.5%.

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

The mice were used only once and were unfamiliar with the test box. The experiments were conducted between 10:00 a.m. and 6:00 p.m. in a sound-attenuating room.

Multidimensional Analysis

At more than 30 min before multidimensional behavioral analyses on each day, mice were selected according to the number of revolutions (range from 125 to 150 per 10 min for criterion) using wheel cages. This was done to reduce individual differences among animals in locomotor activity to limit the variability in results. About 30% of the mice initially were discarded for failing to meet the criterion in the first measurement. These mice were retested in the wheel cages on subsequent days. Eventually, almost all of the mice met the criterion for inclusion in the study. The screening procedure did not result in residual behavioral effects in the multidimensional behavioral analyses that followed. Behavior was observed over a period of 30 min, cumulating counts at a 15-min period, but the results in the latter period (15–30 min) were not used in the analyses because the behavioral effects of apomorphine were not prominent in the period. The Animex II, equipped with an electronic microcomputer, was used for measuring the behavior (6,12). The sensor consisted of three pairs of electrodes and formed a capacitor bridge. Once a mouse was placed in the space (150 × 210 × 140 mm) between the electrodes connected to field detectors, the value of the capacitor then depended upon the location of the mouse within that space. When converting the analog signal received by the detectors to a digital form, the DC voltage movement spectrum analyser classified the movement into nine degrees (1/1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, and 1/256). The surface areas of the cage in which mice showed behavioral responses (ambulation, circling, and rearing) were 490 mm in distance. The 490-mm distance consisted of the length of the cage bottom (210 mm) and the cage walls (140 mm × 2). Thus, the counters corresponded to the following sizes of movements: 1/1 (× 490.0 mm) = 490.0 mm, 1/2 (× 490.0 mm) = 245.0 mm, 1/4 (× 490.0 mm) = 122.5 mm, 1/8 (× 490.0 mm) = 61.3 mm, 1/16 (× 490.0 mm) = 30.6 mm, 1/32 (× 490.0 mm) = 15.3 mm, 1/64 (× 490.0 mm) = 7.7 mm, 1/128 (× 490.0 mm) = 3.8 mm, and 1/256 (× 490.0 mm) = 1.9 mm. The movement of greatest magnitude was principally registered on the 1/1 counter and the movement of the smallest magnitude, such as tremor, on the 1/256 counter. Specific patterns of behavior, induced by a drug, were registered on the counters as follows: linear locomotion on 1/1, circling on 1/4, rearing on 1/16, and grooming on 1/64 (14,18). In particular, circling behavior consisted of walking around left or right along sides in the cage. The sensitivity (%) of the device was adjusted according to the body weight (g) as follows: 20–21 g = 27%, 22–23 g = 26%, 24–25 g = 25%, 26–28 g = 24%, and 29–30 g = 23%. Each value in the figures was labeled "ratio (number of movements) = value of drug-treated animals/mean value of controls."

Drugs and Treatments

Apomorphine hydrochloride (Sigma, St. Louis, MO), SCH 23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepine-7-ol maleate) (Schering Corp., USA), *S*(−)-sulpiride (Research Biochemicals Inc., USA), DAMGO, DPLPE (Peninsula Laboratories, Inc., Belmont, CA), and dynorphin A-(1–13) (Peptide Institute Inc., Minoh, Japan) were employed throughout. The vehicle for SCH 23390 and *S*(−)-sulpiride was 8.5% lactic acid and 1.0 N sodium hydroxide in a 3 : 2 ratio (pH 4–5), while that for apomorphine was 0.9% saline containing 0.1% ascorbic acid. The vehicle injections in lieu of sulpiride or apomorphine were similarly prepared. Apomorphine (SC), SCH 23390 (IP), and *S*(−)-sulpiride (IP) were administered 25, 60, and 60 min before the start of behavioral measurements. The peptides, dissolved in sterile isotonic saline in polypropylene containers, were ICV injected 10 min before the start. The unilateral injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears (3). The injection was made with a 4-mm-long needle attached to a 50-μl Hamilton microsyringe. The needle was inserted perpendicularly through the skull and into the brain of the mouse. The mouse was anesthetized locally with Xylocaine (8% lidocaine) spray (Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan). Solutions were injected in a volume of 10 μl per mouse over a period of 20 s as previously described (6,12). The site was checked by injecting a 1 : 10 dilution of India ink in isotonic saline (0.9% NaCl, pH 7.5). Histological examinations revealed particles of the ink in the lateral and 3rd ventricles but not in the others. Severe tissue damage was never seen in the brain. As previously described (6,12), neither insertion of the needle nor injection of 10 μl of isotonic saline solution had a significant influence on behavioral responses during observation periods, although slight sedation was seen only within a few minutes after ICV injections. Each of the figures had its own control values, but there were no statistically significant differences among them.

Data Analysis

Data for untransformed values were analysed statistically by means of a one-factor analysis of variance (ANOVA), although only transformed data are shown in each of the figures. Post hoc analysis for between-group differences was carried out by the Newman-Keuls method for multiple comparisons (20). Effects were considered statistically significant

TABLE 1

UNTRANSFORMED CONTROL VALUES IN EACH OF THE FIGURES

	Behavioral Responses			
Figures	Linear Locomotion	Circling	Rearing	Grooming
Figure 1A, B	6.5 ± 0.9	11.6 ± 1.5	25.9 ± 2.5	52.1 ± 4.4
Figure 2A	7.2 ± 1.4	9.0 ± 1.1	19.0 ± 2.2	32.9 ± 3.8
Figure 2B	7.9 ± 1.6	11.9 ± 1.9	25.4 ± 4.5	44.3 ± 7.4
Figure 2C	4.9 ± 0.9	9.9 ± 2.0	22.0 ± 4.0	39.8 ± 5.1

Values represent the mean ± SE for 14–16 mice.

if $p < 0.05$. Data in figures indicate ratios derived from actual values for the clearer presentation of results.

RESULTS

Untransformed Control Values

The untransformed control values for each of the dependent measures are displayed in Table 1.

Effects of Dopamine Antagonists

SCH 23390 (0.01 and 0.03 mg/kg) had no effects on behavior, while sulpiride (3.0 mg/kg) produced a marked decrease in circling and rearing (Fig. 1). Apomorphine (0.03 mg/kg) significantly decreased circling and rearing behavior (Fig. 1). Although SCH 23390 (0.03 mg/kg) failed to affect behavior induced by apomorphine (0.03 mg/kg) (Fig. 1A), *S*(−)-sulpiride (3.0 mg/kg) reversed the decrease in circling and rearing induced by apomorphine (0.03 mg/kg) (Fig. 1B).

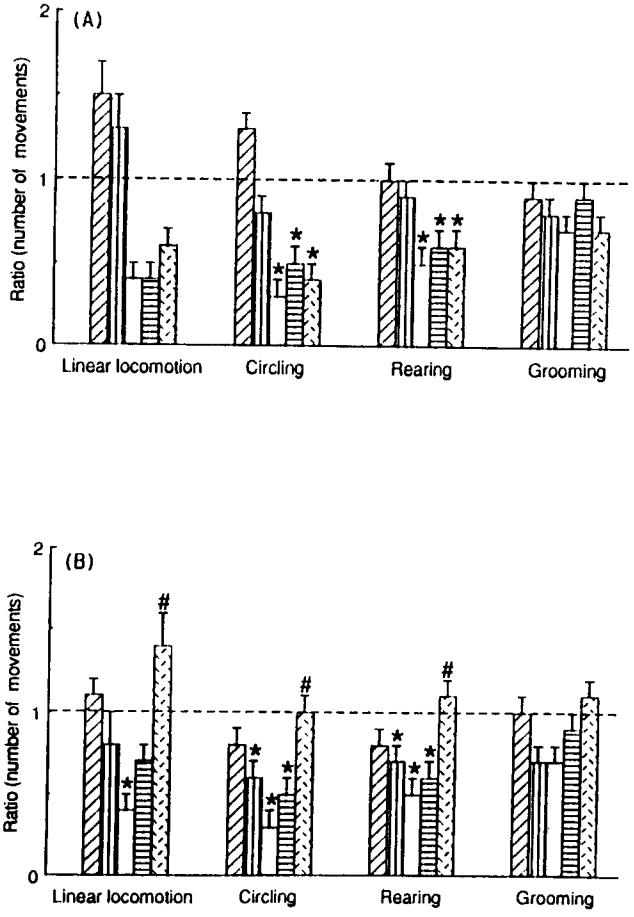

FIG. 1. (A) Movements in mice after the administration of apomorphine (APO), SCH 23390 (SCH), and their combinations. Saline (SC) + saline (IP) (----), saline (SC) + SCH 0.01 mg/kg (IP) (▨), saline (SC) + SCH 0.03 mg/kg (IP) (▥), APO 0.03 mg/kg (SC) + saline (IP) (□), APO 0.03 mg/kg (SC) + SCH 0.01 mg/kg (IP) (▤), APO 0.03 mg/kg (SC) + SCH 0.03 mg/kg (IP) (▩). (B) Movements in mice after the administration of apomorphine (APO), *S*(−)-sulpiride (SUL) and their combinations. Saline (SC) + saline (IP) (----), saline (SC) + SUL 1.0 mg/kg (IP) (▨), saline (SC) + SUL 3.0 mg/kg (IP) (▥), APO 0.03 mg/kg (SC) + saline (IP) (□), APO 0.03 mg/kg (SC) + SUL 1.0 mg/kg (IP) (▤), APO 0.03 mg/kg (SC) + SUL 3.0 mg/kg (IP) (▩). Values represent the mean ± SE for 14 mice. *$p < 0.05$ vs. saline control. #$p < 0.05$ vs. APO 0.03 mg/kg.

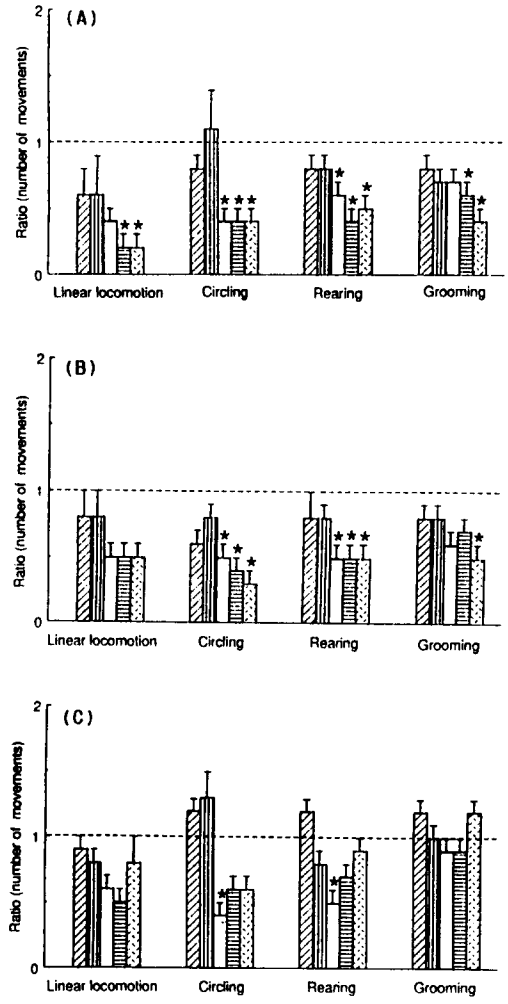

FIG. 2. (A) Movements in mice after the administration of apomorphine (APO), DAMGO, and their combinations. saline (SC) + saline (ICV) (----), saline (SC) + DAMGO 0.003 μg (ICV) (▨), saline (SC) + DAMGO 0.01 μg (ICV) (▥), APO 0.03 mg/kg (SC) + saline (ICV) (□), APO 0.03 mg/kg (SC) + DAMGO 0.003 μg (ICV) (▤), APO 0.03 mg/kg (SC) + DAMGO 0.01 μg (ICV) (▩). Values represent the mean ± SE for 14 mice. (B) Movements in mice after the administration of apomorphine (APO), dynorphin A-(1-13) (DYN), and their combinations. Saline (SC) + saline (ICV) (----), saline (SC) + DYN 3.0 μg (ICV) (▨), saline (SC) + DYN 10.0 μg (ICV) (▥), APO 0.03 mg/kg (SC) + saline (ICV) (□), APO 0.03 mg/kg (SC) + DYN 3.0 μg (ICV) (▤), APO 0.03 mg/kg (SC) + DYN 10.0 μg (ICV) (▩). Values represent the mean ± SE for 14 mice. (C) Movements in mice after the administration of apomorphine (APO), DPLPE, and their combinations. Saline (SC) + saline (ICV) (----), saline (SC) + DPLPE 0.3 μg (ICV) (▨), saline (SC) + DPLPE 1.0 μg (ICV) (▥), APO 0.03 mg/kg (SC) + saline (ICV) (□), APO 0.03 mg/kg (SC) + DPLPE 0.3 μg (ICV) (▤), APO 0.03 mg/kg (SC) + DPLPE 1.0 μg (ICV) (▩). Values represent the mean ± SE for 16 mice. *$p < 0.05$ vs. saline control.

Effects of Opioid Peptides

Apomorphine (0.03 mg/kg) again produced a marked decrease in behavioral responses such as circling and rearing

(Fig. 2). DAMGO (0.003 or 0.01 μg), dynorphin A-(1-13) (3.0 and 10.0 μg), or DPLPE (0.3 and 1.0 μg) alone had no significant effects on behavior, while these opioid peptides no longer affected the decrease in behavior induced by a low dose (0.03 mg/kg) of apomorphine (Fig. 2).

DISCUSSION

Apomorphine reportedly potentiates naltrexone-induced hypodipsia in the rat (13), suggesting the close interaction of endogenous opioid peptides with dopamine neurons in drinking behavior. Moreover, DAMGO (0.003 and 0.01 μg) (14) and dynorphin A-(1-13) (10.0 μg) (16) have been reported to produce an inhibitory effect on the increase in rearing and/or grooming behavior induced by higher doses (0.56 and 1.0 mg/kg) of apomorphine. The effects of DAMGO (14) and dynorphin A-(1-13) (16) are clearly reversed by receptor-selective opioid antagonists. Thus, it is possible that the mu and kappa opioid agonists play an inhibitory role in behavior induced by higher doses of apomorphine by acting on the postsynaptic sites of dopamine neurons. In particular, the effects of DAMGO and dynorphin A-(1-13) seem to be associated with dopamine D_2 receptors. For example, DAMGO (0.003 and 0.01 μg) (11) and dynorphin A-(1-13) (12.5 μg) (17) attenuate the dopamine D_2 agonist RU 24213 (3.0 mg/kg)-induced increase in behavioral responses, such as linear locomotion, circling, rearing, and/or grooming, while these peptides have no significant effects on grooming behavior elicited by the dopamine D_1 agonist SKF 38393 (10.0 mg/kg) (10,17). In contrast, DPLPE (0.3, 1.0, or 1.75 μg) has no significant effects on behavior induced by apomorphine (1.0 mg/kg) (15) or RU 24213 (3.0 mg/kg) (11), although the peptide (1.0 μg) combined with SKF 38393 (10.0 mg/kg) produces a marked increase in linear locomotion and rearing (10).

Feigenbaum et al. (2) present evidence showing antagonistic interactions of morphine with central dopamine receptors. The apparently antagonistic effects of morphine may be explained on the basis of an inhibition of dopamine release through the stimulation of opioid receptors localized on presynaptic dopamine terminals. Apomorphine at a lower dose (0.03 mg/kg) produced a marked decrease in circling and rearing behavior. The effects of apomorphine were clearly inhibited by *S*(−)-sulpiride (3.0 mg/kg) but not SCH 23390 (0.01 and 0.03 mg/kg), indicating that the apomorphine (0.03 mg/kg)-induced decrease in behavior is mediated through dopamine D_2 but not D_1 receptors. This evidence supports the data by Steketee and Kalivas (9), who demonstrate that the activity of mesolimbic dopamine neurons is regulated by the somatodendritic dopamine D_2 but not D_1 autoreceptors. Moreover, it has been reported that higher doses of DAMGO (0.1 and 0.5 μg) and DPDPE (10 and 25 μg) increase dopamine release in the nucleus accumbens, although the dynorphin analog E-2078 (1.0 μg) decreases it according to an in vivo microdialysis study (8). Since each of the opioid receptor types appears to be located substantially on dopamine terminals in the nucleus accumbens and on dopamine cell bodies in the ventral tegmental area, the effects of opioid peptides would be through presynaptic mediation (1). The present study, however, provides the first in vivo demonstration that none of the opioid peptides selective for opioid receptors influenced the decrease in circling or rearing behavior induced by a low dose (0.03 mg/kg) of apomorphine. It is thus unlikely that opioid peptides interact with dopamine D_2 autoreceptors. In contrast, an alternative interpretation of the data is that the doses of opioid peptides used were insufficient for altering the low-dose apomorphine effect. Higher doses of opioid peptides may be required for interaction with presynaptic dopamine D_2 receptors, while opioid receptors associated with presynaptic dopamine D_2 receptors may have higher threshold for stimulation. However, higher doses than those of opioid peptides used in the study were not injected, because higher doses of opioid peptides themselves have been demonstrated to markedly alter behavioral responses in mice (12,18). For example, DAMGO (0.03-0.3 μg) and DPLPE (3 and 10 μg) markedly increase circling behavior (18), whereas dynorphin A-(1-13) (30 μg) decreases it (unpublished observation). In other words, the modulating effects of DAMGO, dynorphin A-(1-13), and DPLPE on behavioral responses induced by dopamine agonists would be mediated exclusively through postsynaptic dopamine receptors (10,14,16), because the doses used in the study have been reported to affect behavioral responses induced by a higher dose of apomorphine (14-16).

ACKNOWLEDGEMENTS

The authors thank Schering Corp. for supplying the sample of SCH 23390. The secretarial work of Tetsuya Kobayashi and Mayumi Mizutani is greatly appreciated.

REFERENCES

1. Cooper, S. J. Interactions between endogenous opioids and dopamine: Implications for reward and aversion. In: Willner, P.; Scheel-Kruger, J., eds. The mesolimbic dopamine system: From motivation to action. Chichester: John Wiley & Sons Ltd.; 1991: 331-366.
2. Feigenbaum, J. J.; Fishman, R. H. B.; Yanai, J. Mechanism of dopamine antagonism by morphine in rodents. Subst. Alcohol Actions Misuse 3:307-324; 1982.
3. Haley, T. J.; McCormick, W. G. Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. Br. J. Pharmacol. 12:12-15; 1957.
4. 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. 217:451-466; 1981.
5. Kalivas, P. W.; Duffy, P. Effect of acute and daily neurotensin and enkephalin treatments on extracellular dopamine in the nucleus accumbens. J. Neurosci. 10:2940-2949; 1990.
6. Kameyama, T.; Ukai, M. Multi-dimensional analyses of behavior in mice treated with morphine, endorphins and [des-tyrosine^1]-γ-endorphin. Pharmacol. Biochem. Behav. 19:671-677; 1983.
7. Locke, K. W.; Holtzman, S. G. Behavioral effects of opioid peptides selective for mu or delta receptors. II. Locomotor activity in nondependent and morphine-dependent rats. J. Pharmacol. Exp. Ther. 238:997-1003; 1986.
8. Spanagel, R.; Herz A.; Shippenberg, T. S. The effects of opioid peptides on dopamine release in the nucleus accumbens: An in vivo microdialysis study. J. Neurochem. 55:1734-1740; 1990.
9. Steketee, J. D.; Kalivas, P. W. Sensitization to psychostimulants and stress after injection of pertussis toxin into the A10 dopamine region. J. Pharmacol. Exp. Ther. 259:916-924; 1991.
10. Toyoshi, T.; Ukai, M.; Kameyama, T. Combination of a δ opioid receptor agonist but not a μ opioid receptor agonist with the D_1-selective dopamine receptor agonist SKF 38393 markedly potentiates different behaviors in mice. Eur. J. Pharmacol. 213:25-30; 1992.
11. Toyoshi, T.; Ukai, M.; Kameyama, T. [D-Ala^2,NMePhe^4,Gly-ol^5]enkephalin, but not [D-Pen^2,L-Pen^5]enkephalin, specifically

inhibits behaviors induced by the dopamine D_2 agonist RU 24213. Eur. J. Pharmacol. 201:41–46; 1991.
12. Ukai, M.; Kameyama, T. The antagonistic effects of naloxone on hypermotility in mice induced by dynorphin-(1–13) using a multi-dimensional behavioral analysis. Neuropharmacology 23: 165–168; 1984.
13. Ukai, M.; Nakayama S.; Kameyama, T. Apomorphine markedly potentiates naltrexone-induced hypodipsia in the rat. Brain Res. 451:357–360; 1988.
14. Ukai, M.; Toyoshi T.; Kameyama, T. DAGO ([D-Ala^2, N-Me-Phe^4,Gly-ol]enkephalin) specifically reverses apomorphine-in duced increase in rearing and grooming behaviors in the mouse. Brain Res. 557:77–82; 1991.
15. Ukai, M.; Toyoshi T.; Kameyama, T. Effects of [D-Ala^2, D-Leu^5]enkephalin and [D-Pen^2,L-Pen^5]enkephalin on apomorphine-induced motor activity in the mouse. Pharmacol. Biochem. Behav. 41:171–176; 1992.
16. Ukai, M.; Toyoshi T.; Kameyama, T. Dynorphin A-(1–13) modulates apomorphine-induced behaviors using multi-dimensional behavioral analyses in the mouse. Brain Res. 499:299–304; 1989.
17. Ukai, M.; Toyoshi, T.; Kameyama, T. Dynorphin A-(1–13) preferentially inhibits behaviors induced by the D_2 dopamine agonist RU 24213 but not by the D_1 dopamine agonist SKF 38393. Pharmacol. Biochem. Behav. 42:755–759; 1992.
18. Ukai, M.; Toyoshi, T.; Kameyama, T. Multi-dimensional analyses of behavior in mice treated with the delta opioid agonists DADL (D-Ala^2-D-Leu^5-enkephalin) and DPLPE (D-Pen^2-L-Pen^5-enkephalin). Neuropharmacology 28:1033–1039; 1989.
19. Werling, L. L.; Frattali, A.; Portoghese, P. S.; Takemori, A. E.; Cox, B. M. Kappa receptor regulation of dopamine release from striatum and cortex of rats and guinea pigs. J. Pharmacol. Exp. Ther. 246:282–286; 1988.
20. Zar, J. H. Biostatistical analysis. Englewood Cliffs, NJ: Prentice-Hall; 1984.