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Dynorphin A(1-13) Preferentially Inhibits Behaviors Induced by The D_2 Dopamine Agonist RU 24213 but Not by the D_1 Dopamine Agonist SK&F 38393

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UKAI, M., T. TOYOSHI AND T. KAMEYAMA. *Dynorphin A(1-13) preferentially inhibits behaviors induced by the D_2 dopamine agonist RU 24213 but not by the D_1 dopamine agonist SK&F 38393.* PHARMACOL BIOCHEM BEHAV **42**(4) 755-759, 1992.—The effects of dynorphin A(1-13) on the D_1 dopamine agonist SK&F 38393- and the D_2 dopamine agonist RU 24213-induced behavioral alterations in the mouse were determined by using multidimensional behavioral analyses based upon a capacitance system. Although dynorphin A(1-13) (3.0 or 12.5 μg) alone did not produce any significant effects on behaviors, the peptide (12.5 μg) caused an inhibitory effect on the RU 24213 (3.0 mg/kg)-induced increase in behavioral patterns such as linear locomotion and circling except rearing and grooming behaviors. The antagonistic effects of dynorphin A(1-313) (12.5 μg) were fully reversed by the opioid antagonist Mr 2266 (10.0 mg/kg). However, dynorphin A(1-13) (3.0 or 12.5 μg) failed to affect behaviors elicited by SK&F 38393 (10.0 mg/kg). These results suggest that dynorphin A(1-13) plays an inhibitory role in behaviors induced by the D_2 dopamine agonist but not by the D_1 dopamine agonist, possibly through the mediation of κ-opioid receptors.

Dynorphin A(1-13) SK&F 38393 RU 24213 D_1 dopamine receptor D_2 dopamine receptor
Locomotor activity Mouse

IT has been reported that the peptides coexist with or localize near classical neurotransmitters. For example, opioid peptides abundantly distribute along with the nigrostriatal dopamine system (1). Thus, there should be the close interaction of the peptides with the dopamine system (4,20). Previously, we analyzed the effects of opioid peptides selective for various opioid receptors on apomorphine-induced alterations in behaviors (17,19). For example, dynorphin A(1-13) and DAMGO {[D-Ala^2, NMePhe^4, Gly(ol)]enkephalin} produce an antagonistic effect on the apomorphine-induced increase in rearing behaviors (17,19).

It has been demonstrated that dopamine neurons have at least two distinct receptors: D_1 and D_2 (11). Among them, D_1 receptors are positively coupled with adenylate cyclase, but in contrast D_2 receptors are not or negatively coupled with the cyclase. Recently, the selective dopamine agonists such as SK&F 38393 for D_1 receptors and RU 24213 or quinpirole for D_2 receptors have been available in pharmacological research (11). For in vivo experiments, SK&F 38393 reportedly elicits marked grooming behaviors, while RU 24213 produces marked sniffing behaviors (7,10). However, there is no further characterization of behaviors induced by each of the selective dopamine agonists and particularly by combinations of the dopamine agonists with opioid peptides.

We demonstrated the behavioral effects of opioid peptides and their related drugs by using multidimensional behavioral analyzers based upon a capacitance system in which nine different sizes of behaviors can simultaneously be detected (5,6,13,15,18). For instance, α- and γ-endorphins produce a marked increase in linear locomotion (5,6), while the opioid antagonists naloxone and Mr 2266 produce a significant decrease in linear locomotion without affecting other behavioral measures (14,16). In addition, β-endorphin and U-50,488H, a κ-selective opioid agonist, produce a significant decrease in almost all of the behaviors (6,13). Specifically, the κ-opioid agonist dynorphin A(1-13) has been reported to produce a biphasic effect, that is, naloxone-reversible marked increase in linear locomotion at lower doses (0.3 and 1.0 μg) and no marked effects at higher doses (3.0 and 10.0 μg) (14).

In the present study, the effects of dynorphin A(1-13) on behaviors induced by the D_1-selective dopamine agonist SK&F 38393 and the D_2-selective agonist RU 24213 were determined

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using multidimensional behavioral analyses. The effects of the opioid antagonist Mr 2266 on the effects of dynorphin A(1-13) were also evaluated to clarify the involvement of opioid receptors.

METHOD

Animals

Male ddY mice (Japan SLC, Inc., Hamamatsu, Shizuoka, Japan), weighing between 20-30 g, were employed in the experiments. Animals were randomly assigned to groups consisting of 8-10 mice per group. Before experiments, 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 ± 5%. Mice were used only once and were unfamiliar with the test box. Experiments were conducted between 10:00 a.m. and 6:00 p.m. in a sound-attenuating room.

Multidimensional Analysis

Immediately before multidimensional behavioral analyses, mice were selected according to the number of revolutions (range from 125-150 per 10 min for criterion), employing wheel cages to exclude individual differences of animals as much as possible. About 30% of the mice purchased were discarded for failing the criterion in the first measurement. Mice discarded were repeatedly put into wheel cages for selection on different days (data not used in this study). Finally, we could use almost all mice purchased in the study. Although behaviors were observed over a period of 30 min with a 15-min interval, the data of the latter 15-min interval were only displayed because most behaviors were not markedly influenced during the former 15-min interval. The AnimexII (LKB Farad, Stockholm, Sweden), equipped with an electronic microcomputer, was used for measuring behavior (5,6,13,15). 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 analyzer classified the movement into nine degrees ($\frac{1}{1}$, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, and $\frac{1}{256}$). The surface areas of the cage in which mice could show behavioral responses (ambulation, rearing, and circling) 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: $\frac{1}{1}$(×490.0 mm) = 490.0 mm, $\frac{1}{2}$(×490.0 mm) = 245.0 mm, $\frac{1}{4}$(×490.0 mm) = 122.5 mm, $\frac{1}{8}$(×490.0 mm) = 61.3 mm, $\frac{1}{16}$(×490.0 mm) = 30.6 mm, $\frac{1}{32}$(×490 mm) = 15.3 mm, $\frac{1}{64}$(×490.0 mm) = 7.7 mm, $\frac{1}{128}$(×490.0 mm) = 3.8 mm, and $\frac{1}{256}$(×490.0 mm) = 1.9 mm. The movement of greatest magnitude was principally registered on the $\frac{1}{1}$ counter and the movement of the smallest magnitude, such as tremor, on the $\frac{1}{256}$ counter. Specific patterns of behavior, induced by a drug, were registered on the counters as follows: linear locomotion on $\frac{1}{1}$, circling on $\frac{1}{4}$, rearing on $\frac{1}{16}$, and grooming on $\frac{1}{128}$. 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

SK&F 38393 (Research Biochemicals Inc., Natick, MA), RU 24213 (Roussel-Uclaf, Romainville, France), dynorphin A(1-13) (Peptide Institute, Inc., Minoh, Osaka, Japan), and Mr 2266 (Boehringer Ingelheim KG, Ingelheim am Rhein, Germany) were employed throughout. SK&F 38393 and RU 24213 were dissolved in isotonic saline (0.9% NaCl, pH 7.5).

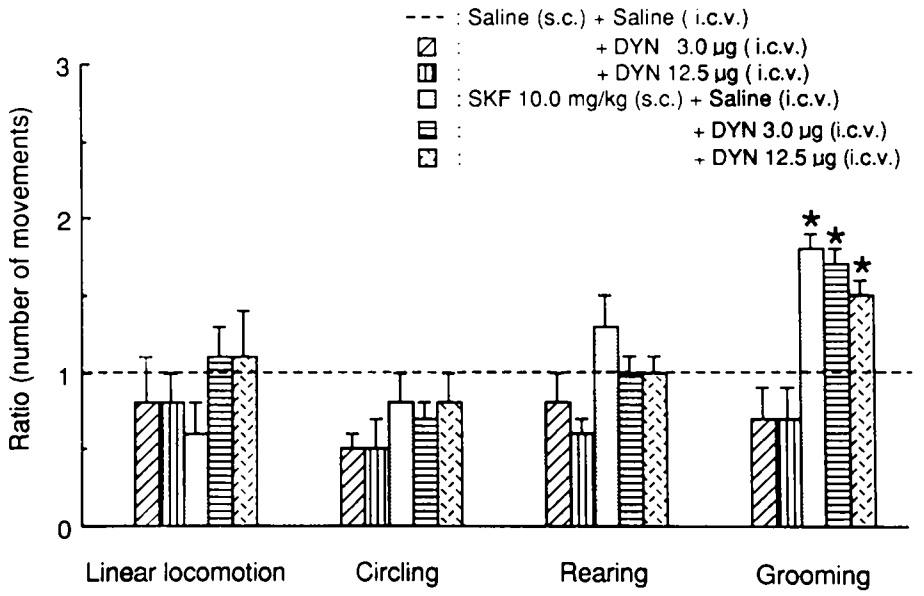

FIG. 1. Spontaneous movements in mice after administration of SK&F (10.0 mg/kg), DYN (3.0 and 12.5 μg), and their combinations. Values represent the mean ± SE for 10 mice. *significant difference from saline control, $p < 0.05$.

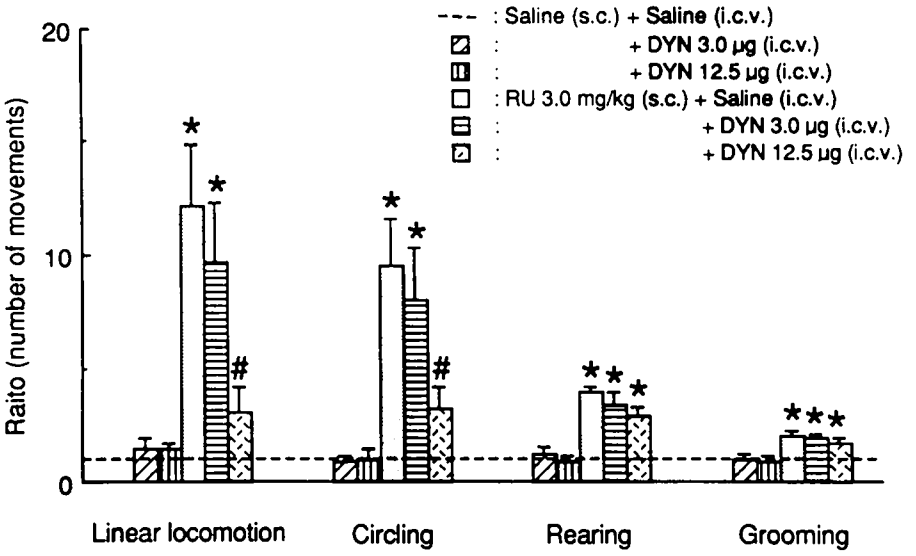

FIG. 2. Spontaneous movements in mice after administration of RU (3.0 mg/kg), DYN (3.0 and 12.5 μg), and their combinations. Values represent the mean ± SE for 10 mice. *significant difference from saline control, $p < 0.05$. #significant difference from RU (3.0 mg/kg) alone, $p < 0.05$.

Mr 2266 was dissolved in 1.0 ml 5% w/v (±)-tartaric acid and the volume was made up with 0.9% saline. SK&F 38393 (SC), RU 24213 (SC), and Mr 2266 (SC) were administered 25, 25, and 15 min before the start of behavioral measurements, respectively. Dynorphin A(1-13) dissolved in sterile isotonic saline in polypropylene containers was injected intracerebroventricularly 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. The injection was made with a 4-mm long needle (30 ga) attached to a 50-μl Hamilton microsyringe. The needle was inserted perpendicularly through the skull and into the brain of the mouse; the drug was injected in a volume of 10 μl per mouse over a period of 20 s as previously described (3,5,6). The site was checked by injecting a 1 : 10 dilution of India ink in isotonic saline. Histological examinations revealed particles of the ink in the lateral and third ventricles but not in the others. As previously described, neither insertion of the needle nor injection of 10 μl isotonic saline solution had a significant influence on behaviors measured by multidimensional behavioral analyses (6).

Data Analysis

Data for actual values were analysed statistically by means of a one-factor analysis of variance (ANOVA). Post-hoc analysis for between-group differences was carried out by the Newman-Keuls method for multiple comparisons (21). Effects were considered statistically significant if $p < 0.05$. Data in figures indicate ratios derived from actual values for the clearer presentation of results.

RESULTS

Effects of Dynorphin A(1-13) on SK&F 38393-Induced Behaviors

ANOVA revealed a significant relation, $F(5, 42) = 10.57$, $p < 0.01$, in grooming (Fig. 1). Dynorphin A(1-13) (3.0 or 12.5 μg) did not affect any behavioral patterns induced by SK&F 38393 (10.0 mg/kg) (Fig. 1).

Effects of Dynorphin A(1-13) on RU 24213-Induced Behaviors

ANOVA showed a significant relation ($p < 0.01$): $F(5, 42) = 8.84$ in linear locomotion, $F(5, 42) = 8.32$ in circling, $F(5, 42) = 15.51$ in rearing, and $F(5, 42) = 6.82$ in grooming (Fig. 2). RU 24213 (3.0 mg/kg) again produced a marked increase in linear locomotion, circling, rearing, and grooming behaviors, although dynorphin A(1-13) (3.0 or 12.5 μg) alone did not affect behaviors (Fig. 2). A 3.0-μg dose of dynorphin A(1-13) did not affect the RU 24213 (3.0 mg/kg)-induced behaviors, while a 12.5-μg dose of the peptide produced an antagonistic effect on the marked increase in linear locomotion and circling behaviors induced by RU 24213 (3.0 mg/kg) without influencing other behavioral parameters such as rearing or grooming behaviors (Fig. 2).

Effects of Mr 2266

ANOVA revealed a significant relation ($p < 0.01$): $F(7, 56) = 13.74$ in linear locomotion, $F(7, 56) = 15.85$ in circling, and $F(7, 56) = 7.47$ in rearing (Fig. 3). The antagonistic effects of dynorphin A(1-13) (12.5 μg) on RU 24213 (3.0 mg/kg)-induced behavioral changes were completely reversed by Mr 2266 (10.0 mg/kg), although Mr 2266 (10.0 mg/kg) alone did not affect any behavioral patterns (Fig. 3).

DISCUSSION

We used multidimensional behavioral analyses to clarify the behavioral effects of opioid peptides and opioid-related compounds (5,6,18). This apparatus can simultaneously analyze and record nine different kinds of behaviors in the mouse based upon a capacitance system, demonstrating the close relationship between actual number of behaviors ob-

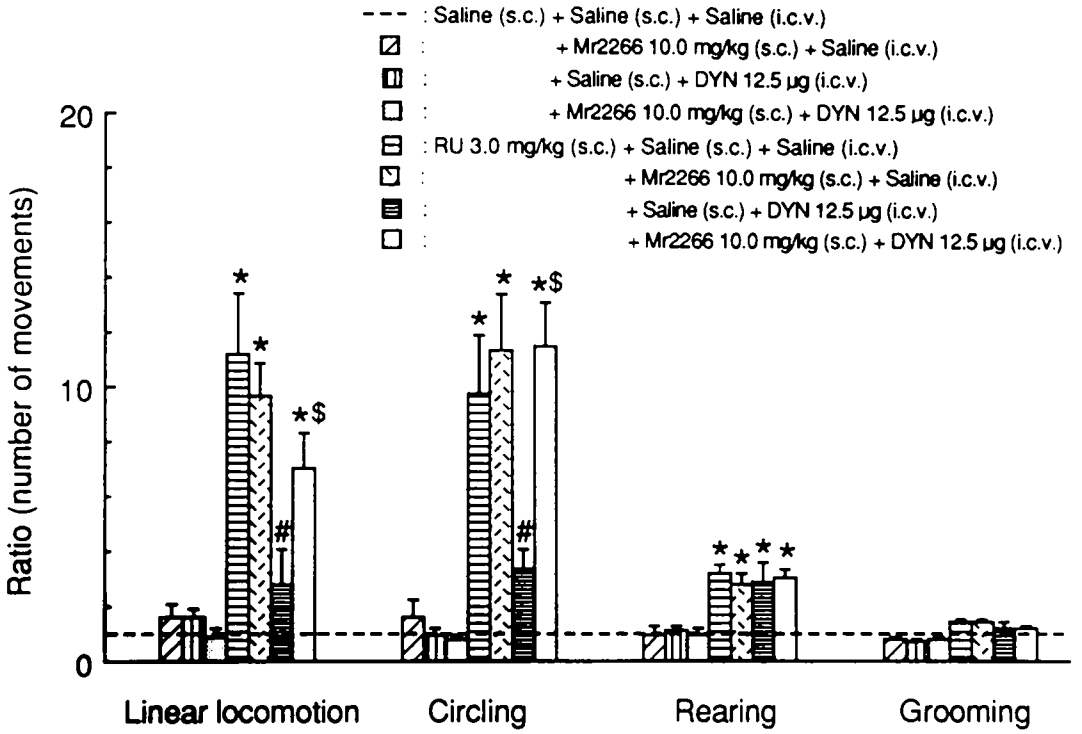

FIG. 3. Spontaneous movements in mice after administration of RU (3.0 mg/kg), DYN (12.5 μg), Mr 2266 (10.0 mg/kg), and their combinations. Values depict the mean ± SE for 10 mice. *significant difference from saline control, $p < 0.05$. #significant difference from RU (3.0 mg/kg) alone, $p < 0.05$. $significant difference from RU (3.0 mg/kg) plus DYN (12.5 μg), $p < 0.05$.

served by experimenters and counts for sizes of movements (17,18).

Recent evidence indicates that the nonspecific dopamine agonist apomorphine (0.56 and 1.0 mg/kg)-induced increase in rearing behaviors is completely inhibited by dynorphin A(1-13) (10.0 μg) (18). The inhibitory effects of dynorphin A(1-13) (10.0 μg) are entirely reversed by the opioid antagonist Mr 2266 (17). On the other hand, it has been demonstrated that RU 24213 strongly reduces the striatal binding of [^{3}H]-spiperone without influencing striatal dopamine-sensitive adenylate cyclase activity. This D_2-selective agonist action seems to be preserved in vivo, where its effects can be reversed by a D_2-selective antagonist without showing any signs of D_1 agonist activity (2,9). This is the first reported study that dynorphin A(1-13) specifically inhibited the behavioral effects of the D_2 dopamine agonist RU 24213. In particular, the present results indicate that although only linear locomotion and circling behaviors induced by RU 24213 were antagonized by a 12.5-μg dose of dynorphin A(1-13) other behaviors such as rearing or grooming behaviors were not antagonized by that dose, thus suggesting that dynorphins play an inhibitory role only in linear locomotion and circling behaviors. In addition, Mr 2266, which possesses relatively high affinity for κ-opioid receptors, almost completely reversed the effects of dynorphin A(1-13) on behaviors induced by RU 24213. It thus seems that the inhibitory effects of dynorphin A(1-13) are elicited, at least in part, through κ-opioid receptors. The lack of effects of dynorphin A(1-13) on rearing behaviors induced by RU 24213 was unexpected since it has been reported that dynorphin A(1-13) (10.0 μg) inhibits such behaviors induced by the nonspecific dopamine agonist apomorphine (17), suggesting that dynorphin A(1-13) inhibits rearing behaviors mediated through both D_1 and D_2 dopamine receptors but not through D_2 alone. Moreover, our recent report has demonstrated that the μ-opioid agonist DAMGO, but not the δ-opioid agonist [D-Pen^2, L-Pen^5]enkephalin, produces an inhibitory effect on behaviors induced by RU 24213 (12). Taken together, it is probable that both μ- and κ-opioid receptors are involved in the inhibition of behaviors induced by RU 24213. However, the inhibitory effects of dynorphin A(1-13) on behaviors induced by RU 24213 would be mediated through κ- but not μ-opioid receptors because the μ-opioid antagonist β-funaltrexamine antagonizes the effects of DAMGO (12) without influencing those of dynorphin A(1-13) (unpublished observation).

Because Mulder et al. (8) demonstrated that κ-opioid agonists inhibit striatal dopamine release, it is possible that the inhibitory effects of dynorphin A(1-13) on behaviors induced by RU 24213 are mediated via the decrease in dopamine release. In contrast with the case of RU 24213, dynorphin A(1-13) (3.0 or 12.5 μg) did not affect behavioral effects induced by SK&F 38393, suggesting that dynorphins do not play a modulatory role in behaviors induced by the selective activation of D_1 dopamine receptors.

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