Opioid Receptor Agonists Selective for μ and κ Receptors Attenuate Methamphetamine-Induced Behavioral Sensitization in the Mouse

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The effects of intracerebroventricular (i.c.v.) injection of the μ -selective opioid receptor agonist [D-Ala², N-MePhe⁴, Gly-ol]enkephalin (DAMGO) and the κ -selective opioid receptor agonist dynorphin A-(1–13) on the development of methamphetamine-induced behavioral sensitization in the mouse were determined using multidimensional behavioral analyses based upon a capacitance system. Methamphetamine (2 mg/kg, s.c.) was administered to mice on 6 occasions at 3- or 4-d intervals. The methamphetamine-induced increase in linear locomotion and circling was markedly augmented by repeated administrations (3 or more times) of the drug, showing behavioral sensitization. Although repeated administrations of DAMGO (0.003 and 0.01 μ g, i.c.v.) or dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) alone did not produce any significant effects on behavior, repeated administrations of DAMGO (0.003 and 0.01 μ g, i.c.v.) and dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) attenuated the behavioral sensitization induced by methamphetamine (2 mg/kg, s.c.). The attenuating effects of DAMGO (0.003 and 0.01 μ g, i.c.v.) and dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) were fully reversed by withdrawal of these drugs for 3 weeks. Additionally, a single administration of DAMGO (0.003 and 0.01 μ g, i.c.v.) or dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) alone did not produce any significant effects on behavior; DAMGO (0.003 and 0.01 μ g, i.c.v.) and dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) only attenuated the behavioral sensitization which had previously been developed by methamphetamine (2 mg/kg, s.c.). These results suggest that opioid receptor agonists selective for μ and κ receptors play an inhibitory role in the development of methamphetamine-induced behavioral sensitization.

Key words methamphetamine; [D-Ala², N-MePhe⁴, Gly-ol]enkephalin; dynorphin A-(1–13); behavioral sensitization; reverse tolerance; mouse

Repeated administrations of psychostimulants, *i.e.* methamphetamine and amphetamine, are known to produce sensitization of the behavioral responses elicited by a small dose of these drugs.¹⁾ The responses sensitized with methamphetamine and amphetamine have been associated with central dopaminergic systems.^{1–8)}

It has previously been reported that methamphetamine produces a marked increase in linear locomotion, circling, rearing and grooming behavior.^{9,10)} The behavioral effects of methamphetamine seem to be indirectly mediated through dopamine D₂ receptors, since the selective dopamine D₂ receptor antagonist S(–)-sulpiride, unlike the selective dopamine D₁ receptor antagonist SCH 23390, fully antagonizes the effects of methamphetamine.⁹⁾ [D-Ala², N-MePhe⁴, Gly-ol]enkephalin (DAMGO) and dynorphin A-(1–13) produce antagonistic effects on methamphetamine-induced behavioral responses.^{9,10)} However, little is known about the involvement of opiodergic systems in methamphetamine-induced behavioral sensitization.

Opioid receptors in the central nervous system can be divided into at least three different types: μ , δ and κ .^{11–13)} Previous reports have demonstrated the pharmacological interactions of μ -, δ - and κ -selective opioid receptor agonists with dopaminergic neurons. For instance, opioid peptide-containing terminals have been demonstrated to provide direct synaptic input into dopaminergic neurons in the rat ventral tegmental area (VTA).¹⁴⁾ Morphine injected into the VTA markedly increases the content of dopamine and its metabolites in the nucleus accumbens.¹⁵⁾

On the other hand, amphetamine- or methamphetamine-induced behavioral sensitization is perhaps considered the best known approach to the experimental

study of schizophrenia.¹⁶⁾ Although Ujike *et al.*¹⁷⁾ have reported that the selective dopamine D₂ receptor antagonist YM-09151-2 attenuates the methamphetamine-induced development of behavioral sensitization, it is of great interest to determine whether μ - or κ -opioid receptor agonists affect the methamphetamine-induced behavioral sensitization.

In the present study, the effects of an intracerebroventricular injection of DAMGO and dynorphin A-(1–13) on the development of methamphetamine-induced behavioral sensitization in the mouse were determined using multidimensional behavioral analyses, which can measure various types of behavior.^{18–21)}

MATERIALS AND METHODS

Animals Male ddY mice (Japan SLC, Inc.) weighing between 30 and 35 g were employed in the experiments. The animals were randomly assigned to groups consisting of 8 to 16 mice. 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 (temperature of 23 $\pm$ 1 °C, relative humidity of 55 $\pm$ 2.5%, light-dark cycle with the light on between 8 a.m. and 8 p.m.). The mice were used only once and were unfamiliar with the test box. The experiments were conducted between 10 a.m. and 6 p.m. in a sound-attenuated room.

Multidimensional Analysis Immediately before multidimensional analyses, mice were selected according to their activity measured by the number of revolutions criterion (range from 125 to 150 per 10 min, employing wheel cages), to exclude individual differences of animals as much as possible. About 30% of the mice purchased were discarded

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for failing the criterion in the first measurement. The discarded mice were repeatedly put into wheel cages for selection on different days, so that eventually we used almost all of the mice purchased in the study. Behavior was observed over a period of 15 min. The Animex II, equipped with a personal computer, was used for measuring the behavior.^{18,22-25} 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, a d.c. voltage movement spectrum analyzer classified the movement into 9 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 could show behavioral responses (ambulation, circling, rearing and grooming) 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 mm) = 490 mm, 1/2 (× 490 mm) = 245 mm, 1/4 (× 490 mm) = 122.5 mm, 1/8 (× 490) = 61.3 mm, 1/16 (× 490 mm) = 30.6 mm, 1/32 (× 490 mm) = 15.3 mm, 1/64 (× 490 mm) = 7.7 mm, 1/128 (× 490 mm) = 3.8 mm, and 1/256 (× 490 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 a 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 1/64.²³ Additionally, the circling behavior involved an animal's movement along every nook and corner of the testing cage in this study. 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 as a ratio mean ± S.E. after calculating the ratios for each of the animals. The ratios for each of the animals = (each of the actual values of drug-treated animals)/(mean actual values of controls).

Drugs and Administration Schedules Methamphetamine hydrochloride (Dainippon Pharmaceutical Co., Osaka, Japan), DAMGO (Peninsula Laboratories, Inc., Belmont, California, U.S.A.) and dynorphin A-(1–13) (Peptide Institute, Inc., Osaka, Japan) were employed throughout. DAMGO, dynorphin A-(1–13) and methamphetamine were dissolved in isotonic saline (0.9% NaCl, pH 6.4). Methamphetamine was administered s.c. 25 min before the start of behavioral observations. DAMGO and dynorphin A-(1–13) were intracerebroventricularly injected 10 min before the start (that is, 15 min after the methamphetamine). The unilateral i.c.v. injection site was 2 mm from either side of the midline on a line drawn through the anterior roots of the ears.²⁶ 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 lateral ventricle of the brain. The mouse was anesthetized with ether. Solutions were injected in a volume of 5 µl per mouse over a period of 20 s as previously described.^{22,23} The site was checked by injecting a 1:10 dilution of India ink in isotonic saline (0.9% NaCl, pH 6.4). Histological examinations revealed particles of the ink in the lateral and 3rd ventricles, but not in the others. As previously described,²³ neither the insertion of the needle nor the injection of 5 µl of isotonic saline solution had a significant influence on behavior as measured by multi-dimensional behavioral analyses. The dose of methamphetamine was fixed at 2 mg/kg, and drug administration was carried out at 3- or 4-d intervals (twice a week). The following drug administrations were carried out.

Repeated Administration of Methamphetamine with DAMGO or Dynorphin A-(1–13) Methamphetamine alone and its combination with DAMGO or dynorphin A-(1–13) were administered to mice on 6 occasions at 3- or 4-d intervals. On the 3rd week after the final (6th) administration, a challenge dose (2 mg/kg, s.c.) of methamphetamine was given to all of the mice (Fig. 1, Exp. 1).

A Single Administration of DAMGO or Dynorphin A-(1–13) in Methamphetamine-Sensitized Mice Methamphetamine was administered to mice on 5 occasions

Fig. 1. Experimental Schedule for the Development of Methamphetamine (MAP)-Induced Behavioral Sensitization in Mice

Abbreviations: ↓, administration; Δ, behavioral measurement. DYN, dynorphin A-(1–13).

at 3- or 4-d intervals. On the 3rd day after the last administration of methamphetamine, all of the mice were challenged with methamphetamine (2 mg/kg, s.c.), DAMGO (0.003 and 0.01 μ g, i.c.v.), dynorphin A-(1–13) (3 and 12.5 μ g, i.c.v.) or their combinations (Fig. 1, Exp. 2).

Data Analysis Time-effect curves were analyzed by two-factor (treatment vs. time) analysis of variance with repeated measures. Data at each time point were analyzed by a one-factor analysis of variance. Post hoc analysis of between-group differences was carried out by the Newman-Keuls test for multiple comparisons²⁷⁾ or the Student's *t*-test. Effects were considered statistically significant if $p < 0.05$. Data in the figures indicate the ratios derived from the actual values for a clearer presentation of the results.

RESULTS

Inhibitory Effects of DAMGO or Dynorphin A-(1–13) on the Development of Methamphetamine-Induced Behavioral Sensitization The methamphetamine (2 mg/kg)-induced increase in linear locomotion and circling, but not

grooming, was markedly augmented by repeated (3 or more) administrations of the drug, showing behavioral sensitization. The methamphetamine (2 mg/kg)-induced behavioral sensitization remained evident even after withdrawal for 3 weeks. Although repeated administrations of DAMGO (0.003 and 0.01 μ g) or dynorphin A-(1–13) (3 and 12.5 μ g) alone were without any significant effects on behavior, repeated administrations of DAMGO (0.003 and 0.01 μ g) and dynorphin A-(1–13) (3 and 12.5 μ g) attenuated the behavioral sensitization induced by methamphetamine. The attenuating effects of DAMGO (0.003 and 0.01 μ g) and dynorphin A-(1–13) (3 and 12.5 μ g) were fully reversed by the withdrawal of these drugs for 3 weeks (Figs. 2 and 3).

Inhibitory Effects of DAMGO or Dynorphin A-(1–13) in Methamphetamine-sensitized Mice Repeated administrations of methamphetamine (2 mg/kg) markedly sensitized behavioral responses such as linear locomotion and circling, but not grooming. Although a single administration of DAMGO (0.003 and 0.01 μ g) or dynorphin A-(1–13) (3 and 12.5 μ g) alone did not produce any significant effect on behavior, DAMGO (0.003 and

Fig. 2. Effects of Repeated Administrations of DAMGO on the Development of Methamphetamine (MAP)-Induced Behavioral Sensitization in Mice

Values represent the mean $\pm$ S.E. for 8–16 mice. * $p < 0.05$, ** $p < 0.01$ vs. values on the 1st administration (ANOVA followed by Student's *t*-test), # $p < 0.05$, ## $p < 0.01$ vs. MAP 2 mg/kg alone (ANOVA followed by Newman-Keuls test).

Fig. 3. Effects of Repeated Administrations of Dynorphin A-(1—13) on the Development of Methamphetamine (MAP)-Induced Behavioral Sensitization in Mice

Values represent the mean $\pm$ S.E. for 8—16 mice. * p < 0.05, ** p < 0.01 vs. values on the 1st administration (ANOVA followed by Student's t -test), # p < 0.05, ** p < 0.01 vs. MAP 2 mg/kg alone (ANOVA followed by Newman-Keuls test).

Fig. 4. Effects of a Single Administration of DAMGO on the Development of Methamphetamine (MAP)-Induced Behavioral Sensitization in Mice

Values represent the mean $\pm$ S.E. for 8—10 mice. * p < 0.05, ** p < 0.01 vs. MAP 2 mg/kg ($\times$ 1) alone (ANOVA followed by Newman-Keuls test), # p < 0.01 vs. MAP 2 mg/kg ($\times$ 6) alone (ANOVA followed by Newman-Keuls test).

Fig. 5. Effects of a Single Administration of Dynorphin A-(1—13) on the Development of Methamphetamine (MAP)-Induced Behavioral Sensitization in Mice

Values represent the mean $\pm$ S.E. for 8—10 mice. * p < 0.05, ** p < 0.01 vs. MAP 2 mg/kg ($\times$ 1) alone (ANOVA followed by Newman-Keuls test), ## p < 0.01 vs. MAP 2 mg/kg ($\times$ 6) alone (ANOVA followed by Newman-Keuls test).

0.01 μ g) and dynorphin A-(1—13) (3 and 12.5 μ g) attenuated the behavioral sensitization, which had previously been developed by methamphetamine (2 mg/kg) (Figs. 4 and 5).

DISCUSSION

It has previously been reported that methamphetamine (1 mg/kg) produces a marked increase in linear locomotion, circling, rearing and grooming behavior.^{9,10} DAMGO (0.003 and 0.01 μ g) and dynorphin A-(1—13) (12.5 μ g) produce antagonistic effects on these methamphetamine (1 mg/kg)-induced behavioral responses.^{9,10} However Ujike *et al.*¹⁷ have reported that the selective dopamine D₁ receptor antagonist SCH 23390, as well as the selective dopamine D₂ receptor antagonist YM-09151-2, attenuate the stereotyped behavior and development of behavioral sensitization induced by methamphetamine. The behavioral effects of methamphetamine (1 mg/kg) seem to be indirectly mediated through dopamine D₂ receptors, since the selective dopamine D₂ receptor antagonist S(–)-sulpiride, unlike SCH 23390, fully antagonizes the effects of methamphetamine.⁹

In the present study, the methamphetamine (2 mg/kg)-induced increase in linear locomotion and circling was markedly augmented by repeated administrations (3 or more times) of the drug, showing behavioral sensitization. Although repeated administrations of DAMGO (0.003 and 0.01 μ g) or dynorphin A-(1—13) (3 and 12.5 μ g) alone did not produce any significant effects on behavior, repeated administrations of DAMGO (0.003 and 0.01 μ g) or dynorphin A-(1—13) (3 and 12.5 μ g) attenuated the behavioral sensitization which had previously been developed by methamphetamine (2 mg/kg). The effects of DAMGO (0.003 and 0.01 μ g) and dynorphin A-(1—13) (3 and 12.5 μ g) were fully reversed by withdrawal of these drugs for 3 weeks. Additionally, a single administration of DAMGO (0.003 and 0.01 μ g) or dynorphin A-(1—13) (3 and 12.5 μ g) alone was without any significant effects on behavior, whereas DAMGO (0.003 and 0.01 μ g) and

dynorphin A-(1—13) (3 and 12.5 μ g) attenuated the behavioral sensitization induced by methamphetamine (2 mg/kg). These results suggest that the activation of μ - and κ -opioid receptors plays an inhibitory role in the development of methamphetamine-induced behavioral sensitization.

Dynorphin A-(1—13) (3 or 12.5 μ g) and DAMGO (0.003 or 0.01 μ g) inhibited the methamphetamine (1 mg/kg)-induced increase in rearing.^{9,10} The action sites of dynorphin A-(1—13) and DAMGO in the inhibitory effects on the behavior mentioned above seem to be postsynaptic in the brain, because either dynorphin A-(1—13) or DAMGO failed to affect the decrease in behavior induced by a low dose of apomorphine, which preferentially affects the presynaptic sites of dopaminergic neurons.²⁸ Also, intrastriatal injection of these opioid receptor agonists inhibits the behavioral sensitization induced by apomorphine in 6-hydroxydopamine-treated mice.^{20,29} Moreover, we have demonstrated that DAMGO (0.003 and/or 0.01 μ g) and dynorphin A-(1—13) (3 and/or 12.5 μ g) inhibited the increase in linear locomotion, circling, rearing and/or grooming behavior induced by the dopamine D₂ receptor agonist RU 24213 (3 mg/kg, s.c.).^{30,31} These findings suggest that DAMGO and dynorphin A-(1—13) play an inhibitory role in the behavioral responses induced by the activation of postsynaptic dopamine D₂ receptors.

Taken together, DAMGO and dynorphin A-(1—13) may be available for the therapeutic treatment of schizophrenia, because amphetamine- or methamphetamine-induced behavioral sensitization appears to be one of the animal models for schizophrenia.¹⁶

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