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Biphasic Effects of Intraaccumbens μ-Opioid Peptide Agonist DAMGO on Locomotor Activities

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MEYER, M. E., B. I. McLAURIN, M. ALLEN AND M. E. MEYER. *Biphasic effects of intraaccumbens μ-opioid agonist DAMGO on locomotor activities.* PHARMACOL BIOCHEM BEHAV 47(4) 827–831, 1994.—The effects of bilateral microinjections of μ-opioid receptor agonist DAMGO (0.00, 0.01, 0.1, or 1.0 μg/side) were tested in rats for 120 min in activity monitors. The horizontal movement, rearing, and stereotypy times in seconds were measured during 12 consecutive 10-min time blocks. DAMGO (0.01, 0.1, and 1.0 μg) resulted in biphasic effects, inhibition followed by activation for each of the three measures. These data replicate the behavioral effects of ICV DAMGO except that the duration of the behavioral effects were longer with Acb injections.

μ-Opioid peptide agonist DAMGO Locomotor activity Horizontal movement Rearing Stereotypy

THERE are at least three major families of pharmacologically different opioid receptor subtypes, μ, δ and κ receptors (4, 10,20,27,38,39). Subclassifications of these three receptor subtypes has been suggested. However, the subclassifications are not well defined in binding assays and there is no convincing evidence for receptor subclassification in bioassays. With the development of selective ligands and their availability in [^{3}H]-labelled form, the μ-binding sites have been labelled (11,18).

The effects of various opioid peptide 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 in an initial suppression followed by hyperactivity (2,13,14,15,31,33). Recent studies with mice using DAMGO, a specific μ-opioid peptide, injected intracerebroventricularly ICV, suggests that DAMGO induced an increase in horizontal activity and a decrease in rearing without the typical biphasic effect (26). On the other hand, ICV-injected DAMGO in rats resulted in biphasic effects across various measures of horizontal movement, rearing, and stereotypy behaviors, and an inhibition of thigmotaxis (21,22).

The nucleus accumbens (Acb) is one of a number of neural sites associated with the μ-opioid receptor subtype. Furthermore, the Acb has been suggested as a limbic system site for the integration of cognitive, motivational, and locomotor processes. Recently, it has been reported that a specific μ-agonist, PL017, in the rat Acb elicited motor inhibition, rigidity, and catalepsy (19). However, when compared to ICV DAMGO and DALDA, Pl017 was less effective in altering locomotor activity (22). On the other hand, microinjections of DAMGO into the Acb has been reported to result in inhibition followed by activation of locomotor activity but not rearing (6).

The present study expands the generality of central infusion of DAMGO to intraaccumbens effects on locomotor activities in rats. We report here our findings of the effects of intraaccumbens injection of μ-opioid peptide agonist, DAMGO, on horizontal activity, rearing, and stereotypy during 12 consecutive 10-min time blocks.

METHOD

Animals

Male Long-Evans rats weighing 200–225 g were obtained from Charles River. The rats were individually housed in stainless steel cages, had food and water ad lib, and main-

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tained on a 12 L : 12 D (0700–1900 h) cycle. The animals were tested in the light phase between 1000–1600 h. The room in which the 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

The animals, while under equithesin anesthesia, were cannulated bilaterally with the use of a stereotaxic instrument. Guide cannulae fabricated from 23 g stainless steel hypodermic needles were permanently fixed to the skull with microscrews and dental cement. The 23 g guide cannulae were implanted for the Acb following the coordinates from Paxinos and Watson (28) with reference to bregma, midline, and skull surface, respectively: Acb, +1.7, ±1.4, −4.0 mm (injection cannulae, −7.0). The animals were allowed 2 weeks recovery before behavioral testing. During recovery, the animals were not handled or transported except for routine cleaning.

Histology

After completing behavioral testing, the rats were administered an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains removed and placed in 10% buffered formalin. The brains were frozen, sectioned, mounted on slides, stained with cresyl violet, and the locations of the injection cannulae verified by two independent observers. Only those rats with bilateral placements in the Acb were used in the data analyses.

Drugs and Drug Administration

The μ-receptor agonist DAMGO (Tyr-D-Ala-Gly N-Methyl-Phe-Gly-ol; Mol. wt. 513.7) was obtained from Bachem (Torrance, CA). All peptides were dissolved in distilled water. Distilled water was also given for the vehicle control injections (0.00 μg). The drug solutions were made up daily to the concentrations of 0.01, 0.1, and 1.0 μg. The 0.5 μl of solution was microinjected through injection 30 g cannulae over a period of 60 s and the cannulae remained in place for another 30 s.

Apparatus

Immediately following bilateral Acb injections, each rat was placed in an Omnitech Digiscan Animal Activity Monitor (Columbus, OH) for 120 min. In this experiment, data were collected every 10 min. The acrylic cage within the monitor measured approximately 42 cm wide by 42 cm long and 30.5 cm high. The monitor was equipped with 16 beams 2.54 cm apart from front to back and from side to side on the lower level, as well as 16 beams 2.54 apart from side to side on the upper level. Every 100 ms, the computer sampled the status of all the beams. The Digiscan analyzer converted the patterns of the beams broken into different measures of locomotor activity. The measures automatically analyzed in this study were the horizontal movement time in seconds (as long as the animal moved, movement time was incremented); rearing time in seconds (as long as the animal was rearing and activated the upper sensors, rearing time was incremented); and stereotypy time in seconds (as long as the animal was repeatedly breaking the same beam or set of beams, the monitor considered the animal was emitting stereotypy behavior; this measurement corresponded to grooming, head bobbling, and weaving, chewing, etc.). These measurements were made during 12 consecutive 10-min time blocks.

Statistics

There were 11 animals in each independent treatment group. The animals were treated only once.

A two-factor mixed design ANOVA was used to analyze the within-measures (12 consecutive 10-min time blocks), between-treatment conditions (four dose levels), and the time by dose interaction effect. Significant interactions for the dose by 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 to be statistically significant.

RESULTS

Horizontal Movement Time

Figure 1A illustrates a biphasic dose-response curve over the 12 consecutive 10-min time block of horizontal movement as measured in s, treated with four dose levels of DAMGO (vehicle, 0.01, 0.1, and 1.0 μg/side) bilaterally injected into the Acb.

The four treatment groups by 12 consecutive 10-min time blocks interaction was highly significant $F(33, 429) = 17.42$, $p < 0.001$. From the subsequent analyses between the 1.0 μg group and the vehicle control group revealed a biphasic effect for DAMGO. At the 10–50-min time blocks, there were significant suppression of horizontal movement time ($ps < 0.05$ and 0.01) and at the 90–120-min time blocks, there were significant potentiation ($ps < 0.01$). The subsequent analyses between 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 were significant suppression ($ps < 0.01$) and at time blocks of 50–110-min blocks significant potentiation ($ps < 0.05$ and 0.01). The subsequent analyses for the 0.01 μg group, revealed significant suppression at time block 10 min ($p < 0.01$) and potentiation at time blocks of 30–70 min ($ps < 0.05$ and 0.01).

Rearing Time

Figure 1B shows the significant interaction effects on rearing between the three dose levels and the vehicle control group over the 12 consecutive 10-min time blocks, $F(33, 429) = 9.26$, $p < 0.001$. The subsequent analyses revealed that across the 12 10-min time blocks, the 1.0 μg group was significantly suppressed at time blocks 10–40 min ($ps < 0.01$) and potentiated from 90–120 min ($ps < 0.05$). The 0.1 μg group was significantly suppressed the 10–30-min blocks ($ps < 0.05$ and 0.01) and was potentiated at 60–90-min time blocks ($ps < 0.05$ and 0.01). At the 10-min time block, the 0.01 μg group was suppressed ($p < 0.01$) and this group was potentiated between time blocks 50–70 min ($ps < 0.05$ and 0.01).

Stereotypy Time

Figure 1C illustrates the biphasic effects of DAMGO on stereotypy time. The ANOVA resulted in a highly significant dose by time block interaction, $F(33, 429) = 9.51$, $p < 0.001$. Between time blocks 10–60 min, the 1.0 μg group stereotypy time was significantly attenuated and potentiated between 90–120-min time blocks ($ps < 0.05$ and 0.01). On the other hand, the stereotypy time for the 0.1 μg group was attenuated between 10–40-min time blocks and potentiated at time blocks 70, 80, and 100 ($ps < 0.01$). Lastly, the 0.01 μg group was significantly attenuated at time blocks 10 and 20 min

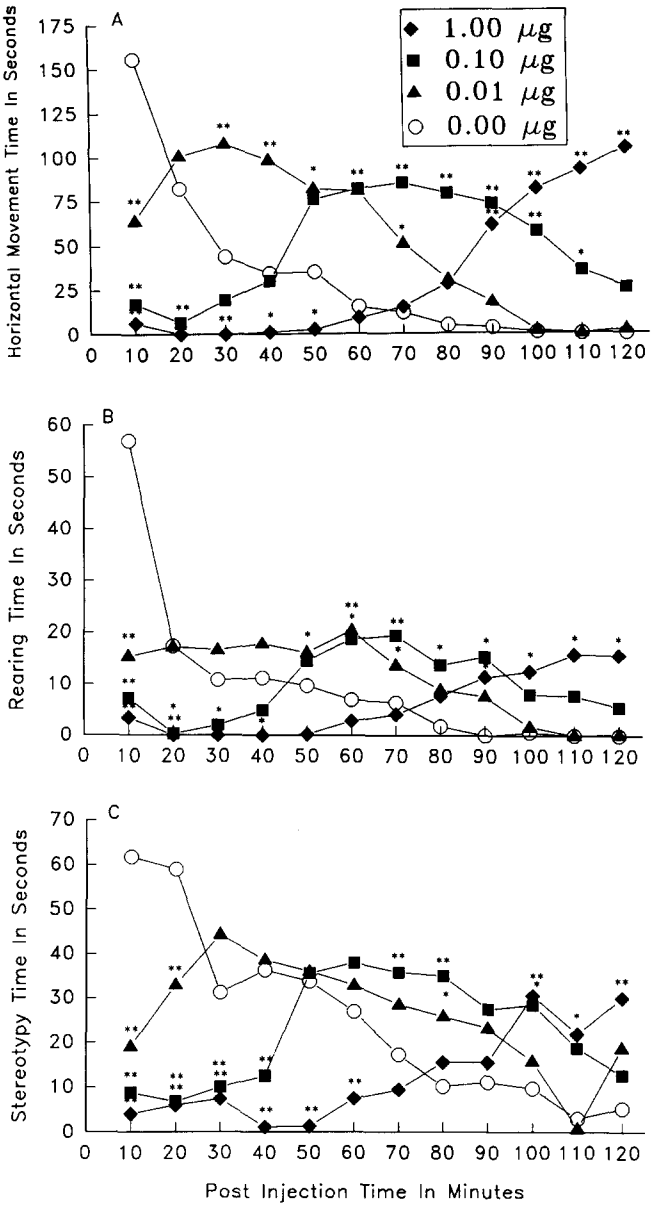

FIG. 1. (A) Significant interaction effects of Acb injected 0.00, 0.01, 0.1, and 1.0 μg dosages of the μ-opioid peptide DAMGO on horizontal movement time in seconds over 12 10-min intervals. (B) Significant interaction effects of Acb injected 0.00, 0.01, 0.1, and 1.0 μg dosages of DAMGO on rearing time in seconds over 12 10-min intervals. (C) Significant interaction effects of Acb injected 0.00, 0.01, 0.1, and 1.0 μg dosages of DAMGO on stereotypy time in seconds over 12 10-min intervals. Significant differences from the vehicle control group (0.00 μg) at each time point: $*p < 0.05$; $**p < 0.01$.

and potentiated at only time block 80 min ($ps < 0.05$ and 0.01).

DISCUSSION

The results presented in this paper provide information on the role of the μ-receptor in mediating changes in locomotor behavior following intraaccumbens administration of the μ-receptor peptide agonist, DAMGO.

Morphine has been considered the μ-receptor prototype agonist as this nonpeptide agonist has a high affinity for the μ-receptor (29,30). Low dosages of morphine typically elicits 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 curves of the μ-receptor peptide agonist, DAMGO, in this present study on locomotor activity, were similar to the effects of morphine in rats (1, 6,13,31). The dose range (0.01-1.0 μg) exerted a significant biphasic effect with an initial suppression followed by significant activation across the three measures of horizontal movement, rearing, and stereotypy times. The differences in behavioral effects among the three dosages of DAMGO were related to the time course of the dose. The largest dose (1.0 μg) resulted in longer initial suppression and later potentiation, whereas, the smallest dosage (0.01 μg) had a short duration of suppression, followed by potentiation, and then no effect during the later time blocks. The 0.1 μg dose had intermediate effects. The present data differs in details from a previous report of intraaccumbens DAMGO (6). It was reported that DAMGO resulted in an initial inhibition followed by activation for activity counts but not for rearing counts, and that only the 1.0 μg dose contributed to the dose by duration interaction. The differences between the studies may be accounted for by the level of measurements associated with the behavioral activity chambers and experimental procedures for habituation.

These biphasic effects were very similar to other reports of ICV DAMGO (21,22) with rats. However, the duration of the effects were longer following Acb injections. On the other hand, the present data differ from those reported with mice where only a single-slope dose hyperactivity were observed (26). Mice show especially significant excitation to opioids, which may explain the differences between rats and mice (13,33).

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 (31). It has been suggested that the locomotor suppression is mediated by μ-receptor, and the activation is a function of the δ-receptor subtype (8,9,12). However, with DAMGO having a very high affinity at the μ-receptor, it is highly unlikely that the two opioid process theory could explain the present biphasic data. On the other hand, it has been established that opiates and opioid peptides induce monophasic suppression if injected in the periaqueductal gray and unidirectional excitation via the striatum (7,16). Furthermore, this biphasic effect may be due to an initial peptide-induced akinesia and to local diffusion of the peptide to various brain regions. For example, DAMGO, microinjected into the ventral tegmental area, elicits hyperactivity (16,37). The diffusion hypothesis is in question, as the most diffusion site would have been the dorsal striatum. It has been shown that the Acb and the dorsal striatum have differential effects when microinjected with the same peptide or transmitter (8,19,36).

It has been hypothesized that biphasic suppression-activation is a function of opioid peptides that modulate locomotor activity primarily within mesolimbic dopaminergic system (3,17,29,37). Recent studies have shown that opioid-elicited locomotor activity can be disrupted by dopaminergic antagonists (3,17,19,29,37,) and that opioid peptides can antagonize dopamine-elicited behaviors (34,35). If the dopamine-opioid peptide hypothesis is correct, the effects are in

all probability complex and indirect. For example, many of the dopamine agonists also elicit biphasic effects of suppression activation (23-25,36).

Perhaps the most important area for future research will be studies that focus upon the anatomical substrates that underlie various behavioral effects elicited by selective opioid receptors agonists and antagonists and are also functionally related to the dopaminergic systems (5,7,9,32).

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