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Intra-Accumbens δ_1-Opioid Agonist, pCl-DPDPE, Differentially Affects Patterns of Locomotor Activity

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MEYER, M. E., B. I. McLAURIN AND M. E. MEYER. *Intra-accumbens δ_1-opioid agonist, pCl-DPDPE, differentially affects patterns of locomotor activity.* PHARMACOL BIOCHEM BEHAV 51(2/3) 359–362, 1995.—The effects of bilateral microinjections of a new potent and highly selective δ-opioid receptor agonist pCl-DPDPE (0.00, 0.1, 1.0, or 2.5 μg/side) were tested in rats for 60 min in activity monitors. The durations of horizontal movement time, rearing time, and stereotypy time were measured during six consecutive 10-min time blocks. The pCl-DPDPE resulted in short-lived biphasic effects of attenuation followed by potentiation for the three measures. These data in part replicate the behavioral effects of other δ-opioid receptor agonists.

δ-Opioid agonist pCl-DPDPE Locomotor activity Horizontal movement Rearing Stereotypy Rats

THERE is evidence that there are at least three major families of pharmacologically different opioid subtypes: μ, δ, and κ receptors. However, δ receptor subtypes have been suggested (6,10). With the development of selective ligands and their availability in ^{3}H -labelled form, the δ_1 binding sites have been labelled (1,14,15).

It has been argued that the opioid peptides are involved in cognitive, motivational, emotional, and motor activities through the limbic and motor dopaminergic systems (7). The nucleus accumbens (Acb) has been thought as a significant site for the integration of the limbic system associated with cognition, motivation/emotion, and locomotor activities. The Acb is one of the neural sites with a substantial density of δ-opioid receptors (9). Bilateral Acb microinjections of [D-Pen^{2,5}]enkephalin, a specific δ_1-opioid receptor agonist, elicited increased locomotor activity (2) and activity and rearing counts; however, the potentiation effects were short-lived (2,4–6). Similarly, [D-Ala^2]deltorphin II (DADELT), a specific δ_2-opioid agonist, has been microinjected into the Acb resulting in a dose-dependent elicitation of locomotion, rearing, and stereotypy behaviors (8).

Recently, pCl-DPDPE (Tyr-D-Pen-Gly-*p*-chloro-Phe-D-Pen), a new highly potent δ_1 receptor agonist, has been developed. This peptide has been reported to have the highest selective binding to the δ receptor (14,15). With this highly specific δ agonist, we have undertaken a study to investigate the effects of pCl-DPDPE on locomotor activity when bilaterally injected into the Acb. In addition, we were interested in the pCl-DPDPE effects upon behaviors in a dose-by-time block design by using computer-based measures of locomotor activities.

METHOD

Animals

Male Long-Evans rats weighing 250–275 g were obtained from Charles River. The rats were individually housed in stainless steel cages, had food and water ad lib, and were maintained on a 12L : 12D (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 (210 ± 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-ga stainless steel hypoder-

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mic needles were permanently fixed to the skull with microscrews and dental cement. The guide cannulae were implanted following the coordinates from Paxinos and Watson (12) with references to bregma, lateral from midline and skull surface, respectively: Acb, +1.7, 1.4, −2.5 mm (injection cannulae, −7.0). The animals were allowed 2 weeks of 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 administrated an overdose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% buffered formalin. The brains were 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 δ_1 receptor agonist, pCl-DPDPE ([D-Pen^{2,5}, *p*-Cl-Phe^4]ENK; Tyr-D-Pen-Gly-*p*-chloro-Phe-D-Pen; mol. wt. 680.2) was obtained from Sigma (St. Louis, MO). The 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 0.10, 1.00, and 2.5 μg. The 0.5 μl of solution was microinjected through 30-ga injection cannulae over a period of 60 s and the cannulae remained in place for an additional 30 s.

Apparatus and Behavioral Testing

Immediately following Acb injections, each rat was placed in an Omnitech Digiscan Animal Activity Monitor (Columbus, OH) for 60 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, as well as 16 beams 2.54 cm 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 beams broken into different measures of locomotor activity. In this study, the measures automatically analyzed by the computer were 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 activating the upper sensors, rearing time was incremented), and stereotypy time in seconds (as long as the animal was repeatedly breaking the same beam of sets of beams, the monitor considered the animal was emitting stereotypy behavior).

Statistical Analyses

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

A two-factor mixed ANOVA was used to analyze the within measures (six consecutive 10 min time blocks), between the treatment conditions (four dose levels), and the dose-by-time block interaction effect. Significant dose effects or treatment conditions and significant treatment-by-time blocks interactions were followed-up within time blocks by Duncan's multiple-range test and by Dunnett's multiple comparison tests between the control group and the treatment groups. Values of $p \leq 0.05$ were judged to be statistically significant.

RESULTS

Horizontal Movement Time

Figure 1A illustrates the dose-response curve over six consecutive 10-min time blocks of horizontal movement as measured in seconds, treated with four dose levels of pCl-DPDPE (0.00 or vehicle, 0.1, 1.0, and 2.5 μg/side) bilaterally injected into the Acb.

The four treatment groups by six consecutive 10-min time blocks interaction was highly significant, $F(15, 220) = 12.02$, $p < 0.001$. From the subsequent analyses, the 1.0- and 2.5-μg groups were significantly attenuated during the 10-min block

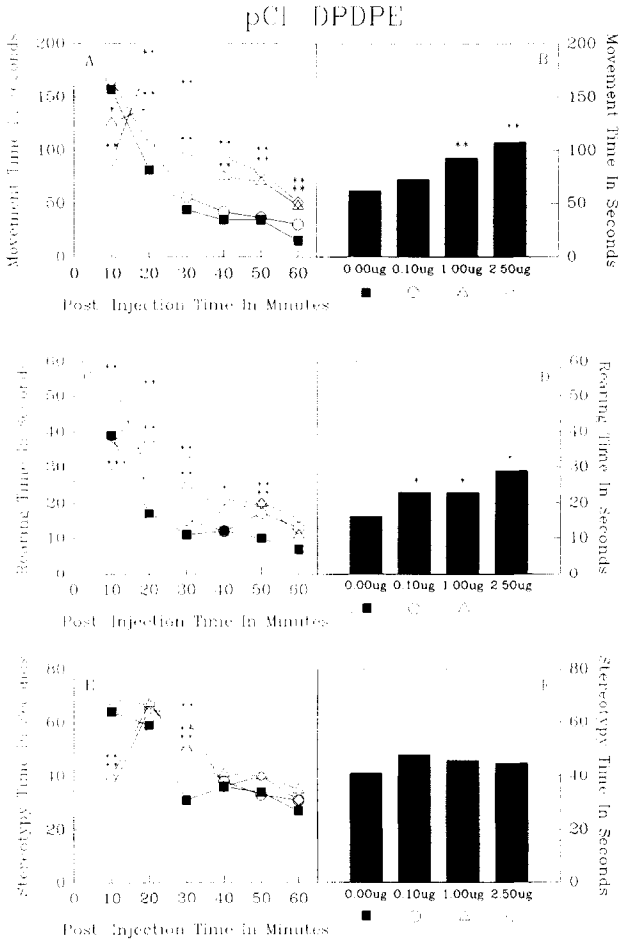

FIG. 1. (A) Significant interaction effects of Acb injected 0.00, 0.1, 1.0, and 2.5 μg/side of the δ-opioid peptide pCl-DPDPE on horizontal movement time in seconds over six 10-min intervals. (B) Significant dose effects. (C) Significant interaction effects of Acb injected 0.00, 0.1, 1.0, and 2.5 μg/side of pCl-DPDPE on rearing time in seconds over six 10-min intervals. (D) Significant dose effects. (E) Significant interaction effects of Acb injected 0.00, 0.1, 1.0, and 2.5 μg/side of pCl-DPDPE on stereotypy time in seconds over six 10-min time intervals. (F) Nonsignificant dose effects. The error bars have been excluded for clarity. Significant differences from the vehicle control group (0.00 μg) at each time point: $^*p < 0.05$; $^{**}p < 0.01$.

and potentiated at time block 20 min through 60 when compared to the vehicle control group ($ps < 0.05$ and 0.01). The 0.10-μg group did not differ from the vehicle control group at any time block ($ps > 0.05$). Figure 1B shows the significant differences between dose effects, $F(3, 44) = 10.34$, $p < 0.001$. The subsequent analyses revealed that the 1.0- and 2.5-μg groups were significantly different from the vehicle control group ($ps < 0.01$).

Rearing Time

Figure 1C shows the significant interaction effects on rearing between the four dose levels over the six consecutive 10 min time blocks, $F(15, 220) = 3.95$, $p < 0.001$. Subsequent analyses revealed that the 1.00-μg group was attenuated and the 0.1-μg group was potentiated during the initial 10-min time block ($p < 0.01$). At time blocks 20 through 50, the 1.0- and 2.5-μg groups were potentiated when compared with the vehicle control group ($ps < 0.05$ and 0.01). There were no significant differences for the 0.1-μg group, except at the post-injection interval of 50 min where there was potentiation of rearing ($p < 0.01$). Figure 1D shows the significant differences between the dose effects, $F(3, 44) = 2.85$, $p < 0.05$. Subsequent analyses of this significant main treatment effect showed significant activation when compared to the vehicle control group ($ps < 0.05$). On the other hand, there were no significant differences between the various dose levels of the peptide ($ps > 0.05$).

Stereotypy Time

Figure 1E presents the significant interaction effects, $F(15, 220) = 3.35$, $p < 0.001$. Subsequent analyses showed significant attenuation in the 1.0- and 2.5-μg groups in the initial 10-min time block, followed by significant potentiation at time block 30 min for the three dosages of the peptide when compared to the vehicle control group ($ps < 0.01$). All other comparisons were not significant. Figure 1F illustrates the nonsignificant main dose effects, $F(3, 44) = 0.62$, $p = 0.39$.

DISCUSSION

The behavioral profile of microinjections of pCl-DPDPE into the Acb in the present study was in part similar to other reports of an increased locomotor activity. In the present study, there was a significant dose-dependent potentiation of horizontal movement that has been reported with various other measures of locomotor activity and with other intra-accumbens δ-opioid receptor agonists (2,4,8,11). Although the main dose-dependent effects are of interest, the dose-by-time course interaction revealed a biphasic function. During the first 10-min time block, the 1.0- and 2.5-μg groups' horizontal movement times were significantly attenuated and followed by potentiation. This biphasic function has also been reported with ICV DPDPE (11).

Although pCl-DPDPE significantly increased rearing time, there were no significant differences among the various 0.1–2.5-μg groups. Like horizontal movement time, the profile for rearing time was also biphasic, attenuation followed by potentiation. Both DPDPE and DADELT, when microinjected into the Acb, increased rearing counts in a monotonic function (4,8). The differences in the rearing profile among the studies may reflect the measures analyzed or may be differences in the agonists.

The effects of Acb pCl-DPDPE on stereotypy time were also biphasic with an initial attenuation followed by potentiation. However, the duration of this δ-opioid agonist on stereotypy time was short-lived, being effective only during the first 30 min. These data on stereotypy time were in marked contrast to the dose-dependent activation effect of Acb DADELT on sniffing and other oral stereotypies (8). In the present experiment, the computer-based stereotypy response measure does not differentiate sniffing, licking, gnawing, or grooming. If the two δ agonists, pCl-DPDPE and DADLET, are found to be associated with different δ receptors, various stereotypy behaviors may become one of the significant differences in the behavioral markers.

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