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Effects of Acute and Chronic Morphine on Rotational Behavior in Nigral-Lesioned Rats

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KIMMEL, H. L., B. E. GARRETT AND S. G. HOLTZMAN. *Effects of acute and chronic morphine on rotational behavior in nigral-lesioned rats.* PHARMACOL BIOCHEM BEHAV **52**(2) 397–401, 1995. Stimulation of mu-opioid receptors located on dopaminergic neurons in the striatum and the nucleus accumbens increases dopamine release, which may account for some of the behavioral effects of morphine. In this study, we examined the effects of acute and chronic morphine treatment on rotational behavior in rats with unilateral 6-hydroxydopamine (6-OHDA)-induced lesions of the nigrostriatal pathway. Rats receiving morphine acutely (0.3–10 mg/kg) did not show a significant bias toward contralateral or ipsilateral turning. Mini osmotic pumps dispensing morphine continuously (20–24 mg/kg/day) were implanted SC in these animals. This treatment induced tolerance to the behavioral depression produced by the highest dose of morphine (10 mg/kg) when it was given acutely. A slight but significant increase in ipsilateral turning occurred over the range of morphine doses examined. The effects of morphine on rotational behavior are slight, and do not correlate well with the reported increase in locomotor activity or extraneural dopamine in the striatum that are produced by doses of morphine similar to the ones tested in this study.

Dopamine Morphine Opioid Rotational behavior Tolerance

THERE is now considerable evidence of opioid modulation of brain dopaminergic systems (26). Dopamine levels in the striatum and nucleus accumbens are influenced by opioid agonists that act upon the dense population of opioid receptors that are located on dopamine neurons in these areas (10,14, 19,27). Mu- and delta-opioid receptor agonists increase extracellular dopamine in these areas after systemic or intracerebral administration, while these effects are blocked by receptor-selective antagonists (7,8,13,20,21). Drugs that act presynaptically to increase synaptic levels of DA in the striatum, such as amphetamine, produce ipsilateral turning in rats with a unilateral 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal tract (22,23). Therefore, morphine might be expected to produce ipsilateral turning by indirectly increasing striatal dopamine release. This possibility was tested in this study.

Morphine has effects on locomotor activity in rats that are presumably due to the increase in dopamine release (12), and these effects are both time and dose related. Low to moderate doses of morphine (e.g., 1.0–5.0 mg/kg) produce an increase in activity that lasts for an hour or two (2,3,6,9). Higher doses, ranging from 10 to 40 mg/kg, have a biphasic effect, with an initial depression of activity that is followed by an increase (3,6,9,15). Chronic morphine administration results in tolerance to the depressant effect on locomotor activity while the stimulatory effects of morphine on locomotor activity and brain dopamine release are enhanced (4,5,12,24). Therefore, to maximize the stimulant component of action of morphine in this study, the drug was administered continuously via a SC osmotic pump. Morphine was infused at a dose that produces tolerance to analgesic and response-rate decreasing effects (1).

METHOD

Subjects

Male Sprague–Dawley rats (Sasco, Inc., Omaha, NE) weighing 300–350 g at the start of the experiment were used. All rats were initially group housed in polycarbonate cages and maintained in a temperature-controlled colony room with a 12 L : 12 D cycle. Food (Purina Rodent Chow, Purina Mills, St. Louis, MO) and water were available ad lib.

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6-Hydroxydopamine Lesions

Rats were given unilateral lesions of the right nigrostriatal pathway by injection of 6-hydroxydopamine (6-OHDA). Rats were anesthetized with 3.3 mg/kg, IP, equithesin and placed into a stereotaxic frame. Stereotaxic coordinates relative to bregma were AP = −4.8, ML = −2.2, DV = −8.0 (17). We used a 25 μl Hamilton syringe to inject 8 μg/4 μl of 6-OHDA into the right substantia nigra at a rate of 1.0 μl/min for 4 min. Upon completion, the injection needle was kept in place for an additional minute to minimize back flow of the solution.

Rotational Activity

Rotational activity was measured with six stainless steel Roto-Rat rotometers (MED Associates, Inc., East Fairfield, VT). Each chamber consisted of a round metal bowl (16″ diameter and 10″ high) with a transparent Plexiglas cover. A recording apparatus, consisting of a spring tether connected to a direction sensitive rotation sensor mounted above the bowl, was attached to the rat by means of a Velcro belt. Rotational activity was recorded by the Roto-Rat Version 1.2 computer program (MED Associates, Inc.). Measurements were taken of partial and full clockwise turns, partial and full counterclockwise turns, and direction changes. The partial counter incremented at every 90°, while the full counter incremented at every 360°. Direction changes were noted when the animal moved from left to right, or vice versa, through an arc of at least 45°. During experimental test sessions, counts were taken in 10-min time intervals for 3 h, resulting in 18 data points per animal for each session.

Rats were allowed to recover from surgery for at least 7 days, then they received 0.3 mg/kg apomorphine SC twice weekly for 2 weeks. Rats exhibiting at least 50 full contralateral turns in each 10-min interval for 1 h were used for further behavioral testing. The amount of turning observed in response to apomorphine has been found to be directly correlated with the extent of the nigral lesion (11).

Drug Administration

Doses of morphine sulfate (0.3, 1.0, 3.0, and 10 mg/kg, SC) were administered to the rats in a random sequence. All drugs were administered 5 min before the start of each test session. Each animal was tested for rotational behavior two times per week, with a 2- or 3-day interval between sessions.

Morphine Infusion

Once the effects of various doses of acute morphine on turning behavior was established, a 7-day Alzet mini-osmotic pump (2ML1; Alza Corp., Palo Alto, CA) was implanted in the rats and the dose effects of morphine were redetermined. Rats were lightly anesthetized with methoxyflurane (Metofane; Pitman-Moore, Inc., Mundelein, IL) and the pumps implanted SC in the midscapular region of the back. The pumps were filled with a morphine sulfate solution and dispensed approximately 20–24 mg/kg/day. Drug concentrations were based on the weight of the animal and the infusion rate of the pump. Once the pumps were implanted, rats were housed individually in polycarbonate cages and maintained in a temperature-controlled cabinet with a 12 L : 12 D cycle. All animals received food ad lib and had scheduled access to their drinking water for 10 min every 6 h, 7 days per week. Three days after the implantation, drug testing resumed. A week after the implantation, the pump was removed and a second pump implanted to achieve a morphine dose of 20–24 mg/kg/day. At the conclusion of the experiment, the pumps were removed.

Data Analysis

The time course data for turning behavior were analyzed using three-factor analysis of variance (ANOVA), with repeated measures on two factors. Total rotational count values were analyzed by two-factor ANOVA, with repeated measures on one factor, followed by *t*-tests protected for multiple pairwise comparisons.

Drugs

Morphine sulfate was obtained from Penick Corp. (Newark, NJ), 6-hydroxydopamine (6-OHDA) hydrobromide from Sigma Chemical Co. (St. Louis, MO), and apomorphine hydrochloride from Research Biochemicals, Inc. (Natick, MA). Morphine was dissolved in 0.9% saline; apomorphine and 6-OHDA were dissolved in a solution of 0.02% ascorbic acid in 0.9% saline. Morphine and apomorphine were administered in a volume of 1.0 ml/kg body weight, with all doses expressed as the free base.

RESULTS

Figure 1 shows the dose- and time-effect relationships of morphine on rotational behavior of lesioned animals receiving only acute morphine injections and the same animals receiving a continuous infusion of the drug. Morphine doses ranging from 0.3 mg/kg to 3.0 mg/kg produced slight but significant increases in full ipsilateral turning, $F(4, 48) = 2.86$, $p = 0.033$, and direction changes, $F(4, 48) = 3.04$, $p = 0.0261$, that were dose related in both treatments. However, these increases were greater when the lesioned animals were receiving chronic morphine. The highest dose of morphine (10 mg/kg) produced minimal activity in the control lesioned animals, those receiving acute morphine only. This dose produced a large initial increase in partial ipsilateral turns in the animals receiving an infusion, then the activity declined but remained higher than that of the control lesioned animals. The animals receiving chronic morphine had increased partial ipsilateral activity and direction changes even when they received a 0.9% saline injection.

Treatment with chronic morphine had a significant effect on both full, $F(1, 12) = 10.73$, $p = 0.007$, and partial ipsilateral turning behavior, $F(1, 14) = 9.55$, $p = 0.008$, but not on direction changes. For these two measures, the interaction between the treatment and the time course of the experiments was also significant: full ipsilateral, $F(17, 204) = 4.34$, $p < 0.0001$, and partial ipsilateral, $F(17, 238) = 3.02$, $p < 0.0001$. Thus, receiving chronic morphine had an effect on the turning response of the animals to acute morphine over the time course.

Total rotational behavior over the 3-h period is compared in Fig. 2. This figure shows that there was no significant difference in full contralateral turning, partial contralateral turning, and direction changes between the control lesioned animals and morphine-treated lesioned animals. There was a significant difference between the two groups in the total number of full ipsilateral turns, $F(1, 6) = 10.25$, $p = 0.02$, as well as in the total number of partial ipsilateral turns, $F(1, 6) = 6.14$, $p = 0.05$. These were, in both cases, greater in lesioned animals receiving the morphine infusion than in those receiving only acute morphine. None of these five measures showed a significant interaction between the treatments and the doses of acute morphine administered to those animals.

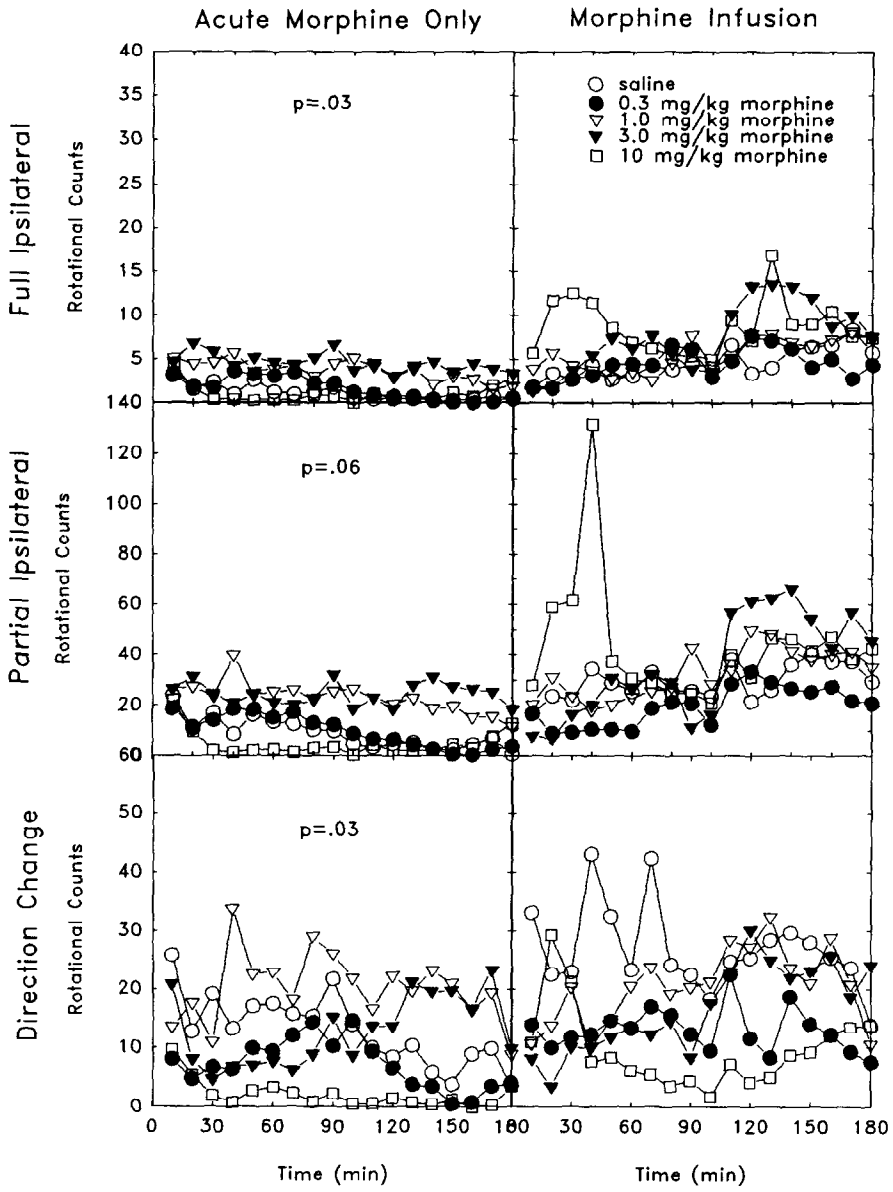

FIG. 1. Rotational counts in rats receiving morphine injections at doses of 0.3, 1.0, 3.0, and 10 mg/kg. Data in the left panels are from control lesioned rats receiving only acute morphine, while data in the right panels are from lesioned rats with osmotic pumps dispensing morphine continuously. Each point is a mean based upon one observation in each of five to eight rats. *p*-Values represent differences between the treatment groups.

DISCUSSION

Despite the fact that morphine increases extraneural levels of dopamine in the striatum and increases locomotor activity over the range of doses tested (see the introductory paragraphs), it failed to produce a large effect on rotational behavior in control lesioned animals and had a marginal effect in animals receiving a continuous infusion of morphine. This lack of increase in net turns in either direction when animals received only acute morphine supports observations previously reported by Von Voigtlander and Moore (25). In lesioned rats receiving chronic morphine, Pert saw an increase in ipsilateral turning in response to 10 mg/kg morphine for a 30-min observation period (18). This report corresponds well with our observation of a large increase in turning for the first half-hour of the time course in lesioned animals receiving chronic morphine. There are several possibilities for this outcome. For example, the increase in synaptic dopamine after the administration of morphine may have been insufficient to initiate turning, failing to reach a threshold level. The quantitative relationship between synaptic concentrations of dopamine and rotational behavior has not been defined. Alternatively, the postsynaptic actions of morphine or effects of morphine on downstream neurotransmitter systems may counteract the effects of the drug on dopamine.

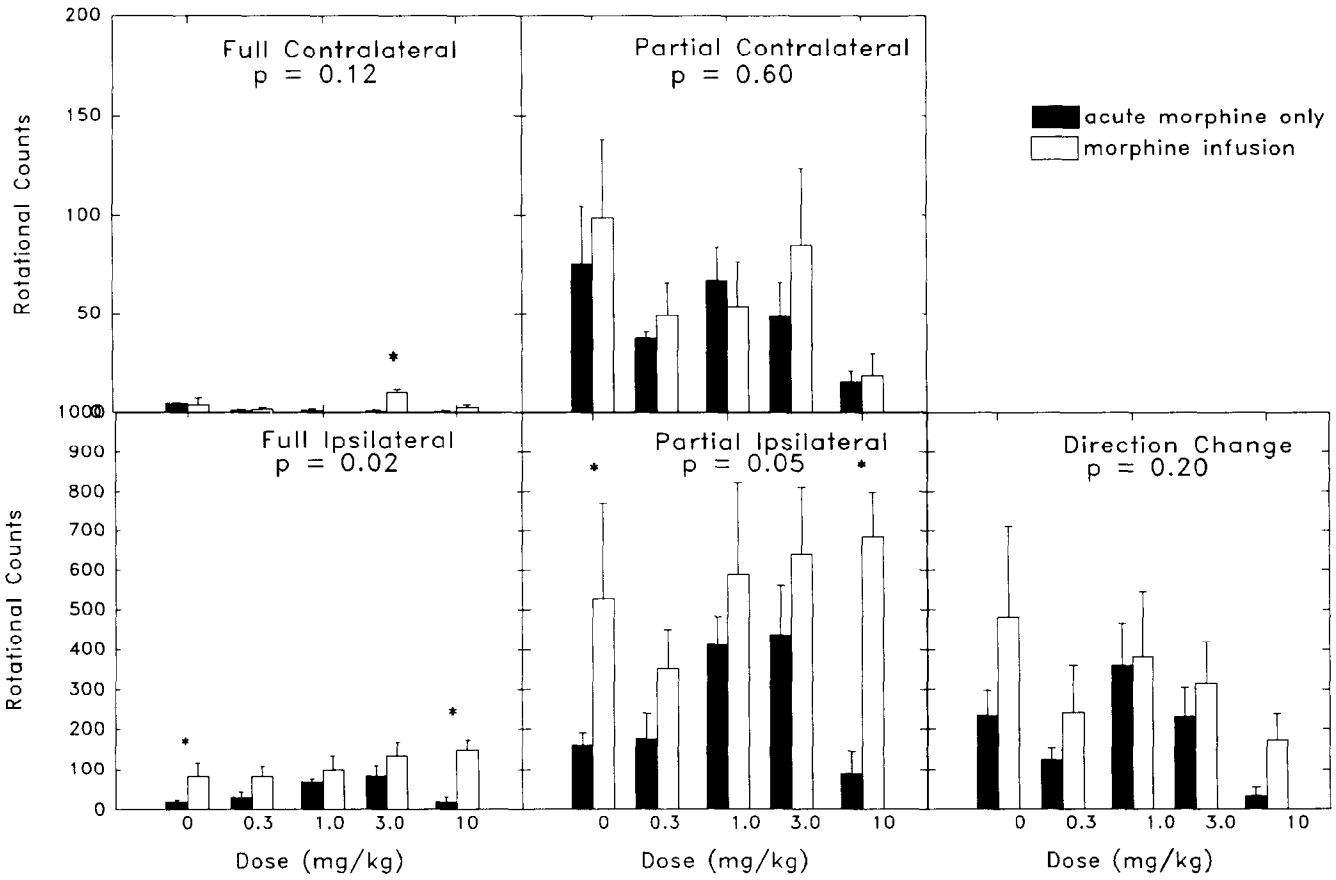

FIG. 2. Total rotational counts over a 3-h testing period, derived from the time effect data in Fig. 1. Each bar represents a mean and 1 SEM. Significant differences between lesioned animals receiving only acute morphine and the same animals with continuous morphine delivered by a mini-osmotic pump, $*p \leq 0.05$, repeated measures ANOVA.

Chronic treatment with morphine produces tolerance to behavioral depressant effects of the drug and enhances the excitatory effects, such as stimulation of locomotor activity (see the introductory paragraphs). The drug treatment regime used in this study was adequate to produce tolerance to analgesic and behavioral depressant effects of morphine (1,16). In the present study, rats were infused with morphine and became tolerant to it, as evidenced by the altered responsiveness to the previously depressant 10 mg/kg dose. Nevertheless, the changes in turning behavior induced by morphine were small. Based upon the results of this study, it appears that the rotational model has limited value for studying the neurochemical-behavioral correlates of the effects of morphine.

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