Mu Opioid Agonists Potentiate Amphetamine- and Cocaine-Induced Rotational Behavior in the Rat

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ABSTRACT
Opioids modulate brain dopaminergic function in various experimental paradigms. This study used the rotational model of behavior in rats with unilateral 6-hydroxydopamine-induced lesions of the nigrostriatal pathway to investigate this interaction. Doses of two presynaptically acting dopaminergic drugs, amphetamine and cocaine, were coadministered with several doses of the mu opioid agonist, morphine. Morphine, at 3.0 mg/kg, potentiated rotational behavior induced by each dose of the stimulants. To determine the receptor specificity of the actions of morphine, the mu opioid agonists buprenorphine, fentanyl, levorphanol, meperidine, and methadone, and dextrorphan, the non-opioid isomer of levorphanol, were administered alone and with 1.0 mg/kg amphetamine. Each of these drugs, as well as morphine, produced circling behavior on its own. All of the mu opioid agonists and dextrorphan increased amphetamine-induced turning; the coadministration of dextrorphan, levorphanol, meperidine, methadone and morphine with amphetamine produced turning greater than predicted by simple additivity. To determine whether an opioid receptor was involved in these interactions, the opioid antagonist, naltrexone, was administered before the amphetamine/mu opioid receptor agonist combination. Naltrexone blocked the potentiating effects of morphine, but not those of the other drugs. Moreover, naltrexone alone dose-dependently increased amphetamine-induced rotational behavior. These studies show that some mu opioid receptor agonists can potentiate stimulant-induced rotational behavior and that blockade of opioid receptors can also produce a potentiation. The role of mu opioid receptors in these effects remains unclear.

Opioids can modulate brain dopamine systems (Wood, 1983). Of the three types of opioid receptors, mu and delta receptor activation increases dopamine release in the mesolimbic and nigrostriatal tracts. Conversely, the stimulation of kappa opioid receptors decreases dopamine levels in these brain regions (Di Chiara and Imperato, 1988a,b). All three opioid receptors have been localized within these dopamine systems (Goodman et al., 1988; Mansour et al., 1995; Sharif and Hughes, 1989; Wood and Iyengar, 1988).

Some effects exhibited by mu opioid agonists reflect increases in dopaminergic activity. In mice, morphine, heroin, levorphanol and meperidine increased locomotor activity (Longoni et al., 1987; Oliverio and Castellano, 1974; Rethy et al., 1971; Shippenberg et al., 1993), which is mediated by dopaminergic neurons in the substantia nigra (Iwatsubo and Clouet, 1977) and in the ventral tegmental area (Matthews and German, 1984). In vivo microdialysis indicated that morphine, methadone and fentanyl increased extracellular dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988b; Kalivas and Stewart, 1991). Behavioral sensitization to repeated administration of opioids was attributed to the indirect stimulation of dopamine cell bodies in the ventral tegmental area and the substantia nigra (Kalivas and Stewart, 1991). These results indicate that the stimulation of opioid receptors influences processes thought to be mediated by brain dopamine systems.

Opioids also can modulate the effects of drugs that act via brain dopamine systems. The effects of cocaine and amphetamine are believed to be mediated largely by dopamine (Kalivas and Stewart, 1991). Behavioral interactions between mu opioid agonists and psychomotor stimulants have been studied with various experimental paradigms. However, the results have not always been consistent among these different techniques. In drug discrimination studies, heroin substituted for cocaine in some rhesus monkeys (Mello et al., 1995), and morphine potentiated the discriminative stimulus effects of cocaine in squirrel monkeys (Spealman and Bergman, 1994). Chronic treatment with the partial mu opioid receptor agonist buprenorphine reduced cocaine self-administration in rats (Carroll and Lac, 1992) and rhesus monkeys (Mello et al., 1992, 1993, 1995). Doses of buprenorphine and cocaine, which were ineffective by themselves, induced con-
ditioned-place preference in rats when administered together (Brown et al., 1991). In studies of analgesia, cocaine potentiated the effects of morphine and the mu opioid receptor-selective ligand DAMGO in mice (Sierra et al., 1992) and rats (Kauppila et al., 1992), and the effects of morphine and nalbuphine in rhesus monkeys (Gatch et al., 1995). The co-administration of stimulants and morphine-like compounds, commonly known as “speedballing,” is frequently seen in drug abusers (Kosten et al., 1986; Kreek, 1987). Whether the coadministration of the drugs enhances their euphoric effects or attenuates their dysphoric effects is not known. In summary, mu opioid receptor agonists influence the effects of cocaine, but the direction of the change in the behavioral response to cocaine is difficult to predict.

The effects of opioid antagonists on the behavioral effects of cocaine and amphetamine have also been studied. In many cases, the antagonists did not alter the effects of stimulants. When the antagonists had an effect, it was usually to reduce the effects of the stimulant drugs. For example, naltrexone, two nonspecific opioid receptor antagonists, attenuated amphetamine-induced locomotor activity in a variety of animal species (Adams et al., 1981; Andrews and Holtzman, 1978; Dettmar et al., 1978; Hitzemann et al., 1982; Holtzman, 1974; Schad et al., 1995; Winslow and Miczek, 1988), and blocked cocaine- and amphetamine-induced conditioned place preference in rats (Gerrits et al., 1995; Sala et al., 1995; Trujillo et al., 1991). These two opioid receptor antagonists also attenuated cocaine self-administration (Corrigall and Coen, 1991; Ramsey and van Ree, 1991). Sensitization to repeated cocaine injections was attenuated by naltrexone (Sala et al., 1995). As the opioid antagonists presumably were blocking effects of endogenous opioids, the results of the experiments cited above suggest that endogenous opioids modulate the effects of stimulants, probably via the dopaminergic system.

To study further the relationship between opioids and dopaminergic neurons in the brain, we used the rotational model of behavior in the rat. This is a method of studying the nigrostriatal dopaminergic system in which rats are given a unilateral lesion of the nigrostriatal tract with 6-OHDA, thus creating a postsynaptic dopamine receptor supersensitivity (Ungerstedt, 1971). These animals circle toward the lesion in response to presynaptically acting dopamine agonists (i.e., amphetamine) (Lynch and Carey, 1989; Ungerstedt and Arbuthnot, 1970; Zetterstrom et al., 1986a). This paradigm has been used infrequently to investigate interactions between the opioid and dopaminergic systems of the rat brain, and results have not been consistent across studies. Morphine produced rotational behavior in one study (Cowan et al., 1975; Pert, 1978), but amphetamine was used to determine the validity of the 6-OHDA-induced lesion in this study. Apomorphine-induced turning has been a more accurate indicator of the magnitude of the 6-OHDA-induced lesion (Hudson et al., 1993). In other studies, injections of morphine produced significant turning only in those rats that had been made tolerant to the depressant effects of the drug by repeated exposure to morphine (Kimmel et al., 1995; Pert 1978). In one study, amphetamine-induced turning was reduced by morphine (Blundell et al., 1976), whereas in another, it was reduced by naloxone (Dettmar et al., 1978). Based on the effects of mu opioid agonists on brain dopamine and on the dopamine-mediated behaviors cited above, we hypothesized that these drugs would potentiate the effects of psychomotor stimulants upon rotational behavior, and would do so by activating the mu opioid receptor.

In this study, we first examined the effects of morphine on amphetamine- and cocaine-induced rotational behavior. To do this, we administered various doses of morphine immediately before doses of amphetamine or cocaine. Because combinations of the highest doses of morphine and cocaine were lethal in some rats, subsequent experiments were carried out using only amphetamine. To determine the receptor specificity of the effects of morphine on turning induced by amphetamine, we tested the mu opioid receptor agonists fentanyl, levorphanol, meperidine and methadone. We also examined the effects of dextrorphan, the optical isomer of levorphanol, to investigate the stereoselectivity of this interaction. We measured rotational behavior for a 4-hr period to capture the full time course of drug action.

Biochemical and behavioral effects of opioids that involve mesolimbic and nigrostriatal dopamine systems can be blocked by the general opioid antagonists naloxone and naltrexone (Di Chiara and Imperato, 1988b; Spanagel et al., 1990). Thus, to determine whether the effects of these mu opioid agonists were mediated by an opioid receptor, we administered naltrexone before amphetamine alone or the combination of amphetamine with the mu opioid agonists.

Methods

Subjects. Male Sprague-Dawley rats (Sasco, Inc., Omaha, NE) weighing 300 to 350 g at the time of surgery were used. All rats were group housed in polycarbonate cages and maintained in a temperature-controlled colony room with a 12 hr light:12 hr dark lighting cycle, beginning with lights on at 7:00 A.M. Food (Purina Rodent Chow, Purina Mills, St. Louis, MO) and water were available ad libitum.

Stereotaxic surgery. Rats were given unilateral lesions of the right nigrostriatal pathway by a single injection of 6-OHDA. They were first anesthetized with 3.3 mg/kg i.p. Equithesin and then placed into a stereotaxic frame. Stereotaxic coordinates relative to bregma were AP = −4.8, ML = −2.2, DV = −8.0 (Paxinos and Watson, 1986). A 25-μl Hamilton syringe was used to inject 8 μg/4 μl of 6-OHDA in a solution of 0.02% ascorbic acid and 0.9% saline 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 backflow of the solution. No desmethyliapramine pretreatment was used before the surgeries, so norepinephrine nerve terminals may have been lesioned. However, norepinephrine innervation of the striatum is relatively low, so this should not have affected the experimental results.

Rotational behavior. Rotational activity was measured in stainless steel rotometer stations (MED Associates, Inc., East Fairfield, VT). Each of eight stations consisted of a round stainless steel bowl (40.6 cm diameter and 25.4 cm high) in a transparent Plexiglas cover. 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). Measurements were taken of full (360°) turns in both the clockwise and counterclockwise direction. During experiments, counts were taken in 15-min intervals for 4 hr, resulting in 16 time points per animal for each session. All sessions were conducted during the light phase of the lighting cycle. Rats were allowed to recover from surgery for at least 14 days, then they received 0.3 mg/kg R(−)-amphetamine s.c. twice weekly for 2 weeks. Animals exhibiting at least 50 full contralateral turns/10 min for 1 hr were used for further behavioral testing. The amount of turning observed in response to apomorphine is directly correlated
with the extent of the nigral lesion (Hudson et al., 1993). The rats used in this study exhibited a mean (± S.E.M.) of 471 ± 56 turns/hr in tests with 0.3 mg/kg apomorphine.

Drug administration and behavioral testing. On test days, animals were weighed and placed into the test chambers and allowed to habituate for approximately 5 min before drug injection. Rats (n = 16) then received a 5-min pretreatment of doses of saline or morphine (0.03–10 mg/kg) administered in a random sequence. Five minutes later, half of the rats received saline or amphetamine (0.1–1.0 mg/kg) and the other half received saline or cocaine (3.0–30 mg/kg), with the dose of each drug and saline administered in a random order. Measurements of rotational behavior began 5 min after the second injection. Each animal was tested twice a week, with a 3- to 4-day interval between sessions, so that each animal received every possible combination of opioid and either amphetamine or cocaine. Morphine, amphetamine and saline were administered s.c., and cocaine was administered i.p.

Based on results obtained in the experiments above, a 1.0 mg/kg dose of amphetamine was selected for testing in combination with other μ opioid receptor agonists and dextrophan. Buprenorphine (0.01–1.0 mg/kg), dextrophan (1.0–10 mg/kg), fentanyl (0.01–0.056 mg/kg), levorphanol (0.1–1.0 mg/kg), meperidine (3.0–30 mg/kg) and methadone (0.3–3.0 mg/kg) were injected s.c., with doses of each drug given in a random sequence. Amphetamine was injected 5 min later. Testing was conducted in the same manner as described earlier.

To determine whether the interactions observed between the μ opioid agonists and amphetamine could be blocked by naltrexone, one group of animals was given 0.1 mg/kg naltrexone followed by 3.0 mg/kg morphine and 1.0 mg/kg amphetamine. The dose of each of the other agonists that produced the greatest amount of turning in combination with amphetamine was then tested with 1.0 mg/kg naltrexone and 1.0 mg/kg amphetamine, injected in the following order: naltrexone, μ opioid agonist and amphetamine. Because this dose of naltrexone was ineffective in combination with nearly all of the agonists, 10 mg/kg naltrexone was tested in combination with 1.0 mg/kg methadone and 1.0 mg/kg amphetamine.

Statistical analysis. Total rotational count data in the initial experiments with morphine, amphetamine and cocaine were ana-

Fig. 1. Morphine (0.03–10 mg/kg) potentiated ipsilateral turning behavior induced by amphetamine (0.1–1.0 mg/kg) in rats with unilateral lesions of the nigrostriatal tract. Points on the time-course curves (A) represent the mean number of turns per 15 min. Only the saline curves and the 3.0 mg/kg morphine curves are shown for simplicity. A three-way ANOVA of these data revealed significant F values for all factors and interactions: amphetamine: F(3,21) = 30.78, P < .0001; morphine: F(6,42) = 8.02, P < .0001; time: F(15,105) = 27.24, P < .0001; amphetamine × morphine: F(18,126) = 3.95, P < .0001; amphetamine × time: F(45,315) = 13.98, P < .0001; morphine × time: F(80,630) = 5.13, P < .0001; amphetamine × morphine × time: F(270,1890) = 1.92, P < .0001. Dose-response data (B) show total amount of turning for the entire 4-hr session (mean ± S.E.). A two-way ANOVA of these data revealed a significant effect by drug as well as a significant interaction between the two drugs (amphetamine: F(3,28) = 12.87, P < .0001; morphine: F(6,168) = 16.88, P < .0001; and amphetamine × morphine interaction: F(18,168) = 3.29, P < .0001). Asterisks indicate significant differences from saline control plus that same dose of amphetamine (points above sal), **P < .01, *P < .05. Daggers indicate significant differences of amphetamine or morphine alone from saline + saline, + + P < .01, + P < .05.
lyzed using a two-way ANOVA with repeated measures on all three factors (morphine dose, stimulant dose, and time period). Results were considered to be statistically significant if the P value was \( \leq .05 \).

**Drugs.** Buprenorphine hydrochloride (National Institute on Drug Abuse, Rockville, MD), dextrorphan tartrate and levorphanol tartrate (Roche Laboratories, Nutley, NJ) were dissolved in distilled water. \( d \)-Amphetamine sulfate, and naltrexone hydrochloride (Sigma Chemical Co., St. Louis, MO), cocaine hydrochloride (National Institute on Drug Abuse), fentanyl citrate (McNeil Laboratories, Fort Washington, PA), methadone hydrochloride (Mallinkrodt, St. Louis, MO), meperidine hydrochloride and morphine sulfate (Penick Corp., Newark, NJ) were dissolved in 0.9% saline. \( R(-) \)-Apomorphine hydrochloride was dissolved in 0.02 M NaOH.

Fig. 2. Morphine (0.1–10 mg/kg) potentiated ipsilateral turning behavior induced by cocaine (3.0–30 mg/kg). A three-way ANOVA of the time-course values (A) showed that all three variables as well as all two- and three-factor interactions were significant: cocaine: \( F(3,21) = 19.39, P < .0001 \); morphine: \( F(5,35) = 21.00, P < .0001 \); time: \( F(15,105) = 7.94, P < .0001 \); cocaine \( \times \) morphine: \( F(15,105) = 2.75, P < .0013 \); cocaine \( \times \) time: \( F(45,315) = 5.33, P < .0001 \); morphine \( \times \) time: \( F(75,825) = 5.33, P < .0001 \); cocaine \( \times \) morphine \( \times \) time: \( F(225,1575) = 2.77, P < .0001 \). Total full ipsilateral turning during the 4-hr experimental period (B) was influenced by cocaine (\( F(3,28) = 16.55, P < .0001 \)) and morphine (\( F(5,191) = 34.85, P < .0001 \)) as well as their interaction (\( F(15,191) = 2.29, P = .062 \)). Other details are as in figure 1.

Fig. 3. Turning was induced by \( \mu \) opioid agonists and dextrorphan, but not naltrexone. Asterisks represent drug doses that produced turning significantly greater than the vehicle control, **\( P < .01 \), *\( P < .05 \). Data obtained in the experiments with additional \( \mu \) opioid agonists and dextrorphan were analyzed by a two-way ANOVA with repeated measures on both the agonist dose and the amphetamine dose. Tukey’s protected \( t \) tests were performed on these data following the statistical analysis. Two-hour rotation totals were subjected to a one-way repeated measures ANOVA. The Friedmann's ANOVA was used to compare the overall theoretical additive values of the \( \mu \) opioid in combination with amphetamine to the observed behavior. The Wilcoxon matched-pairs test was then performed on each pair of theoretical and observed values for each of the \( \mu \) agonist doses. In the antagonism experiments, data were analyzed with a repeated measures ANOVA with one factor, followed by a Tukey's protected \( t \) test. All time-course data were analyzed by a three-way ANOVA with repeated measures on all three factors (morphine dose, stimulant dose, time period). Results were considered to be statistically significant if the P value was \( \leq .05 \).
drochloride (Research Biochemicals, Inc., Natick, MA) and 6-OHDA hydrobromide (Sigma) were dissolved in a solution of 0.02% ascorbic acid in 0.9% saline. All drugs except for 6-OHDA and 30 mg/kg of meperidine were administered in a volume of 1.0 ml/kg b.wt., with all doses expressed as the free base. The highest dose of meperidine (30 mg/kg) was given in 3.0 ml/kg b.wt. to avoid skin lesions, which occurred with concentrations of meperidine higher than 10 mg/ml.

Results

Effects of morphine on stimulant-induced turning behavior. Amphetamine alone produced dose-dependent ipsilateral turning (fig. 1). The lowest doses of the drug (0.1 and 0.3 mg/kg) had only a slight effect on circling behavior, whereas 1.0 mg/kg produced approximately 250 full ipsilateral turns in a 4-hr observation period, significantly greater than saline (P < .01). In these animals, morphine alone produced significant ipsilateral turning as an overall drug effect; however, no single dose elicited turning greater than turning after saline (fig. 1B).

Morphine modified amphetamine-induced circling in a dose-dependent manner that was more pronounced as the dose of amphetamine increased. When 1.0 mg/kg amphetamine was administered with 3.0 mg/kg morphine, the amount of full ipsilateral turning during 4 hr increased from 250 to nearly 1000 turns (fig. 1B). The time course of effects is illustrated for the 3.0 mg/kg dose of morphine in figure 1A. The combination of 3.0 mg/kg morphine and 1.0 mg/kg amphetamine shifted the peak circling effect from 30 min after injection to 90 min after injection, and increased the maximum turnover in a 15-min interval from 45 turns to 110 turns. In this experiment and those that follow, no contralateral turning was observed.

Cocaine alone also produced ipsilateral turning in a dose-dependent manner (fig. 2). At 30 mg/kg, the highest dose studied, 400 turns were recorded during the 4-hr observation period, slightly more than the effects produced by 1.0 mg/kg amphetamine. This dose of cocaine was the only one examined that produced turning significantly greater than saline alone (P < .05). Morphine produced significant ipsilateral turning, and 3.0 mg/kg produced turning significantly greater than saline did (P < .01) (fig. 2B), in contrast to the results described in figure 1. The effect of this dose of morphine had a plateau of approximately 45 turns/15 min for 150 min, which tapered off during the final 90 min (fig. 2A). The effects of cocaine were dose-dependently affected by morphine. The combination of 3.0 mg/kg morphine and 30 mg/kg

Fig. 4. Methadone (0.3–3.0 mg/kg) potentiated amphetamine-induced ipsilateral turning, with a significant effect of its own. Part A shows the time-course effects of the saline control and one test dose of methadone (1.0 mg/kg). (The others were omitted for clarity.) Each data point represents the mean full ipsilateral counts during that 15-min time block (n = 8). The P value shown represents the amphetamine × methadone × time interaction determined by a three-factor ANOVA, with all doses of methadone tested. Part B shows the same data as in A, but collapsed into 2-hr time blocks in the first two panels, and then total ipsilateral turns during the entire 4-hr observation time in the third panel. In the first two panels of B, asterisks denote a significant difference between that dose of methadone and the saline control (within the saline group and the amphetamine group). **P < .01, *P < .05. In the third panel, asterisks denote a significant difference between amphetamine alone and the amphetamine = methadone combination. **P < .01, *P < .05. The P values shown here were determined by a two-factor ANOVA (methadone dose × amphetamine dose).

![Graph showing the effects of methadone on amphetamine-induced turning.](image-url)
cocaine was the most effective of the doses examined in producing ipsilateral circling. The 3.0 mg/kg morphine dose did not shift the overall peak effect of 30 mg/kg cocaine from the first 15-min period, but it increased the maximum turning observed during this 15-min period from 75 to 125 turns (fig. 2A). Turning was also prolonged, lasting the entire 240 min, rather than ceasing after 150 min, as did after 30 mg/kg of cocaine alone. This drug combination produced 1400 turns/4 hr, greater than a 3-fold increase in the amount of turning seen with this dose of cocaine alone (fig. 2B). The combination of a higher dose of cocaine (56 mg/kg) and 3.0 mg/kg morphine was lethal in some animals (data not shown), so we discontinued testing this pair of drug doses.

Effects of mu opioid receptor agonists alone and on amphetamine-induced turning. When doses of the mu agonists were tested alone, each one induced significant ipsilateral turning across all doses during the 4-hr experimental session (fig. 3). Buprenorphine at 0.1 and 1.0 mg/kg produced turning greater than that occurring after saline (P < .01) and the most of any of the mu opioid receptor agonists examined, with a peak effect of nearly 500 turns/4 hr. Levorphanol at 0.3 and 1.0 mg/kg also produced significant turning (P < .01), as did the 30 mg/kg dose of meperidine (P < .05). These two mu opioid agonists produced a maximum effect of approximately 250 turns/4 hr. No single dose of fentanyl, methadone and morphine produced turning significantly greater than saline + saline, although there was a significant overall effect. Unlike the other drugs tested, fentanyl produced severe catalepsy and rigidity. Dextrophan produced less turning than did its optical isomer, levorphanol, even at a dose that was 10 to 30 times higher than doses of levorphanol that produced turning. The nonselective opioid receptor antagonist, naltrexone, did not produce rotational behavior at doses of 0.1 to 10 mg/kg.

Several doses of each of the mu opioid agonists were administered along with 1.0 mg/kg amphetamine. The time-course data were analyzed to determine whether there was a significant interaction between the mu opioid agonist of interest and amphetamine. The interactions between time and amphetamine and methadone (fig. 4A) and morphine (fig. 5A) were significant, as determined by a three-factor ANOVA (mu opioid agonist dose x amphetamine dose x time). However, in the cases of buprenorphine (fig. 6A), fentanyl (fig. 7A), meperidine (fig. 8A) and levorphanol (fig. 9A) and its stereoisomer dextrophan (fig. 10A), this interaction term was not significant. Nevertheless, with the exception of fentanyl, these drugs increased the duration of action of amphetamine from approximately 180 min to more than 240 min. The data presented in figure 5 for morphine are derived from those in figure 1, and they are shown here to facilitate comparison with the other agonists studied.

We examined the effects of the mu opioid agonists alone and combined with amphetamine on full ipsilateral turning during 2-hr time blocks as well as during the full 4-hr observation. These data are the same as those in the time-course graphs, but they are collapsed into 2-hr and 4-hr blocks,
respectively, for further analysis. When amphetamine was preceded by methadone (fig. 4B), morphine (fig. 5B), buprenorphine (fig. 6B) and dextrorphan (fig. 10B), turning was augmented significantly during both 2-hr periods. However, the effects of fentanyl (fig. 7B) and meperidine (fig. 8B) occurred only during the final 2 hr. Levorphanol did not significantly increase amphetamine-induced turning in either 2-hr time block, although there was a trend in that direction (fig. 9B).

A two-factor ANOVA (\(\mu\) opioid agonist \(\times\) amphetamine) indicated that there was a significant main effect of amphetamine alone, the \(\mu\) opioid agonists alone and dextrorphan alone upon turning over the full 4 hr. However, the interaction term with amphetamine was significant only for methadone (fig. 4B) and morphine (fig. 5B), which indicated that amphetamine changed the shape of the dose-response curves of these two \(\mu\) opioid agonists. To examine whether these drugs significantly potentiated the effects of amphetamine, other statistical methods must be used.

**Synergism of \(\mu\) opioid agonists with amphetamine.**

To determine whether the observed potentiation of amphetamine-induced turning by the \(\mu\) opioid agonists and dextrorphan was a simple additive effect or an effect that was greater than additive (i.e., synergistic), we summed the total turns produced by each \(\mu\) opioid agonist and dextrorphan alone with the total turns produced by amphetamine alone for each individual animal. These sums were averaged to produce a mean and standard error for each drug combination (table 1). Because the variance of the means in the table was not homogeneous, we used nonparametric statistical methods to analyze these data. These values were compared with the total of full ipsilateral turns observed when the two drugs were actually coadministered.

A Friedman’s ANOVA (calculated vs. observed rotations \(\times\) dose) on these data indicated there was an overall significant difference between the predicted and observed amounts of turning induced by the drugs tested in combination with 1.0 mg/kg amphetamine, with the exception of buprenorphine and fentanyl (table 1). A follow-up analysis of individual doses with a Wilcoxon matched-pairs test revealed that the middle doses of dextrorphan (3.0 mg/kg), levorphanol (0.3 mg/kg), meperidine (10 mg/kg), methadone (1.0 mg/kg) and morphine (3.0 mg/kg), as well as the higher dose of dextrorphan (10 mg/kg) in combination with amphetamine, produced turning greater than predicted, \(P < .05\) (table 1).

**Antagonism of \(\mu\) opioid agonist effects.** Naltrexone (1.0 mg/kg) blocked the effects of 3.0 mg/kg morphine upon amphetamine-induced rotational behavior (fig. 11). Post hoc tests revealed that there was no statistical difference between turning induced by amphetamine alone and by naltrexone combined with morphine and amphetamine. Further testing revealed that the effects of 3.0 mg/kg morphine on 1.0 mg/kg amphetamine were blocked by a lower dose of naltrexone, 0.1 mg/kg. However, 1.0 mg/kg naltrexone did not statistically alter the effects of the other \(\mu\) opioid agonists or dextrorphan on amphetamine-induced turning. We tested a
higher dose of naltrexone, 10 mg/kg, with the combination of 1.0 mg/kg methadone and 1.0 mg/kg amphetamine, but it did not significantly alter rotational behavior induced by this drug combination (data not shown).

Inspection of the data in figure 11 suggested that the combination of 1.0 mg/kg naltrexone and 1.0 mg/kg amphetamine often resulted in greater turning than occurred after amphetamine alone. This apparent interaction was not statistically significant within each individual drug series. However, when data were pooled from all 24 subjects, 1.0 mg/kg naltrexone significantly potentiated turning induced by 1.0 mg/kg amphetamine ($P < .05$) (fig. 12). The highest dose of naltrexone (10 mg/kg) also potentiated amphetamine-induced circling ($P < .01$), but the lowest (0.1 mg/kg) did not (fig. 12). None of these doses of naltrexone alone produced turning behavior (fig. 3).

**Discussion**

The initial experiments in this study showed that morphine potentiated rotational behavior induced by both amphetamine and cocaine by 3- to 4-fold. Both of these stimulant drugs are indirect dopamine agonists with different mechanisms of action. Amphetamine preferentially releases newly synthesized dopamine from presynaptic cells (Arbuthnott et al., 1991; Kuczenski and Segal, 1989; Zetterstrom et al., 1986b). Cocaine acts by blocking the presynaptic dopamine transporter, thus blocking the reuptake of synaptic dopamine. Some differences have been found in the modulation of locomotor effects of these two drugs by the nonselective opioid receptor antagonist naloxone (Jones and Holtzman, 1994), which suggests that modulation of the effects of amphetamine by endogenous opioids also differs from that of cocaine. However, our data indicate that the effects of both amphetamine and cocaine are enhanced by $\mu$ opioid receptor agonists, although the exact mechanism(s) by which that occurs is not clear. The results correspond with those of several other studies in which there was an apparent synergy between cocaine and morphine-like opioids (see the introduction).

To determine whether the effects of morphine on stimulant-induced rotational behavior were specific to this drug or general to agonists at the $\mu$ opioid receptor, we examined the effects of several other $\mu$ opioid agonists. Each of these drugs produced ipsilateral turning when administered alone, as reflected in the significant main effect of dose. $\mu$ opioid receptors are localized on dopaminergic nerve terminals in the striatum (Mansour et al., 1995), and increase the release of dopamine from these cells upon stimulation (Kalivas and Stewart, 1991). Presumably, these $\mu$ opioid agonists produced ipsilateral turning by increasing synaptic levels of dopamine in an indirect fashion. The order of potency of these drugs in producing turning paralleled their order of potency in other assays of drug interactions with the $\mu$ opioid receptor (Holtzman and Locke, 1988). Morphine alone pro-
duced relatively little turning in the series of experiments with amphetamine, in agreement with previous results (Kimmel et al., 1995). However, morphine had a larger effect in the series of experiments with cocaine. Each animal in that group was exposed to cocaine 24 times during the randomized sequence of tests with cocaine alone, morphine alone and morphine-cocaine combinations. It is possible that this exposure to cocaine sensitized the animals to the effects of morphine more than did comparable exposure to amphetamine, thus accounting for the different outcomes.

Methadone had a significant overall effect on rotational behavior, although no single dose produced significant turning, and increased amphetamine-induced rotational behavior. A dose of 1.0 mg/kg produced effects greater than predicted by simple additivity. In the conditioned place preference paradigm, methadone enhanced the reinforcing properties of cocaine in rats (Bilsky et al., 1992). Similarly, in humans, cocaine use was greater in methadone-treated opioid addicts than in buprenorphine- or naltrexone-treated patients (Kosten et al., 1989). The discriminative stimulus effects of cocaine in squirrel monkeys were also enhanced by methadone (Speelman and Bergman, 1992, 1994). This mu opioid agonist increases synaptic dopamine levels in the nucleus accumbens (Di Chiara and Imperato, 1988b). The data in the present study concur with the findings that methadone enhances dopaminergic functions.

Buprenorphine is a partial agonist at the mu opioid receptor and an antagonist at the kappa receptor (Cowan et al., 1977; Leander, 1987; Negus and Dykstra, 1988; Negus et al., 1990). Evidence for interactions of buprenorphine with drugs that act via the dopaminergic system has been conflicting. For example, buprenorphine attenuated cocaine self-administration in humans (Foltin and Fischman, 1994), rhesus monkeys (Mello et al., 1992, 1993) and rats (Brown et al., 1991; Carroll and Lac, 1992; Dykstra et al., 1992), and attenuated cocaine conditioned place preference in rats in two studies (Kosten et al., 1991; Suzuki et al., 1992), but augmented cocaine-induced conditioned place preference in another study (Brown et al., 1991). Buprenorphine alone increased locomotor activity in mice, but did not potentiate or attenuate locomotor activity induced by cocaine (Jackson et al., 1993). Both cocaine and buprenorphine increased dopamine release in the nucleus accumbens when administered separately and together (Brown et al., 1991). In the present experiments, buprenorphine produced rotational behavior alone and increased amphetamine-induced circling. These results lend support to the studies which indicate that buprenorphine increases dopamine release, producing behaviors that are associated with this neurochemical event. This augmented release of dopamine is possibly a result of buprenorphine’s actions on both the mu and kappa opioid receptors. Based on microdialysis data, stimulation of the mu opioid receptor would result in increased synaptic dopamine levels, whereas inhibiting the kappa opioid receptor would remove its inhibitory effects upon dopamine release (Di Chiara and Imperato, 1988b). Perhaps it is because of its dual

![Fig. 8. Meperidine (3.0–30 mg/kg) augmented amphetamine-induced turning behavior and induced turning by itself. Graphs are as described in figure 4.](image-url)
actions at mu and kappa opioid receptors that buprenorphine produced the most turning of any of the opioids that we tested.

Fentanyl did not increase the effects of amphetamine in the present study, and in the rat drug discrimination paradigm, it did not substitute for cocaine or alter the effects of cocaine when given as a pretreatment (Broadbent et al., 1995). However, in monkeys, fentanyl potentiated the discriminative effects of cocaine, as evidenced by a leftward shift of the cocaine stimulus curve (Spealman and Bergman, 1992, 1994). In vivo microdialysis studies showed that this opioid agonist increased dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988b).

In addition to examining the effects of mu opioid agonists upon rotational behavior, we tested dextrophan, the optical isomer of levorphanol. Dextrophan has little affinity for the mu opioid receptors and lacks significant analgesic and other opioid effects (Jaffe and Martin, 1985), and alone produced fewer rotations than did levorphanol alone. Surprisingly, in combination with amphetamine, dextrophan produced slightly more turning behavior than did levorphanol. The effect of dextrophan on stimulant-induced turning may be because of its interaction at the PCP recognition site of the NMDA glutamate receptor (Murray and Leid, 1984; Sun et al., 1986).

Based on the model of simple arithmetic additivity, dextrophan, levorphanol, meperidine, methadone and morphine potentiated the effects of amphetamine on circling. Although this “effect-addition” model is not without limitations (Woolverton, 1987), it is a reasonable way to approach the question of drug synergy in the absence of more specific statistical methods. The fact that the mu opioid agonists often increased the effects of amphetamine at times when the opioid itself had little or no effect (e.g., during the second half of the 4-hr session in the case of most of the drugs, figs. 4B, 5B, 6B, 7B, 8B) suggests a true synergy.

Naltrexone, a nonspecific opioid receptor antagonist, blocked the interaction between morphine and amphetamine, but not that between the other mu opioid agonists and amphetamine. The reason for this is not clear. However, the outcomes might have been confounded by an interaction between naltrexone and amphetamine. When 0.1 mg/kg naltrexone was administered with amphetamine, there were no significant effects on the stimulant-induced turning behavior. However, higher doses potentiated amphetamine-induced circling. This observation conflicts with findings that the opioid antagonist naloxone can decrease amphetamine-induced locomotor activity (Hooks et al., 1992; Jones and Holtzman, 1992, 1994) and dopamine release in rats (Schad et al., 1995). Kappa opioid receptor activation decreases dopamine release (Di Chiara and Imperato, 1988b). Perhaps by blocking kappa opioid receptors, naltrexone augments the release of dopamine. Thus, naltrexone might have blocked the potentiating effect of the mu opioid agonist while increas-
The dopamine response to amphetamine, which resulted in no net change in turning. However, even if this speculation was to prove correct, it would leave unresolved the reason that naltrexone did block the amphetamine-potentiating effect of morphine.

In summary, morphine increases rotational behavior induced by amphetamine and cocaine. However, the role of opioid receptors in this interaction is unresolved. Results with other mu opioid agonists were consistent among drugs in that they all increased amphetamine-induced circling, but this interaction was not greater than additive in all cases. In addition, each of the mu opioid agonists studied induced turning by themselves, although not always at any one dose. The opioid antagonist naltrexone blocked the effects of morphine but not those of the other mu opioids. Although we cannot draw conclusions about the role of opioid receptors in the effects of mu opioid agonists on turning induced by psychomotor stimulant drugs, it is, nevertheless, clear that these opioids can enhance a dopamine-mediated behavioral effect of amphetamine and cocaine.

### Acknowledgments

The authors extend their appreciation for the generous contribution of dextrorphan tartrate and levorphanol tartrate by Roche Lab-

![Fig. 10. Dextrorphan (1.0–10 mg/kg), a stereoisomer of levorphanol, potentiated amphetamine-induced ipsilateral turning and had an effect on circling behavior alone. Graphs are as described in figure 4.](image)

**TABLE 1**

Synergism of mu opioid agonists and amphetamine

<table>
<thead>
<tr>
<th>Calculated Observed</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calculated</strong></td>
<td><strong>Observed</strong></td>
</tr>
<tr>
<td>Buprenorphine</td>
<td>559 ± 149</td>
</tr>
<tr>
<td>Dextrorphan</td>
<td>522 ± 115</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>209 ± 46</td>
</tr>
<tr>
<td>Levorphanol</td>
<td>369 ± 84</td>
</tr>
<tr>
<td>Meperidine</td>
<td>504 ± 106</td>
</tr>
<tr>
<td>Methadone</td>
<td>343 ± 70</td>
</tr>
<tr>
<td>Morphine</td>
<td>359 ± 132</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>800 ± 180</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td>702 ± 135</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>305 ± 68</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>617 ± 79</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td>476 ± 112</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>493 ± 72</td>
</tr>
<tr>
<td><strong>Low</strong></td>
<td>652 ± 157</td>
</tr>
<tr>
<td><strong>Medium</strong></td>
<td>421 ± 83</td>
</tr>
<tr>
<td><strong>High</strong></td>
<td>421 ± 83</td>
</tr>
</tbody>
</table>

* Significantly greater than calculated, P < .05.
Fig. 11. Naltrexone did not block potentiation of the effects of amphetamine by dextrorphan or mu opioid agonists, with the exception of 3.0 mg/kg morphine. The asterisk denotes a significant difference between the mu agonist + amphetamine + naltrexone group and the mu agonist + 1.0 amphetamine group, *P < .05.

Fig. 12. Naltrexone (0.1–1.0 mg/kg) did not induce turning behavior by itself, but it potentiated amphetamine-induced circling in a dose-dependent fashion, with the two higher doses producing significant effects. Numbers above each pair of bars represent the number of animals in that particular group. The 1.0 mg/kg naltrexone group data are pooled from the data shown in figure 11. Asterisks represent a significant difference from the corresponding 1.0 mg/kg amphetamine alone group, **P < .01, *P < .05.

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HOLTZMAN, S. G. AND LOCKE, K. W.: Neural mechanisms of drug stimuli: experimental approaches. In Transduction Mechanisms of Drug Stimuli, ed. by...