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Opioid receptor agonists and antagonists alter GBR12909-induced turning in the rat

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Abstract

We extended observations on cocaine-induced turning and its interactions with μ-opioid receptor agonists in nigrally-lesioned rats to GBR12909 (1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenyl-propyl]-piperazine), a selective dopamine reuptake inhibitor. GBR12909 produced turning that was potentiated by the μ-opioid receptor agonists morphine and methadone. The effects of these opioids were blocked by the general opioid receptor antagonist naloxone, which did not affect the action of GBR12909. The reuptake inhibitors nisoxetine (norepinephrine) and fluoxetine (serotonin) did not produce turning alone or in combination with morphine. Antagonists selective for each opioid receptor subtype did not alter GBR12909-induced turning. However, naltrexone, another general opioid receptor antagonist, potentiated turning induced by GBR12909. This was blocked by naloxone, suggesting that naltrexone has opioid receptor agonist actions, in contrast to naloxone. These results indicate that cocaine-induced turning and its potentiation by μ-opioid receptor agonists are dependent upon the inhibition of dopamine reuptake. © 1998 Elsevier Science B.V.

Keywords: GBR12909; Morphine; Naloxone; Naltrexone; Nigrostriatal lesion; Rotational behavior

1. Introduction

Rats given a unilateral lesion of the nigrostriatal dopamine tract exhibit a postsynaptic dopamine receptor supersensitivity in the lesioned striatum as a result of the destruction of presynaptic dopamine neurons (Robinson and Becker, 1983). Cocaine inhibits the reuptake of dopamine, and the resulting increase of synaptic levels of this neurotransmitter stimulates dopamine receptors in the intact nigrostriatal tract. Since the neurons on one side of the brain control movement on the opposite side of this body, the administration of cocaine produces turning ipsilateral to the lesion (Ungerstedt and Arbuthnott, 1970). The effects of cocaine on turning behavior in unilaterally lesioned rats can be modified by opioids. The μ-opioid receptor agonists buprenorphine and morphine potentiated cocaine-induced turning behavior in nigrally lesioned rats (Kimmel and Holtzman, 1997; Kimmel et al., 1997).

Turning induced by cocaine is due to its inhibition of dopamine reuptake, but it is not clear if the interactions with μ-opioid receptor agonists also depends upon this action of cocaine. In addition to inhibiting dopamine reuptake, cocaine also inhibits the reuptake of norepinephrine and serotonin. In contrast to cocaine, 1-[2-[bis(4-fluorophenyl)methoxy]ethyl]-4-[3-phenyl-propyl]-piperazine (GBR12909) is a selective dopamine uptake inhibitor. GBR12909 inhibited, with high affinity and selectivity, dopamine uptake in rat striatal slices (Van der Zee et al., 1980; Heikkila and Manzino, 1984; Rothman et al., 1989; Anderson, 1989) and increased [^{3}H] dopamine efflux (Gifford et al., 1993). In vivo microdialysis studies in rats showed that GBR12909 produced a slow and sustained increase in extracellular dopamine in the nucleus accumbens (Baumann et al., 1994) and in the striatum (Rothman et al., 1991; Nakachi et al., 1995) of rats. These studies suggest that GBR12909 inhibits the dopamine transporter selectively and produces increases in extracellular dopamine.

Behavioral studies have shown further similarities between the effects of cocaine and of GBR12909. In rhesus monkeys trained to distinguish cocaine from saline, GBR12909 produced cocaine-appropriate responding (Kleven et al., 1990). GBR12909 produced a long-lasting dose-dependent increase in locomotor activity in intact rats

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(Kelley and Lang, 1989; Nakachi et al., 1995) as well as ipsilateral circling in rats with a unilateral nigrostriatal lesion (Heikkila and Manzino, 1984). Both of these effects were attenuated by haloperidol pretreatment (Heikkila and Manzino, 1984), indicating that they were mediated by dopamine (Kelley and Lang, 1989).

The objective of this study was to characterize the effects of GBR12909 on turning in unilaterally lesioned rats. We examined the turning effects of a range of doses of GBR12909 alone, and in combination with the μ-opioid receptor agonist, morphine. To show that the effects of morphine upon GBR12909-induced turning could be generalized to other μ-opioid receptor agonists, we also tested GBR12909 in combination with the μ-opioid receptor agonist, methadone. The non-selective opioid receptor antagonist naloxone was administered prior to the μ-opioid receptor agonist and GBR12909 combination to show that effects of the μ-opioid receptor agonists upon GBR12909-induced turning were mediated by an opioid receptor. Naltrexone, another non-selective opioid receptor antagonist, potentiated amphetamine-induced rotational behavior in an earlier study (Kimmel and Holtzman, 1997); therefore we tested a range of doses of naltrexone in combination with GBR12909 and, for comparison, with cocaine. To determine which opiate receptors might be involved in turning induced by 10 mg/kg GBR12909, we administered the selective opiate receptor antagonists, β-funaltrexamine (μ), naltrindole (δ) and norbinaltorphimine (κ), prior to GBR12909. Finally, to examine the role of serotonin and norepinephrine in cocaine-induced turning, and its potentiation by morphine, we administered the selective reuptake inhibitors fluoxetine (serotonin) and nisoxetine (norepinephrine) alone and in combination with morphine.

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley rats (Charles River Laboratories, Raleigh, NC) weighing 240–260 g were used. All rats were group housed in polycarbonate cages and maintained in a temperature-controlled colony room with a 12L:12D light cycle. Rats had free access to food (Purina Rodent Chow, Purina Mills, St. Louis, MO) and water.

2.2. Stereotaxic surgery

All rats were given a unilateral lesion of the right nigrostriatal pathway by a single injection of 6-hydroxydopamine. Rats were anesthetized with 3.3 mg/kg Equithesin (i.p.) and placed into a stereotaxic frame. Stereotaxic coordinates used relative to bregma were: AP = −4.5, ML = −2.3, DV = −7.1 (Paxinos and Watson, 1986). A 25 μl Hamilton syringe was used to inject 8 μg/4 μl of 6-hydroxydopamine 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 min to minimize back flow of the solution.

2.3. Rotational behavior

Rotational activity was measured in eight stainless steel rotometer stations (MED Associates, East Fairfield, VT). Each station consisted of a round metal bowl (40.6 cm diameter and 25.4 cm high) with 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°) clockwise and counterclockwise turns. During experimental test sessions, counts were taken in 15-min time intervals for 4 h, resulting in 16 time points per animal per session. Rats were allowed to recover from surgery for at least 14 days, then they received 0.3 mg/kg $R(-)$-apomorphine s.c. twice weekly for 2 weeks. Rats exhibiting an average of at least 50 contralateral turns in each 10-min interval for 1 h were used for further experiments. The amount of turning in response to apomorphine has been found to be directly correlated with the extent of the nigral lesion (Hudson et al., 1993). Rats meeting the inclusion criterion have greater than 90% depletion of dopamine in the striatum on the side of the lesion (Kimmel et al., 1997). Behavioral testing did not begin until at least 1 week following the last apomorphine administration, to prevent any possible carry-over effects of the drug.

2.4. 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 injections. Measurements of rotational behavior began 5 min after the final injection. Each animal was tested twice weekly with a 3–4 day interval between testing. When multiple doses of a drug were tested, the doses were administered in a random sequence to prevent any order effects.

Rats ($n = 7$) received GBR12909 (3.0–30 mg/kg, i.p.) alone and in combination with morphine (0.1–3.0 mg/kg, s.c.). Rats ($n = 8$) were then injected with 3.0 mg/kg s.c. naloxone followed by 3.0 mg/kg s.c. morphine and 3.0 mg/kg i.p. GBR12909. Another group of rats ($n = 7$), received methadone (0.3–3.0 mg/kg, s.c.) prior to 3.0 mg/kg i.p. GBR12909. Rats ($n = 7$) were then injected with 0.3 mg/kg s.c. naloxone followed by 3.0 mg/kg s.c. methadone and 3.0 mg/kg i.p. GBR12909. We tested naloxone (0.3–3.0 mg/kg, s.c.) in combination with 10 mg/kg i.p. GBR12909 ($n = 8$). Following this, we tested naltrexone (0.3–3.0 mg/kg, s.c.) with GBR12909 (3.0–30 mg/kg i.p.) ($n = 8$), then we tested 3.0 mg/kg s.c. nalox-

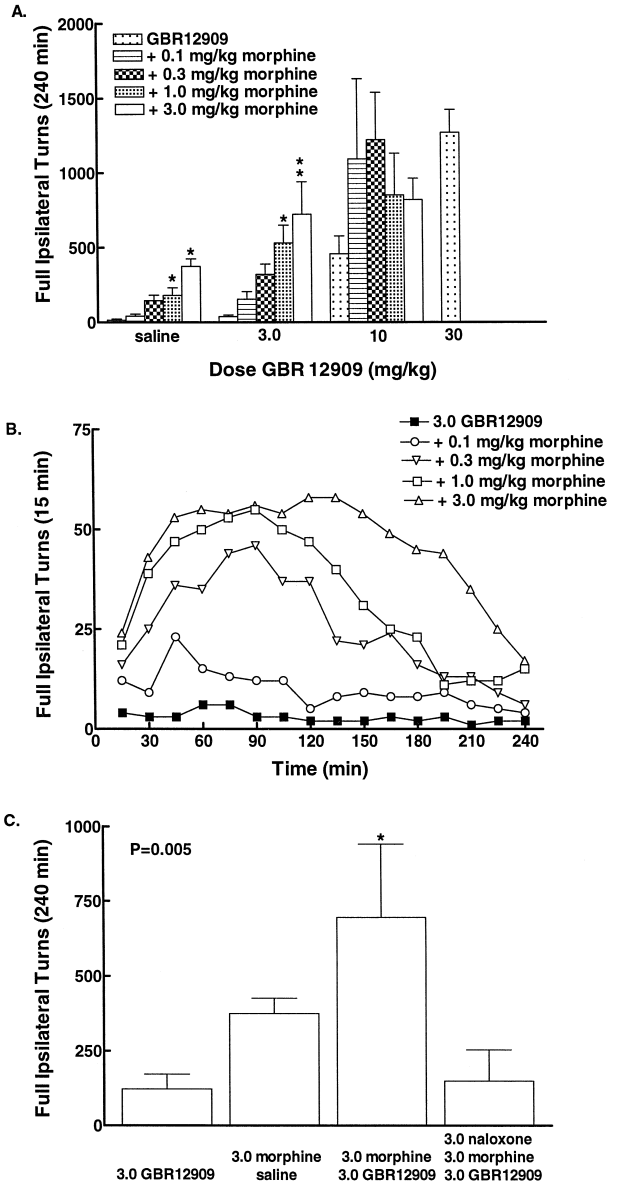

Fig. 1. GBR12909 (3.0–30 mg/kg) produced robust ipsilateral turning that was potentiated by morphine (0.1–3.0 mg/kg). (A) Each bar represents the mean and S.E.M. for 7 animals. A two-way ANOVA revealed a significant F value for GBR12909 $F(2, 12) = 18.7$, $P < 0.0001$, but not for morphine or the interaction term. However, when the data for only 3.0 mg/kg GBR12909 alone and with morphine (0.1–3.0 mg/kg) were analyzed by a one-way ANOVA, a significant effect of morphine was revealed: $F(4, 24) = 19.8$, $P < 0.0001$. Asterisks indicate significant differences between that dose of GBR12909 alone and in combination with morphine, $^{**}P < 0.01$, $^{*}P < 0.05$. (B) Time-course of turning induced by 3.0 mg/kg GBR12909 and its potentiation by 0.1–3.0 mg/kg morphine. The points on the time-course curves represent the mean number of turns/15 min. (C) Naloxone (3.0 mg/kg) blocked the potentiating effects of morphine on GBR12909-induced turning. A one-factor ANOVA revealed significant differences among the treatments, $F(3, 21) = 5.62$, $P = 0.005$. The asterisk indicates a significant difference between that treatment and all others, $^{*}P < 0.05$.

one with 3.0 mg/kg s.c. naltrexone and 10 mg/kg i.p. GBR12909 ($n = 7$). We also tested naltrexone (0.3–10 mg/kg s.c.) with 10 mg/kg i.p. cocaine ($n = 8$). Rats were anesthetized lightly under a 2:1 methoxyflurane:halothane mixture, then administered 3.0–30 μg intracisternal (i.c.) β-funaltrexamine, 24 h before testing ($n = 8$), 3.0–30 μg i.c. naltrindole, 1 h before testing ($n = 8$), or 3.0–30 μg i.c. norbinaltorphimine, 24 h before testing ($n = 7$) with GBR12909. Fluoxetine (3.0–10

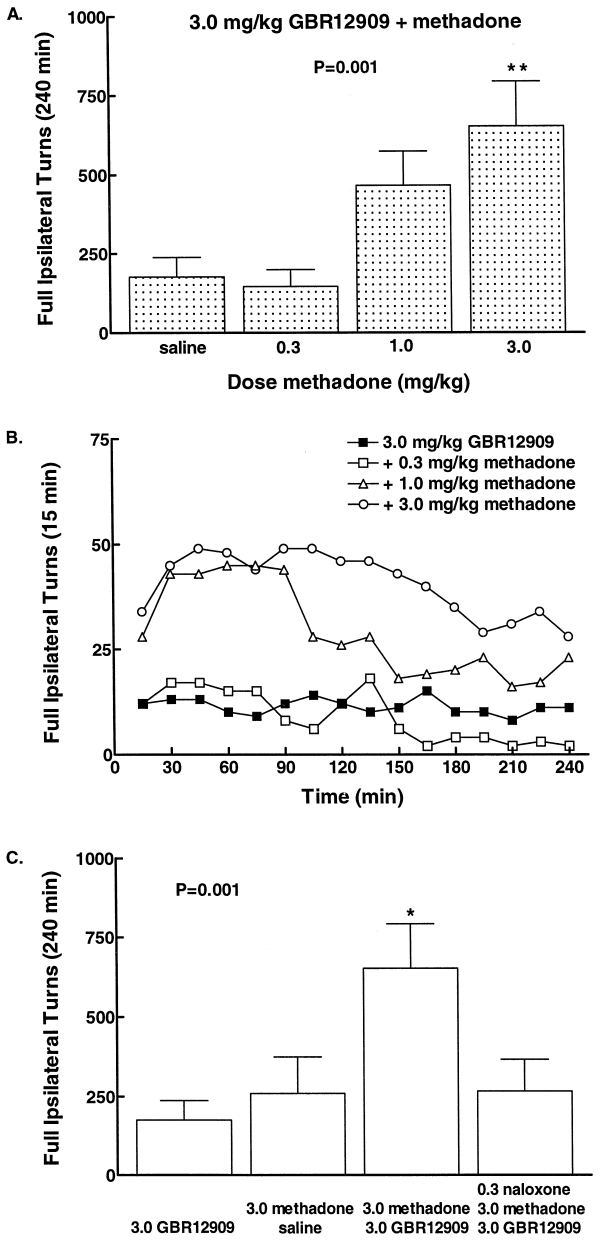

Fig. 2. Methadone (0.3–3.0 mg/kg) potentiated rotational behavior induced by 3.0 mg/kg GBR12909. (A) A one-factor ANOVA revealed a significant effect of methadone, $F(3, 18) = 9.44$, $P = 0.001$. Asterisks represent a significant difference between GBR12909 alone and in combination with that dose of methadone, $^{**}P < 0.01$. Other details as in Fig. 1A. (B) Time-course of turning induced by 3.0 mg/kg GBR12909 and its potentiation by 0.3–3.0 mg/kg methadone. Details as in Fig. 1B. (C) Naloxone (0.3 mg/kg) blocked the potentiating effects of methadone upon GBR12909-induced turning. A one-factor ANOVA revealed significant differences among the treatments $F(3, 18) = 8.59$, $P = 0.001$. Other details as in Fig. 1C.

mg/kg s.c.) ($n = 7$) and nisoxetine (3.0–10 mg/kg s.c.) ($n = 7$) were tested alone and in combination with morphine (1.0 mg/kg s.c.).

2.5. Statistical analysis

The effects of GBR12909, morphine, and naltrexone alone upon ipsilateral turning were analyzed using a one-factor analysis of variance (ANOVA). Drug combinations were then analyzed using a one-factor ANOVA or a two-factor ANOVA (first drug × second drug) with repeated measures on both factors to determine the presence of interactions. When three drugs were given together (i.e. naloxone + morphine + GBR12909), a one-factor ANOVA was used. When appropriate, Tukey's protected *t*-test for multiple pair-wise comparisons was used as a post-hoc test. In the present experiments, no contralateral turning was detected, so this data was not shown.

2.6. Drugs

GBR12909 hydrochloride (Research Biochemicals, Natick, MA) was dissolved in a solution of 30% dimethyl sulfoxide (DMSO) and 70% distilled water. Cocaine hydrochloride (National Institute on Drug Abuse, Rockville, MD) methadone hydrochloride (Mallinkrodt, St. Louis, MO), morphine sulfate (Penick, Newark, NJ) and naloxone hydrochloride (Sigma Chemical, St. Louis, MO), naltrexone hydrochloride (Sigma Chemical) were dissolved in 0.9% saline. *R*(−)-apomorphine hydrochloride (Research Biochemicals) and 6-hydroxydopamine hydrobromide (Sigma Chemical) were dissolved in a solution of 0.1% ascorbic acid in 0.9% saline. Fluoxetine hydrochloride and nisoxetine hydrochloride (Eli Lilly, Indianapolis, IN) and β-funaltrexamine hydrochloride, naltrindole hydrochloride, and norbinaltorphimine dihydrochloride (NIDA) were dissolved in distilled water. All drugs except for 6-hydroxydopamine, β-funaltrexamine, naltrindole, and norbinaltorphimine were administered in a volume of 1 ml/kg body weight. β-Funaltrexamine, naltrindole, and norbinaltorphimine were administered intracisternally in a 5 μl volume.

3. Results

3.1. μ-Opioid receptor agonists and GBR12909

GBR12909 (3.0–30 mg/kg) produced robust ipsilateral turning in a dose-dependent manner (Fig. 1A). The lowest dose of GBR12909 (3.0 mg/kg) produced 43 ± 12 turns/4 h (mean ± S.E.M.), whereas 30 mg/kg produced 1270 ± 154 turns/4 h. GBR12909 did not produce contralateral turning in these lesioned animals, so this data is not shown. Morphine (0.1–3.0 mg/kg) also produced significant turning ($F(4, 24) = 18.3$, $P < 0.0001$). The lowest dose of morphine (0.1 mg/kg) produced 43 ± 13 ipsilateral turns/4 h, while 3.0 mg/kg produced 375 ± 43 turns/4 h. When morphine (0.1–3.0 mg/kg) was administered immediately prior to GBR12909, no significant interaction was observed across all doses of GBR12909 and of morphine. However, morphine potentiated the effect of 3.0 mg/kg GBR12909 significantly ($P < 0.0001$), such that the combinations of GBR12909 with 1.0 mg/kg and 3.0 mg/kg morphine produced turning that was significantly greater than was produced by this dose of GBR12909 alone ($P < 0.05$ and $P < 0.01$, respectively). Turning after 10 mg/kg GBR12909 and morphine was also higher than after GBR12909 alone, but high variability prevented this from reaching statistical significance. There was a significant main effect of GBR12909 dose ($P < 0.0001$), but not of morphine dose in the two-way ANOVA performed on the drug combinations. Fig. 1B shows the time-course of turning produced by 3.0 mg/kg GBR12909 alone and in combination with 3.0 mg/kg morphine. The effects of 3.0 mg/kg morphine upon 3.0 mg/kg GBR12909 were blocked by 3.0 mg/kg naloxone (Fig. 1C). A one-way ANOVA indicated a significant difference among the four treatment groups ($P = 0.005$), and the post-hoc test revealed that the morphine + GBR12909 treatment differed significantly from both the GBR12909 alone and the

Table 1
Synergism of μ-opioid receptor agonists and GBR12909

	Calculated				Observed				*P* value
	0.1	0.3	1.0	3.0	0.1	0.3	1.0	3.0	
Morphine									
+3 mg/kg GBR12909	102 ± 24	241 ± 45	246 ± 66	413 ± 54	205 ± 69	392 ± 72[a]	588 ± 127[a]	804 ± 245	0.0005
+10 mg/kg GBR12909	555 ± 127	690 ± 129	681 ± 149	875 ± 160	1424 ± 672	1498 ± 353[a]	849 ± 280	812 ± 146	0.0542
Methadone									
+3 mg/kg GBR12909		95 ± 29	204 ± 67	288 ± 107		145 ± 53	465 ± 107	651 ± 141[a]	0.0008

Theoretical number of full ipsilateral turns expected when the μ-opioid receptor agonist morphine or methadone was administered with GBR12909 vs. observed turning with the combination. All values represent the mean ± S.E.M. *P* values in last column denote statistical difference between the calculated and observed values at all doses of the μ-opioid receptor agonist plus GBR12909 as determined by Freidman two-way ANOVA by rank analyses.

[a] Significantly greater than calculated, $P \leq 0.05$.

naloxone + morphine + GBR12909 treatment. In addition, the response to GBR12909 alone and to the naloxone + morphine + GBR12909 combination did not differ from each other. These results suggest that the effects of morphine upon GBR12909-induced turning were mediated by an opiate receptor.

To confirm the effects of morphine, we administered methadone (0.3–3.0 mg/kg) prior to 3.0 mg/kg GBR12909 (Fig. 2A). Methadone had a significant effect upon GBR12909-induced turning ($P = 0.001$) and 3.0 mg/kg methadone combined with 3.0 mg/kg GBR12909 produced turning greater GBR12909 alone did ($P < 0.01$). Fig. 2B shows the time-course of 3.0 mg/kg GBR12909 alone and with 3.0 mg/kg methadone. To determine if the effects of methadone upon GBR12909 were mediated by an opiate receptor, we administered naloxone (0.3 mg/kg) prior to methadone (3.0 mg/kg) and GBR12909 (3.0 mg/kg) (Fig. 2C). A one-way ANOVA indicated a significant difference among the four treatment groups ($P = 0.001$), and the post-hoc test revealed that the methadone + GBR12909 combination differed from the other three groups. Thus, naloxone blocked the effects of methadone upon GBR12909-induced turning, suggesting that the effects of methadone were mediated by an opiate receptor.

To determine whether the observed interaction of GBR12909 and the μ-opioid receptor agonists was a simple additive effect or an effect that was greater than additive, we summed the total turns produced by each dose of the μ-opioid receptor agonists alone with the total turns produced by GBR12909 alone for each animal. These sums were averaged to produce a mean and standard error for each drug combination (Table 1) and were compared to the total number of full ipsilateral turns observed when the two drugs were actually co-administered. Since the variance of these means was not homogenous, we used non-parametric statistical methods to analyze these data. A Freidman's ANOVA (calculated vs. observed rotations × dose) indicated that there were overall differences between the predicted and observed amounts of turning induced by 3.0 mg/kg GBR12909 combined with morphine and with methadone. However, the predicted and observed amounts of turning induced by 10 mg/kg GBR12909 and morphine fell just short of being significantly different from each other. A follow-up analysis of individual doses with a Wilcoxon matched-pairs test revealed that 0.3 mg/kg morphine with 3.0 mg/kg and 10 mg/kg GBR12909, 1.0 mg/kg morphine with 3.0 mg/kg GBR12909, and 3.0 mg/kg methadone with 3.0 mg/kg GBR12909 produced turning greater than predicted, $P < 0.05$ (Table 1).

3.2. Opioid receptor antagonists and GBR12909

Naltrexone alone (0.3–3.0 mg/kg), had no significant effect upon turning. When naltrexone and GBR12909 were administered together, there was a significant main effect of dose for both GBR12909 and naltrexone ($P < 0.0001$ and $P = 0.016$, respectively), and the interaction between the two factors was also significant ($P = 0.03$) (Fig. 3A). Fig. 3B shows the time-course of the effects of 3.0 mg/kg naltrexone upon turning induced by 10 mg/kg GBR12909. To determine if the effects of naltrexone upon GBR12909-induced turning were mediated by an opiate

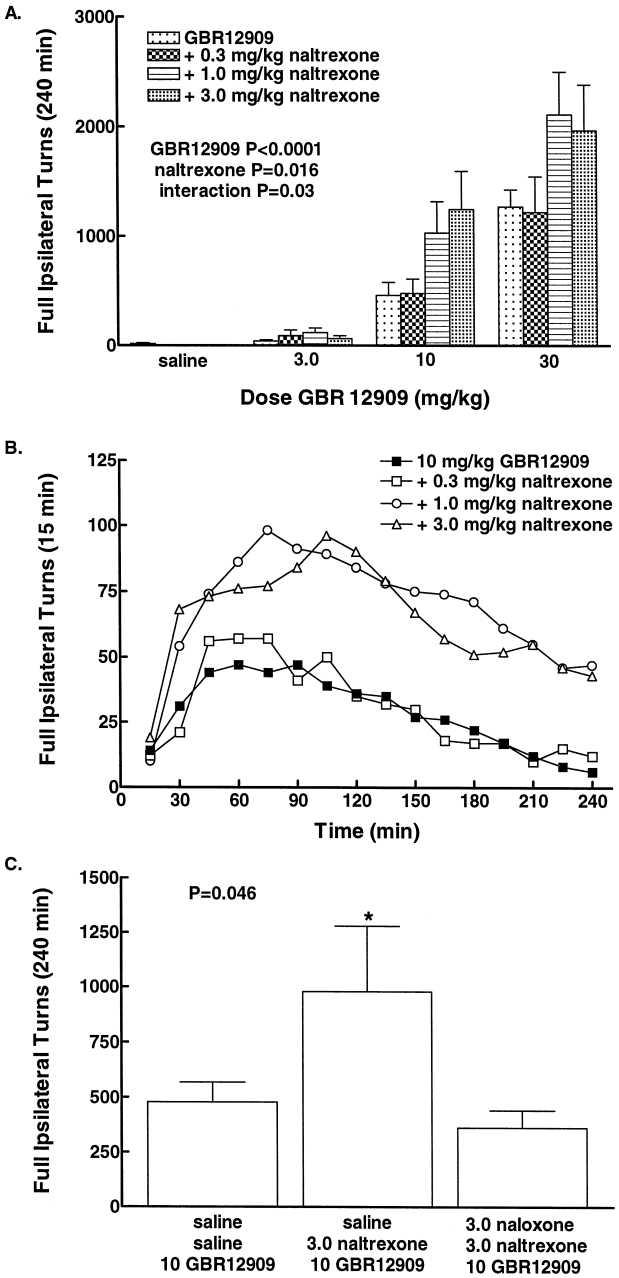

Fig. 3. Naltrexone (0.3–3.0 mg/kg) potentiated turning induced by GBR12909 (3.0–30 mg/kg). (A) A two-way ANOVA revealed significant main effects of GBR12909 dose, $F(3, 21) = 42.5$, $P < 0.0001$ and of naltrexone dose, $F(3, 21) = 4.33$, $P = 0.016$, and a significant interaction between those two factors, $F(9, 63) = 2.25$, $P = 0.03$. Other details as in Fig. 1A. (B) Time-course of turning induced by 10 mg/kg GBR12909 and its potentiation by 0.3–3.0 mg/kg naltrexone. Other details as in Fig. 1B. (C) A one-way ANOVA revealed significant differences among the treatments ($F(2, 6) = 4.014$, $P = 0.046$). Other details as in Fig. 1C.

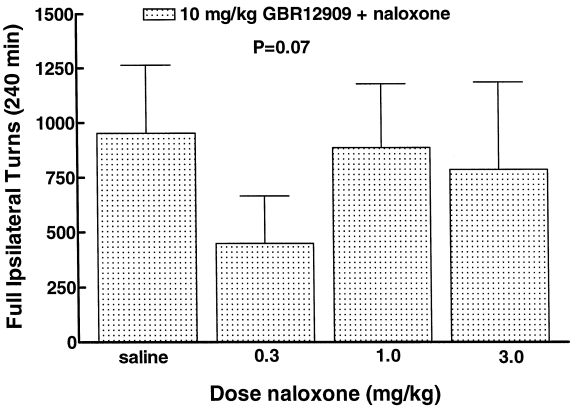

Fig. 4. Naloxone (0.3–3.0 mg/kg) did not alter turning induced by 10 mg/kg GBR12909. A one-factor ANOVA showed that there were no significant differences between turning induced by 10 mg/kg GBR12909 alone and that induced by 10 mg/kg GBR12909 in combination 0.3–3.0 mg/kg of naloxone ($F(3, 21) = 1.79$, $P = 0.07$). Details as in Fig. 1A.

receptor, we administered naloxone (3.0 mg/kg) prior to naltrexone (3.0 mg/kg) and GBR12909 (10 mg/kg) (Fig. 3C). A one-way ANOVA revealed significant differences among the three treatment groups ($P = 0.046$). A post-hoc test revealed that the naltrexone + GBR12909 group differed significantly from the naloxone + naltrexone + GBR12909 group ($P < 0.05$), but that the GBR12909 group and the naloxone + naltrexone + GBR12909 groups did not differ from each other. This suggests that the effects of naltrexone were mediated by an opiate receptor. To determine if naloxone had any effect upon GBR12909-induced turning alone, we administered naloxone (0.3–3.0 mg/kg) prior to 10 mg/kg GBR12909 (Fig. 4). Naloxone did not significantly alter GBR12909-induced turning. In addition, we administered naltrexone (0.3–10 mg/kg) with 10 mg/kg cocaine (Fig. 5). Naltrexone, at any dose tested, did not alter cocaine-induced rotational behavior.

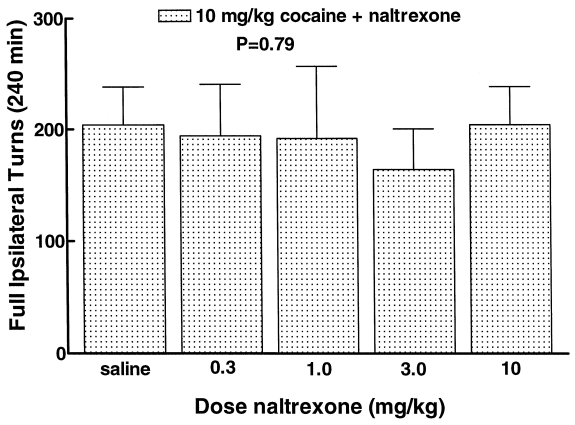

Fig. 5. Naltrexone (0.3–10 mg/kg) did not alter turning induced by cocaine. A one-factor ANOVA showed that there were no significant differences between turning induced by 10 mg/kg cocaine alone and by 10 mg/kg cocaine in combination with 0.3–10 mg/kg naltrexone ($F(4, 28) = 0.43$, $P = 0.79$). Details as in Fig. 1A.

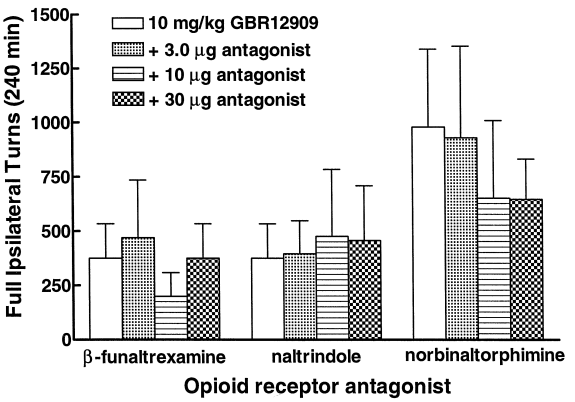

Fig. 6. The opioid-receptor selective antagonists β-funaltrexamine (3.0–30 μg), naltrindole (3.0–30 μg), and norbinaltorphimine (3.0–30 μg) did not alter turning induced by 10 mg/kg GBR12909. A one-way ANOVA revealed that turning induced by 10 mg/kg GBR12909 was not altered by 3.0–30 μg β-funaltrexamine ($F(3, 21) = 1.05$, $P = 0.39$), nor by 3.0–30 μg naltrindole ($F(3, 21) = 0.52$, $P = 0.67$), or by norbinaltorphimine. ($F(3, 18) = 1.32$, $P = 0.30$). Details as in Fig. 1A.

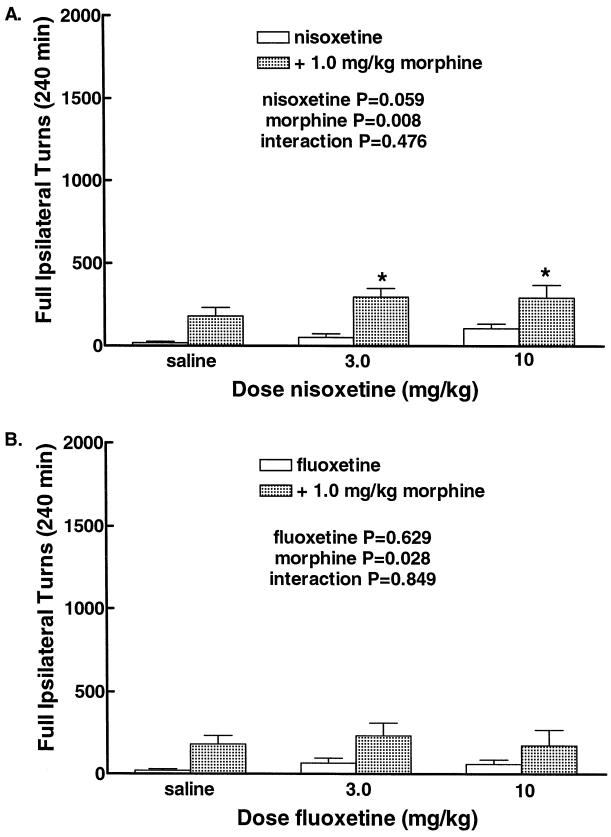

Fig. 7. The selective monoamine transporter inhibitors were tested alone and in combination with 1.0 mg/kg morphine. (A) Nisoxetine (3.0–10 mg/kg), a norepinephrine transporter inhibitor, did not produce turning on its own or have a significant interaction with morphine. However, morphine had a significant main effect ($F(1, 6) = 15.34$, $P = 0.008$) and potentiated the effects of nisoxetine alone. (B) Fluoxetine (3.0–10 mg/kg), a serotonin transporter inhibitor, did not produce significant rotational behavior alone or in combination with 1.0 mg/kg morphine. However, morphine had a significant main effect ($F(1, 6) = 8.309$, $P = 0.028$). Asterisks represent those points which are greater than the transporter inhibitor alone, $^{*}P < 0.05$.

To determine which opioid receptor might be involved in the effects of naltrexone upon GBR12909-induced turning, we administered selective opioid receptor antagonists prior to 10 mg/kg GBR12909 (Fig. 6). The group of animals receiving norbinaltorphimine had a higher baseline response to 10 mg/kg GBR12909 than those animals receiving β-funaltrexamine or naltrindole. Neither β-funaltrexamine (3.0–30 μg), naltrindole (3.0–30 μg), or norbinaltorphimine (3.0–30 μg) significantly altered GBR12909-induced turning.

3.3. Morphine and other monoamine uptake inhibitors

To determine if other monoamine uptake inhibitors had effects on turning similar to those of GBR12909, we administered the norepinephrine uptake inhibitor nisoxetine (3.0–10 mg/kg) and the serotonin uptake inhibitor fluoxetine (3.0–10 mg/kg) alone and in combination with morphine (1.0 mg/kg). A two-way ANOVA showed that nisoxetine did not produce turning on its own, but there was a significant main effect of morphine dose ($F(1, 6) = 15.43$, $P = 0.008$) (Fig. 7A). No significant interaction between nisoxetine and morphine was found. Morphine (1.0 mg/kg), in combination with both doses of nisoxetine, produced turning greater than that dose of nisoxetine alone ($P < 0.05$), but not greater than the turning observed after morphine alone. Similarly, fluoxetine did not produce turning on its own and there was a significant main effect of morphine dose ($F(1, 6) = 8.31$, $P = 0.028$) (Fig. 7B). There was no significant interaction between fluoxetine and morphine, and morphine in combination with fluoxetine did not produce turning greater than morphine alone did.

4. Discussion

The μ-opioid receptor agonists morphine and methadone dose-dependently potentiated GBR12909-induced turning. The effects of these μ-opioid receptor agonists were blocked by the nonspecific opioid receptor antagonist naloxone, suggesting that their actions were mediated by an opiate receptor. In earlier studies in this laboratory, the μ-opioid receptor agonists levorphanol, methadone, meperidine, and morphine potentiated amphetamine-induced rotational behavior (Kimmel and Holtzman, 1997) and the μ-opioid receptor agonist buprenorphine potentiated cocaine-induced rotational behavior (Kimmel et al., 1997). Mu-opioid receptors are localized upon inhibitory gamma-aminobutyric acid (GABA) neurons projecting from the striatum (Mansour et al., 1995), and the stimulation of these receptors inhibits GABAergic activity thus increasing dopamine release from striatonigral dopamine neurons (Kalivas and Stewart, 1991). Thus, one possible mechanism underlying the present finding is that stimulation of these μ-opioid receptors potentiates dopamine release, leading to a potentiation of dopamine-mediated behaviors. This occurs regardless of whether the dopaminergic drug is a dopamine reuptake inhibitor, such as cocaine, or a dopamine releaser, such as amphetamine.

Naltrexone, but not naloxone, dose-dependently potentiated GBR12909-induced turning. These data for naltrexone are consistent with those that showed that naltrexone dose-dependently potentiated amphetamine-induced turning (Kimmel and Holtzman, 1997). Naloxone blocked the potentiating effects of naltrexone upon GBR12909-induced turning, suggesting that naltrexone is acting via opioid receptors, but as an agonist. These data suggest a fundamental difference between the actions of naloxone and naltrexone. Other studies have found agonist actions of naltrexone that are not apparent with naloxone. In studies of cocaine lethality in rats, naltrexone, but not naloxone, decreased the lethal dose of cocaine, thus potentiating the effects of cocaine (Patterson and Holtzman, 1997). Binding studies using rat brain membrane preparations showed that naltrexone had a higher affinity for the μ- and δ-opioid receptors than did naloxone, with a similar affinity for the κ-opioid receptor (Shaham et al., 1996; Wood et al., 1981). However, none of the specific opioid receptor antagonists, β-funaltrexamine, norbinaltorphimine, or naltrindole, altered GBR12909-mediated turning. The doses of β-funaltrexamine (i.c.) and norbinaltorphimine (i.c.) used in this study were greater than the doses that blocked morphine- and U69593-induced analgesia in the rat (Paronis et al., 1993). The amphetamine-induced increase in extracellular dopamine in the striatum of rats was attenuated by a dose of naltrindole (i.c.) equivalent to that used in the present study. Similarly, a dose of β-funaltrexamine (i.c.) equivalent to that used in the present study attenuated amphetamine-induced increase in extracellular dopamine in the nucleus accumbens (Schad et al., 1996). That these behaviorally active doses did not alter GBR12909-mediated turning suggests that endogenous opioids do not play a role this effect and that the differences between naltrexone and naloxone do not involve their antagonist actions at a single opioid receptor. It is possible that simultaneous antagonism of more than one opioid receptor subtype is necessary to produce the effects of naltrexone. We are unaware of any reports of opioid receptor agonist effects of moderate doses of naltrexone. However, it is possible that either naltrexone or one of its metabolites has agonist activity that is manifested only under certain experimental conditions, such as those present in this study.

Another puzzling finding is that although naltrexone potentiated GBR12909-induced turning in this study and amphetamine-induced turning in an earlier study (Kimmel and Holtzman, 1997), it did not potentiate cocaine-induced turning. GBR12909 and cocaine both inhibit dopamine uptake and produce robust rotational behavior, so it would be expected that naltrexone would have a similar effect on both of these drugs. Amphetamine also inhibits dopamine

uptake, although it is mainly a dopamine releaser. Although inhibition of serotonin and norepinephrine transport did not produce turning, these neurotransmitters may have a modulatory effect upon turning. The effects of cocaine may be dampened by increased activity of norepinephrine and serotonin. This effect would not be apparent with GBR12909, since its actions are selective for the dopamine transporter. In addition, amphetamine and GBR12909 both have a relatively long duration of action, reaching a peak 60 min after administration (Fig. 3B), while the effects of cocaine peak immediately after administration. The peak effect of naltrexone may occur after the peak effect of cocaine, thus any effects of naltrexone upon cocaine-induced turning would not be apparent.

In the present experiments, we observed a dose-dependent increase in ipsilateral turning induced by the selective dopamine reuptake inhibitor GBR12909, similar to that observed in an earlier study (Heikkila and Manzino, 1984). However, nisoxetine and fluoxetine did not induce rotational behavior on their own. In several studies, nisoxetine and fluoxetine did not produce locomotor activity in rats and in mice (Tyler and Tessel, 1980; Callaway et al., 1990; Rempel et al., 1993; Horowitz et al., 1997), and the present results support these findings. These results suggest that inhibition of the dopamine transporter, but not of the norepinephrine or serotonin transporters, produces turning behavior that can be modified by μ-opioid receptor agonists. The results of this study suggest that turning induced by amphetamine and cocaine and the potentiation observed when these compounds are combined with μ-opioid receptor agonists are due to increased extrasynaptic levels of dopamine.

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