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Effects of selective D_1 and D_2 dopamine antagonists on the development of behavioral sensitization to apomorphine*

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Abstract. The objective of the present study was to determine whether the development of behavioral sensitization to apomorphine could be blocked by either D_1 or D_2 selective dopamine antagonists. In three experiments, male rats received 10–21 daily injections of a selective D_1 (SCH 23390; 0 or 0.5 mg/kg IP) or D_2 (sulpiride; 0, 30, or 100 mg/kg IP) antagonist followed by an apomorphine (0 or 1.0 mg/kg SC) injection. In two experiments, the rats were tested for locomotor activity in photocell arenas after the daily injections. In all experiments, the rats were tested for sensitization to apomorphine following the training phase. The results indicated that apomorphine produced a progressively greater increase in locomotor activity with each injection, and this apomorphine-induced increase in activity was completely blocked by both sulpiride and SCH 23390 treatments. However, although both sulpiride and SCH 23390 blocked apomorphine-induced activity, only SCH 23390 injections prevented the development of sensitization to apomorphine. That is, rats pretreated with sulpiride and apomorphine displayed significant sensitization when subsequently tested with a challenge dose of apomorphine alone. These findings suggest that the development of behavioral sensitization to apomorphine is related specifically to the stimulation of dopamine D_1 receptors.

Key words: Behavioral sensitization – Apomorphine – Sulpiride – SCH 23390 – Locomotor activity

The acute administration of direct (e.g., apomorphine) and indirect (e.g., amphetamine, cocaine) dopamine agonists in rats often produces an increase in locomotor activity and the induction of various stereotyped oral movements (sniffing, licking, and gnawing). The repeated administration of these drugs results in the development of behavioral sensitization, characterized by a progressive augmentation of these drug-induced motor behaviors. In humans, repeated exposure to drugs which directly or indirectly stimulate dopamine receptors often results in the delayed appearance of several severe, and sometimes long-lasting, behavioral side-effects (e.g., amphetamine psychosis). It is widely assumed that the neurobiological mechanisms mediating behavioral sensitization in animals are the same as those responsible for the side-effects observed in humans (see Robinson and Becker 1986; Kalivas and Weber 1988). Although it is clear that the development of behavioral sensitization requires the stimulation of dopamine receptors (Kuczenski and Leith 1981; Mattingly and Rowlett 1989; Peris and Zahniser 1989), the specific drug-induced neurobiological changes mediating the development of behavioral sensitization are unknown.

Dopamine receptors exist in at least two distinct subtypes possessing unique pharmacologic and biochemical properties (see Breese and Creese 1986; Clark and White 1987, for review). Dopamine D_1 receptors stimulate adenylate cyclase activity, whereas dopamine D_2 receptors are either unlinked to, or inhibit, this enzyme. Dopamine agonists which induce behavioral sensitization (e.g., apomorphine, amphetamine, cocaine) result in an increased stimulation of both D_1 and D_2 dopamine receptor subtypes. At present, there is still considerable disagreement regarding the involvement of specific dopamine receptor subtypes in the development of behavioral sensitization. For example, Stewart and Vezina (1989; Vezina and Stewart 1989) have concluded that the development of sensitization to amphetamine is the result of increased D_1 receptor stimulation, whereas Levy et al. (1988) suggest that amphetamine-induced sensitization is the result of D_2 receptor stimulation. In contrast, there is some evidence which suggests that the concurrent stimulation of both D_1 and D_2 receptors may be necessary for the development of sensitization to cocaine (Peris and

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Zahniser 1989) and methamphetamine (Ujike et al. 1989). Thus, although the overt behavioral effects of these indirect dopamine agonists are similar, the neurochemical mechanisms mediating the development of behavioral sensitization to these agents may differ.

The objective of the present study was to determine the involvement of specific dopamine receptor subtypes in the development of behavioral sensitization to the direct dopamine receptor agonist, apomorphine. Consequently, in three experiments rats were treated daily with apomorphine in combination with either a selective D_1 or D_2 antagonist and then tested for sensitization following a challenge dose of apomorphine.

Experiment 1

Repeated apomorphine treatments in doses equal to or greater than 1.0 mg/kg produce a progressively greater increase in locomotor activity when administered intermittently (Castro et al. 1985; Mattingly et al. 1988a, 1988b). This progressive increase occurs regardless of whether the apomorphine injection is paired with the activity testing environment, but is generally larger if such pairing occurs (Mattingly and Gotsick 1989). Moreover, the development of sensitization to apomorphine is completely blocked by the concurrent administration of the mixed D_1/D_2 dopamine receptor antagonist, haloperidol (Mattingly and Rowlett 1989). The purpose of experiment 1, therefore, was to determine whether the development of sensitization to apomorphine would also be prevented by concurrent administration of the selective D_2 dopamine receptor antagonist, sulpiride.

Materials and methods

Subjects. Seventy-two male Wistar albino rats (Harlan Industries, Indianapolis, IN) weighing between 250 and 300 g served as subjects. All rats were housed individually in a colony room with a 12-h light-dark cycle and maintained with food and water available continuously. All behavioral testing was conducted during the light phase of the cycle.

Apparatus. Activity measures were taken in two BRS/Lehigh Valley cylindrical activity drums (Model 145–03) that were 60 cm in diameter and 43 cm high. The interior of each drum was painted flat black, and the floor was made of 4 cm diamond-shaped wire mesh. Each drum was located in a separate sound-attenuated experimental cubicle that was kept totally dark throughout testing.

Two banks of three infrared photocells were mounted on the outside of each drum. The photocells were approximately 12 cm apart and 2.5 cm above the drum floor. The photocell banks were connected to back-path eliminator diodes. Movement of the rat through a photocell beam sent a single pulse to the counters. Simultaneous pulses (i.e., pulses spaced less than 0.05 s apart) such as might occur when two beams are broken near their intersection were recorded as a single count by this method. Thus, activity was operationalized as the cumulative number of photobeam interruptions per unit time.

Drugs. Apomorphine hydrochloride (Sigma) was dissolved daily in 0.001 N HCL. It was injected SC in a volume of 0.5 ml/kg. Sulpiride (Sigma) was mixed daily in a 1% glacial acetic acid solution and administered IP in a volume of 1.0 ml/kg. Control injections were given using the appropriate vehicle using the same route and volume as the corresponding drug injection.

Design and procedure. At the beginning of the experiment, the rats were randomly assigned, in equal numbers, to one of six groups comprising a two (agonist dose) × three (antagonist dose) factorial design. On each of the first 21 days of the experiment each rat was first injected with either 0 (vehicle), 30, or 100 mg/kg sulpiride and then about 30 min later injected with either 0 (vehicle) or 1.0 mg/kg apomorphine. The rats were returned to their home cage after the daily injections without behavioral testing. Following this training phase the rats were left undisturbed in their home cages for 6 days prior to sensitization testing. On each of the 4 sensitization test days, all rats received an injection of apomorphine (5.0 mg/kg) prior to activity testing. Activity testing began 15 min following the injection and was conducted for 20 min.

Results and discussion

The results of the sensitization test phase are shown in Fig. 1. These data were analysed with a three-factor mixed analysis of variance (ANOVA) using antagonist dose and agonist dose as between factors and activity session as a within factor. As may be seen in Fig. 1, the three groups of rats pretreated with apomorphine (VEH-APO, 30 SUL-APO, 100 SUL-APO) for 21 days displayed a much greater increase in activity in response to apomorphine than did rats receiving apomorphine for the first time (VEH-VEH, 30 SUL-VEH, 100 SUL-VEH), agonist effect, $F(1, 66) = 17.12$, $P < 0.0001$. More important, this sensitization effect was not blocked by

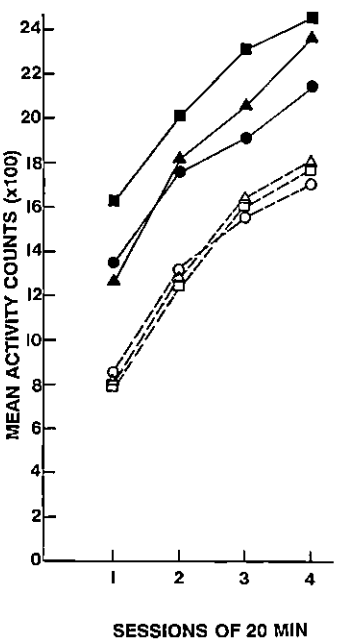

Fig. 1. Mean activity counts across four 20 min sensitization test sessions for rats chronically pretreated with sulpiride (0 (VEH), 30, or 100 mg/kg SUL) and apomorphine (0 (VEH) or 1.0 mg/kg, APO). All rats received 5.0 mg/kg apomorphine 15 min before each test session. ■–■ 100 SUL-APO; □– – –□ 100 SUL-VEH; ▲–▲ 30 SUL-APO; △– – –△ 30 SUL-VEH; ●–● VEH-APO; ○– – –○ VEH-VEH

concurrent sulpiride treatments. That is, the apomorphine-induced activity response of rats pretreated with sulpiride and apomorphine did not differ from that of rats pretreated with only apomorphine (i.e., VEH-APO group). Likewise, it is clear from Fig. 1 that repeated treatments of sulpiride without apomorphine did not significantly affect subsequent sensitivity to apomorphine in this test. The ANOVA performed on these data indicated that neither the main effect of antagonist dose nor the Antagonist dose × Agonist dose interaction was significant. The main effect of test session, however, was significant [$F(3, 198) = 120.68$, $P < 0.0001$], as all groups displayed a progressively greater increase in activity in response to apomorphine across the four test sessions.

Experiment 2

In experiment 1, rats pretreated with apomorphine displayed significant sensitization and this sensitization was not blocked by the D_2 antagonist sulpiride. From the results of experiment 1, however, we have absolutely no evidence that sulpiride was effective in blocking dopamine receptors. The purpose of experiment 2, therefore, was to systematically replicate experiment 1 and to determine the acute effect of sulpiride on apomorphine-induced locomotor activity. Consequently, four groups of rats were injected daily with sulpiride and/or apomorphine and tested for locomotor activity for 7 days. Following this brief subchronic training phase, all rats were tested for activity following an apomorphine injection for four additional days. Experiment 2 differed from experiment 1 in the following ways: 1) only the 100 mg/kg dose of sulpiride was used; 2) the rats were tested for activity during the training phase; 3) the training phase was only conducted for 7 days; and 4) a 1.0 mg/kg dose of apomorphine was used in both the training phase and the sensitization test phase.

Materials and methods

Subjects, apparatus, and drugs. The subjects were 35 male Wistar albino rats (Harlan Industries, Indianapolis, IN) experimentally naive and weighing between 250 and 350 g at the beginning of the experiment. They were housed and maintained as in experiment 1. The photocell activity drums used were also the same as in experiment 1. Likewise, the drugs were obtained, prepared, and administered as in experiment 1.

Design and procedure. The rats were randomly assigned to one of four groups ($n = 8$–9 each) comprising the 2 (antagonist dose: 0 or 100 mg/kg sulpiride) × 2 (agonist dose: 0 or 1.0 mg/kg apomorphine) factorial design. On day 1 of the training phase each rat was first injected IP with either sulpiride or vehicle and returned to its homecage. Thirty minutes later apomorphine or vehicle was injected SC and the animal was again returned to its homecage. Fifteen minutes following the second injection each rat was placed in the activity drum and activity was measured for 20 min. This injection-test procedure was repeated daily for 7 days. Sensitization testing began 24 h after the last training day and was conducted for 4 days. On each of these days all rats were tested for locomotor activity 15 min after a challenge dose of 1.0 mg/kg apomorphine.

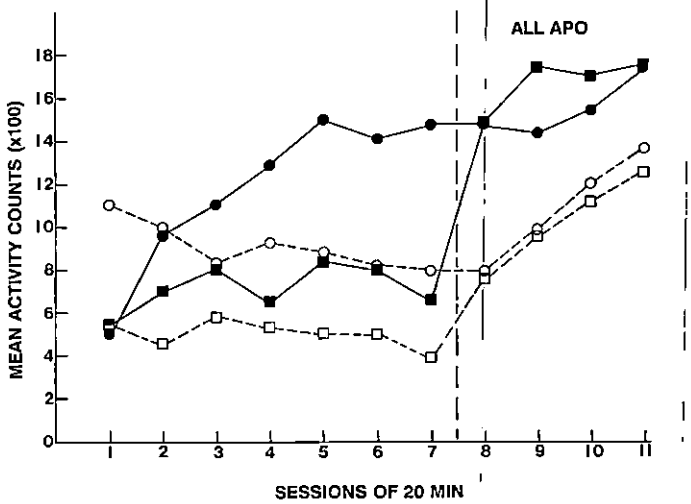

Fig. 2. Mean activity counts across the training (1–7) and sensitization test (8–11) sessions for rats pretreated with 100 mg/kg sulpiride (*100 SUL*) or vehicle (*VEH*) and 1.0 mg/kg apomorphine (*APO*) or VEH. All rats received a single injection of 1.0 mg/kg APO prior to each sensitization test session. For symbols see legend of Fig. 1

Results and discussion

Training sessions 1–7. The mean activity counts for the groups during the training phase (sessions 1–7) are shown in the left panel of Fig. 2. As shown in this figure, rats injected with apomorphine only (VEH-APO) displayed a progressively greater increase in locomotor activity across the 7 test days and were more active than control rats (VEH-VEH) across sessions 4–7. In contrast, rats treated with sulpiride only (100 SUL-VEH) were less active than control rats across the seven sessions. More important, as may seen in Fig. 2, sulpiride given concurrently with apomorphine completely blocked the activity increasing effect of repeated apomorphine treatments. Indeed, the activity of rats given sulpiride and apomorphine (100 SUL-APO) appeared comparable to that of the vehicle control rats (VEH-VEH) across sessions 3–7. As expected from inspection of Fig. 2, the mixed factor ANOVA performed on these data revealed significant main effects of agonist dose, antagonist dose, and a significant Agonist × Antagonist × Day interaction [$F(1, 31) = 9.89$, $P < 0.01$, $F(1, 31) = 36.22$, $P < 0.0001$, and $F(6, 186) = 4.72$, $P < 0.001$, respectively].

Sensitization test sessions 8–11. As may be seen in Fig. 2, rats pretreated with sulpiride for 7 days increased activity on session 8 when given only an apomorphine injection. This increase, however, was greater for sulpiride-treated rats which had also received apomorphine pretreatments. Indeed, the rats given concurrent sulpiride-apomorphine (100 SUL-APO) pretreatments were as active on session 8 as rats given only apomorphine (VEH-APO) during the training phase. In contrast, rats pretreated with only sulpiride (100 SUL-VEH) increased activity to a level comparable to that of the vehicle control rats (VEH-VEH) on session 8. The hyperactivity of the apomorphine pretreated rats was maintained across sessions

9–11 even though the rats pretreated with vehicle (100 SUL-VEH, VEH-VEH) progressively increased activity across these sessions as they too became more sensitive to apomorphine. These findings suggest that concurrent sulpiride treatments did not block the development of behavioral sensitization to apomorphine. Consistent with this interpretation, the mixed factor ANOVA performed on the activity counts across sessions 8–11 revealed only a significant main effect of agonist and a significant main effect of session [$F(1, 31) = 9.51$, $P < 0.01$, and $F(3, 93) = 14.71$, $P < 0.0001$, respectively]. Neither the main effect of antagonist nor any of the interactions containing antagonist as a factor were significant.

Experiment 3

It is evident from the results of experiment 2 that the D_2 antagonist sulpiride blocks the progressive increase in locomotor activity induced by repeated treatments with the mixed dopamine receptor agonist apomorphine. However, consistent with experiment 1, the results of experiment 2 clearly indicate that sulpiride does not prevent the development of behavioral sensitization to apomorphine. In contrast, concurrent treatment with the mixed D_1/D_2 dopamine antagonist haloperidol completely blocks the development of sensitization to apomorphine (Mattingly and Rowlett 1989). Taken together, these results suggest that the development of behavioral sensitization to apomorphine is either exclusively related to the stimulation of D_1 dopamine receptors, or that repeated stimulation of either D_1 or D_2 receptors might be sufficient to induce sensitization. The purpose of experiment 3, therefore, was to determine whether the development of behavioral sensitization to apomorphine could be prevented by a selective blockade of dopamine D_1 receptors. Consequently, four groups of rats were injected daily with apomorphine and/or the selective D_1 dopamine receptor antagonist SCH 23390 and tested for changes in locomotor activity. The design and procedure was the same as in experiment 2, except SCH 23390 rather than sulpiride was used.

Materials and methods

Subjects, design and procedure. Forty male Wistar albino rats (Harlan Industries, Indianapolis, IN) weighing between 250 and 350 g served as subjects. The rats were randomly assigned, in equal numbers, to one of four training groups comprising the 2 (antagonist dose: 0 or 0.5 mg/kg SCH 23390, Research Biochemicals) × 2 (agonist dose: 0 or 1.0 mg/kg apomorphine) factorial design. The apparatus and procedure was the same as in experiment 2. SCH 23390 was dissolved daily in distilled H_2O and injected IP in a volume of 1.0 ml/kg. Apomorphine was obtained, prepared, and administered as described previously.

Results and discussion

Training sessions 1–7. The mean activity counts of the four groups across the seven training sessions are presented in Fig. 3. As shown in this figure, repeated apomorphine treatments alone (VEH-APO group) resulted in a progressively greater increase in activity with each injection. This progressive increase in apomorphine-induced activity, however, was completely blocked by concurrent SCH 23390 treatments (see SCH-APO group). Indeed, SCH 23390 treatments greatly depressed activity compared to the vehicle control group (VEH-VEH), regardless of whether apomorphine was also given (see SCH-VEH, SCH-APO groups). As expected, the three-factor mixed ANOVA performed on these data revealed significant main effects of agonist dose, antagonist dose and a significant Agonist × Antagonist interaction [$F(1, 36) = 5.27$, $P < 0.05$, $F(1, 36) = 110.56$, $P < 0.0001$, and $F(1, 36) = 9.21$, $P < 0.01$, respectively]. The session effect was also significant [$F(6, 216) = 4.01$, $P < 0.001$], as was the Agonist × Session [$F(6, 216) = 19.58$, $P < 0.0001$], and the Agonist × Antagonist × Session interaction [$F(6, 216) = 9.06$, $P < 0.0001$. These significant interactions reflect the fact that only the VEH-APO groups displayed a significant increase in activity across the seven sessions, and the activity level of the VEH-APO group did not increase above that of the VEH-VEH control group until the third activity test session.

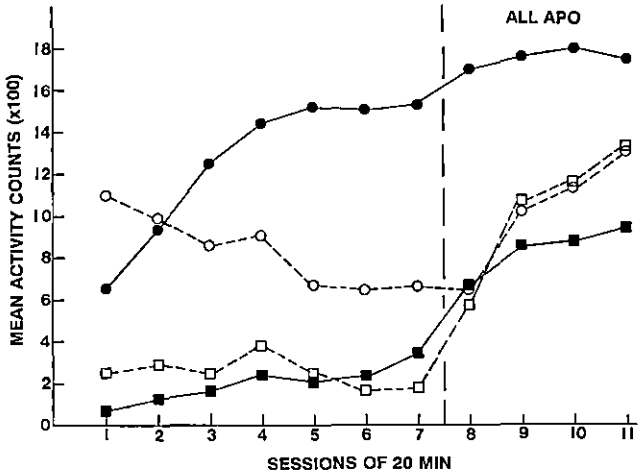

Fig. 3. Mean activity counts across the training (1–7) and sensitization test (8–11) sessions for rats pretreated with 0.5 mg/kg SCH 23390 (*SCH*) or vehicle (*VEH*) and 1.0 mg/kg apomorphine (*APO*) or VEH. All rats received a single injection of 1.0 mg/kg APO prior to each sensitization test session. ■–■ SCH-APO; □– –□ SCH-VEH; ●–● VEH-APO; ○– – –○ VEH-VEH

Sensitization test sessions 8–11. As may be seen in Fig. 3, the SCH 23390-pretreated rats (SCH-VEH, SCH-APO) increased activity on session 8, when given only an injection of apomorphine, to a level comparable to that of rats previously treated only with vehicle (VEH-VEH). Moreover, this increase was the same for both SCH 23390 pretreatment groups. That is, rats pretreated with apomorphine and SCH 23390 (SCH-APO) during the training phase were no more sensitive to apomorphine on session 8 than rats pretreated with only SCH 23390 (SCH-VEH). In other words, concurrent SCH 23390 treatments along with apomorphine completely blocked

the development of sensitization to apomorphine. The mixed factor ANOVA performed on the mean activity counts across these four sensitization test sessions indicated a significant main effect of antagonist [$F(1, 36) = 5.73$, $P < 0.05$] and a significant Antagonist × Agonist interaction [$F(1, 36) = 5.98$, $P < 0.05$]. This latter interaction was further analysed using a Newman-Keuls post hoc test to compare the mean activity counts of the four groups collapsed across the four sensitization test sessions. The results of this analysis indicated that overall, the rats pretreated with only apomorphine (VEH-APO) during the training phase were significantly more active than the other three pretreatment groups [$P < 0.05$ in each case]. In contrast, this analysis indicated that overall, the rats pretreated with SCH 23390 and apomorphine (SCH-APO) were significantly less active than the other three groups [$P < 0.05$ in each case]. The apomorphine-induced activity level of rats pretreated with only SCH 23390 (SCH-VEH) did not differ across these sessions from that of the rats pretreated with only vehicle (VEH-VEH).

The ANOVA performed on these data also revealed a significant session effect [$F(3, 108) = 19.23$, $P < 0.0001$], as overall, the rats tended to increase activity across sessions. This increase, however, was greater for rats receiving apomorphine for the first time than for rats which were pretreated with apomorphine [Agonist × Session interaction, $F(3, 108) = 8.58$, $P < 0.01$].

In summary, the results of experiment 3 indicate that SCH 23390 significantly depressed locomotor activity in control rats and acutely blocked the activity-increasing effect of repeated apomorphine treatments. More important, concurrent SCH 23390 treatments completely prevented the development of behavioral sensitization to apomorphine.

Discussion

It is evident from the present results that repeated treatment of rats with the direct dopamine receptor agonist, apomorphine, results in the development of behavioral sensitization. This finding is consistent with previous work (e.g., Castro et al. 1985; Mattingly et. al 1988b). Further, the results of the present experiments clearly indicate that the activating effects of apomorphine on locomotor activity may be blocked by concurrent treatment with either a selective D_1 or D_2 antagonist. In experiment 2, the D_2 antagonist, sulpiride, blocked the effects of apomorphine on locomotor activity, and in experiment 3, the activity-increasing effects of apomorphine were prevented by the selective D_1 antagonist SCH 23390. Taken together, these findings suggest that the concurrent stimulation of both D_1 and D_2 dopamine receptors is neccessary for the expression of apomorphine-induced locomotor activity. Consistent with these results, recent evidence suggests that the expression of other dopamine agonist-induced behavioral and electrophysiological effects also requires the concomitant stimulation of both D_1 and D_2 dopamine receptors (Amalric et al. 1986; Carlson et al. 1987; Plaznik et al. 1989; Ross et al. 1989; Bergman et al. 1990; Moore and Axton 1990).

In contrast to the involvement of both D_1 and D_2 receptors in the expression of apomorphine-induced locomotor activity, the development of behavioral sensitization to apomorphine appears to require the repeated stimulation of only the D_1 receptor. Indeed, in both experiments 1 and 2, the concurrent administration of the selective D_2 antagonist, sulpiride, in doses which blocked apomorphine-induced increases in activity, did not prevent the development of behavioral sensitization. That is, rats pretreated daily with sulpiride and apomorphine combined displayed an enhanced locomotor activity response to a challenge dose of apomorphine comparable to that observed in rats pretreated with only apomorphine. However, in experiment 3, the concurrent administration of the D_1 selective dopamine receptor antagonist, SCH 23390, blocked both the expression and the development of behavioral sensitization to apomorphine. Rats given both SCH 23390 and apomorphine in this experiment responded to a subsequent challenge dose of apomorphine in a manner similar to rats pretreated with only vehicle. These data suggest, of course, that the development of behavioral sensitization to apomorphine is mediated by dopamine D_1 receptor stimulation.

In agreement with these findings, Vezina and Stewart (1989; Stewart and Vezina 1989) have recently reported that although both D_1 and D_2 selective antagonists blocked the acute locomotor activating effects of amphetamine, only the D_1 receptor antagonist SCH 23390 blocked the development of behavioral sensitization to amphetamine. Thus, like apomorphine, the development of behavioral sensitization to amphetamine appears to be related to D_1 receptor stimulation. Based upon both behavioral and electrophysiological data, other researchers have also concluded that repeated dopamine D_1 receptor stimulation may be the crucial factor neccessary for the induction of agonist-induced behavioral sensitization (e.g., Braun and Chase 1988; Criswell et al. 1989; Henry and White 1989). But, as noted previously, not all investigators share this view (e.g., Levy et al. 1988). Peris and Zahniser (1989), for example, found that the augmentation in amphetamine-induced ^{3}H-dopamine release from striatal slices observed after a single pretreatment with cocaine could be blocked by either the D_1 selective antagonist, SCH 23390, or the D_2 selective antagonist, sulpiride. Similarly, Ujike et al. (1989) reported that the augmentation of locomotor activity and stereotypic behavior observed in rats following repeated methamphetamine administration was blocked by concurrent treatments with either SCH 23390 or the D_2 antagonist, YM 09151–2. These results, of course, implicate both D_1 and D_2 receptors in the development of behavioral sensitization. At present, the basis for these discrepancies is unknown. Since apomorphine, amphetamine, and cocaine enhance dopaminergic activity through different mechanisms, it is possible that the neurochemical mechanisms underlying the development of behavioral sensitization to each of these drugs may differ (cf Rowlett et al. 1991). However, it is not clear why different mech-

anisms would mediate the development of behavioral sensitization to amphetamine and methamphetamine (cf Stewart and Vezina 1989; Ujike et al. 1989), since these drugs have similar mechanisms of action. This latter discrepancy is probably related to the differences in behavioral measures and/or the use of different selective antagonists among the studies.

Several hypotheses have been proposed to account for the development of behavioral sensitization to dopamine agonists, including conditioning, autoreceptor tolerance, and an augmented agonist-induced release of dopamine (see Robinson and Becker 1986). The present results are inconsistent with each of these views. A conditioning explanation of behavioral sensitization, for example, suggests that the progressive increase in apomorphine-induced locomotor activity is related to the development of a conditioned locomotor activity response to the environmental stimuli associated with drug exposure. But in the present study, behavioral sensitization still developed to apomorphine in experiment 2 even though the activating effects of repeated apomorphine treatments were completely blocked by sulpiride during the training phase. Although this finding alone does not completely rule out the involvement of conditioning mechanisms, it is consistent with other work which suggests that behavioral sensitization to apomorphine develops through both associative and nonassociative processes (cf Gold et al. 1988; Mattingly et al. 1988; Mattingly and Gotsick 1989).

The autoreceptor tolerance explanation of behavioral sensitization suggests that dopamine autoreceptors which appear to be inhibitory with respect to locomotor activity, become subsensitive with repeated exposure to non-selective agonists such as apomorphine and amphetamine. Thus, with repeated agonist treatments the inhibitory effects of autoreceptor stimulation on dopamine synthesis, release, and firing rate progressively decrease and consequently, agonist-induced locomotor activity increases (see Robinson and Becker 1986, for review). In the present study, doses of the D_2 antagonist sulpiride large enough to block both pre- and postsynaptic D_2 receptors (cf Vezina and Stewart 1989), did not prevent the development of behavioral sensitization. Moreover, the dose of the D_1 receptor antagonist SCH 23390 which prevented the development of behavioral sensitization in experiment 3, does not interact with dopamine autoreceptor function (Lappalainen et al. 1990). Thus, although a significant amount of evidence suggests that autoreceptors do become less sensitive with repeated agonist treatments, autoreceptor tolerance alone cannot account for the development of behavioral sensitization (see Ackerman and White 1989; Braun and Chase 1988; Mattingly et al. 1988; Vezina and Stewart 1989).

Finally, much recent evidence suggests that repeated amphetamine treatments result in an augmented amphetamine-induced release of dopamine (Kuzcenski and Segal 1988, 1989; Robinson et al. 1988) which coincides with the development of behavioral sensitization to amphetamine. Similarly, the development of behavioral sensitization to cocaine appears to be related to an increase in extracellular dopamine levels in the nucleus accumbens (Kalivas and Duffy 1990). But while these presynaptic effects may account for the development of behavioral sensitization to indirect dopamine agonists such as amphetamine and cocaine, which exert their effects primarily by inducing the release and/or blocking the re-uptake of dopamine, these presynaptic effects cannot explain the development of sensitization to direct dopamine receptor agonists such as apomorphine (cf Braun and Chase 1988; Zahniser et al. 1988; Vaughn et al. 1990). At present, the only presynaptic effect reported which may be related to the development of sensitization to apomorphine is an increase in steady-state dopamine synthesis (Vaughn et al. 1990; Rowlett et al. 1991).

In conclusion, the present results clearly indicate that the expression of dopamine agonist-induced behavioral effects requires some minimal level of stimulation of both D_1 and D_2 dopamine receptors. In contrast, the development of behavioral sensitization to apomorphine appears to require the repeated stimulation of only the D_1 receptor. This finding with apomorphine is consistent with recent work with amphetamine. Finally, the results of the present experiments are inconsistent with conditioning and autoreceptor tolerance explanations of apomorphine-induced behavioral sensitization.

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Excitatory amino acids and morphine withdrawal: differential effects of central and peripheral kynurenic acid administration

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Abstract. The non-selective excitatory amino acid antagonist kynurenic acid, which does not readily cross the blood-brain barrier, dose-dependently attenuated the behavioral signs of naltrexone-precipitated withdrawal in morphine-dependent rats following both intraventricular and subcutaneous administration. However, intraventricular and subcutaneous administration of kynurenic acid had different effects on individual withdrawal behaviors. Moreover, single unit recordings in anesthetized animals showed that intraventricular, but not subcutaneous, kynurenic acid administration attenuated the withdrawal-induced increased firing of locus coeruleus neurons. These studies indicate that: (1) both central and peripheral excitatory amino acid receptors may play an important role in opiate withdrawal; and (2) excitatory amino acid antagonist treatments might be developed to reduce opiate abstinence symptoms in man.

Key words: Kynurenic acid – Locus coeruleus – Excitatory amino acid – Morphine – Opiate withdrawal

The mechanism of opiate withdrawal is not completely understood. In man, opiate withdrawal is characterized by nausea, anxiety, insomnia, hot and cold flashes, muscle aches, perspiration, diarrhea, and craving for the drug (Kolb and Himmelsbach 1938). A variety of neurotransmitter systems have been hypothesized to play a role in opiate withdrawal including the brain noradrenergic system (see Redmond and Krystal 1984). The role of the brain noradrenergic system in opiate withdrawal is supported in part by the finding that the noradrenergic cells of the locus coeruleus (LC) greatly increase their activity during antagonist-precipitated withdrawal (Aghajanian 1978; Valentino and Wehby 1989) and that this increased activity correlates temporally with withdrawal behavior (Rasmussen et al. 1990). In addition, clonidine, an alpha-2 agonist, decreases the activity of LC neurons during morphine withdrawal (Aghajanian 1978), suppresses withdrawal behaviors after direct infusion into the LC (Taylor et al. 1988), and is used to minimize opiate-withdrawal symptoms in man (Gold et al. 1978). However, ST–91, an alpha-2 agonist that does not readily cross the blood-brain barrier, can also suppress morphine-withdrawal behaviors after either peripheral or central administration, indicating that both central and peripheral noradrenergic systems play a role in the expression of many opiate-withdrawal behaviors (Taylor et al. 1988).

Kynurenic acid, a naturally occurring metabolite of tryptophan that is found in brain tissue (Stone et al. 1987), is a non-selective excitatory amino acid antagonist (Perkins and Stone 1982) that does not readily cross the blood-brain barrier (Swartz et al. 1990). Recent studies have shown that intraventricular administration of kynurenic acid greatly attenuates the withdrawal-induced activation of LC neurons (Rasmussen and Aghajanian 1989; Tung et al. 1990). Therefore, the present study was conducted to evaluate the effects of intraventricular administration of kynurenic acid on morphine-withdrawal behaviors, and further, to evaluate the effects of systemic administration of kynurenic acid on both the behavioral signs of morphine withdrawal and the withdrawal-induced increase in firing of LC neurons.

Materials and methods

Opiate dependence was induced in male Sprague-Dawley rats (Charles River, 250–350 g) by the subcutaneous pellet implantation method (Way et al. 1969; Blassig et al. 1973). Under halothane anesthesia, animals were implanted with either morphine pellets (NIDA: 75 mg morphine base) or sham pellets. One pellet was implanted daily for 2 days. Withdrawal was induced 48 h after the last pellet implantation; the pellets were removed under halothane anesthesia 2–3 h before precipitating withdrawal. Withdrawal was induced by administering the opiate antagonist naltrexone HCl (10 mg/kg; Sigma) subcutaneously (SC).

The severity and time course of opiate withdrawal was assessed as described previously (Rasmussen et al. 1990). Briefly, animals were studied in pairs in clear plexiglass cages (11 in × 7 in × 5 in) and

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