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Dopamine receptor antagonists prevent expression, but not development, of morphine sensitization

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Abstract

The present experiments determined the effects of selective dopamine receptor antagonists on the initiation and expression of sensitization to the locomotor-stimulating effects of morphine in rats. Although both the dopamine D_1 receptor antagonist *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390, 0.25 mg/kg) and the dopamine D_2 receptor antagonist eticlopride (0.1 mg/kg) suppressed the ability of morphine (10 mg/kg) to elicit sensitized locomotor activity during the course of a 12 day treatment schedule, subsequent tests with morphine alone revealed significant sensitization. Sensitization in the SCH 23390 + morphine group could not be attributed to dopamine D_1 receptor supersensitivity caused by repeated SCH 23390 administration because electrophysiological recordings indicated that nucleus accumbens neurons in SCH 23390-treated rats were not more sensitive to the inhibitory effects of either dopamine or a dopamine D_1 receptor-selective agonist. Thus, dopamine receptor stimulation may be involved in expression, but not development, of morphine sensitization.

Keywords: Behavioral sensitization; Dopamine D_1 receptor; Dopamine D_2 receptor; Nucleus accumbens; Ventral tegmental area; Drug addiction

1. Introduction

Acute administration of a moderate dose of morphine ($\leq$ 10.0 mg/kg) elicits increased locomotor activity in rats. Although there has been considerable agreement that this response involves actions within the ventral striatal region known as the nucleus accumbens, the extent to which the response is dependent upon innervation of this structure by A10 dopamine neurons of the ventral tegmental area has been debated (see Kalivas and Stewart, 1991 for review). Morphine is known to activate ventral tegmental area dopamine neurons indirectly (disinhibition) as a consequence of inhibiting non-dopamine, presumably γ-aminobutyric acid (GABA), neurons of the ventral tegmental area (Gysling and Wang, 1983; Matthews and German, 1984; Lacey et al., 1989), leading to increased dopamine release in the nucleus accumbens (Di Chiara and Imperato, 1988). Direct infusions of morphine or μ-opioid receptor-selective peptides into the ventral tegmental area elicit locomotion which can be blocked by dopamine receptor antagonist administration into the nucleus accumbens (Broekkamp et al., 1979; Joyce and Iversen, 1979; Kelley et al., 1980; Kalivas et al., 1983), and which is also accompanied by increased dopamine release in the nucleus accumbens (Kalivas et al., 1983; Kalivas and Duffy, 1990). Similar infusions into the nucleus accumbens also result in locomotion, but this effect appears to be dependent upon μ-opioid, rather than dopamine, receptor stimulation since it is blocked by opioid, but not dopamine, receptor antagonists (Pert and Sivit, 1977; Kalivas et al., 1983). Moreover, whereas either systemic or intra-nucleus accumbens infusion of μ-opioid receptor antagonists completely prevent opioid-induced locomotion (Amalric and Koob, 1985; Kalivas and Duffy, 1987), similar experiments with dopamine receptor antagonists have yielded inconsistent results (Eidelberg

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and Erspamer, 1975; Iwamoto, 1981; Longoni et al., 1987; Vaccarino et al., 1986).

Following repeated administration of opioids, there is a marked increase in the induction of locomotor activity, i.e. behavioral sensitization develops (Kalivas and Stewart, 1991). As with the acute locomotor-stimulating effects of morphine, there is general agreement that the mesoaccumbens dopamine system is the anatomical locus for sensitized locomotion, although the precise mechanisms involved have not been resolved. Sensitized locomotion produced by repeated morphine administration can be reproduced by repeated infusions of the opioid directly into the ventral tegmental area (Joyce and Iversen, 1979; Vezina and Stewart, 1984; Vezina and Stewart, 1989), but not the nucleus accumbens (Vezina et al., 1987), suggesting that the underlying processes responsible for the *development* of sensitization to opioids occur within the ventral tegmental area. However, the dopamine innervation of nucleus accumbens appears to be involved in the *expression* of sensitization since morphine-induced dopamine release is augmented in opioid-sensitized rats (Kalivas and Stewart, 1991; Acquas and Di Chiara, 1992; Spanagel et al., 1993). Although antagonism experiments have clearly shown that μ-opioid receptor stimulation is necessary for the development of sensitization (Kalivas and Duffy, 1987), similar studies investigating the importance of dopamine receptors in opioid-induced behavioral sensitization remain inconclusive (Kalivas et al., 1985; Vezina and Stewart, 1984; Vezina and Stewart, 1989; Kalivas and Stewart, 1991).

The present experiments have re-addressed the role of dopamine receptor subtypes in morphine sensitization. Specifically, we have tested the ability of *R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH 23390), an antagonist with selectivity for the dopamine D_1 receptor subfamily (D_1, D_5), and eticlopride, an antagonist with selectivity for the dopamine D_2 receptor subfamily (D_2, D_3, D_4), on both the development and expression of sensitization to repeated systemic administration of morphine.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlen, Indianapolis, IN), weighing 200–240 g at the beginning of treatment, were used for all experiments. The rats were housed two/cage in a vivarium maintained at a constant temperature (21–23°C) and humidity (40–50%) and kept on a 12:12 h light/dark cycle (07:00–19:00). These cages served as home cages, in which the animals remained except during behavioral testing. All animal procedures were conducted in strict accordance with the *Principles of Laboratory Animal Care* as endorsed by the National Institutes of Health (Publication No. 85-23).

2.2. Motor activity measurements

Experiments were conducted in a separate room within the vivarium. Standard polypropylene rat cages (30×50 cm) were placed inside adjustable frames equipped with three infrared photobeams (San Diego Instruments, San Diego, CA). The photoelectric beams were placed at a height of 2.5 cm at equal distances along the length of the cage. For the present experiments, interruptions of two adjacent beams in succession were quantified as ambulation counts by the programmed software. Ambulation counts reflect horizontal locomotor activity more accurately than the sum total of all beam interruptions. On test days, rats were administered pretreatment injections of an antagonist or drug vehicle, if necessary, and placed in the test cages for 30 min. Rats were then challenged with morphine or drug vehicle and the test was begun. Activity was measured continuously for a 2 h period between 13:00–17:00 during the light phase of the daily cycle.

2.3. Drug treatments

The drugs used for systemic injections in these experiments included morphine sulfate (Sigma Chemical Co., St. Louis, MO), SCH 23390 hydrochloride (Research Biochemicals, RBI, Natick, MA) and eticlopride hydrochloride (RBI). All drugs were dissolved in deionized water and injected i.p. in a volume of 1.0 ml/kg. The morphine dose was chosen on the basis of its marked locomotor-activating properties. The doses of antagonists were chosen based on pilot data, using identical test conditions, which demonstrated that they were at least 2-fold higher than those effective at preventing hyperactivity produced by cocaine (10 mg/kg, i.p.) and *d*-amphetamine (2.5 mg/kg, s.c.; unpublished observations – see Discussion for additional references).

For each of the behavioral experiments investigating the effects of the dopamine D_1 receptor antagonist SCH 23390 (0.25 mg/kg, i.p.) or the dopamine D_2 receptor antagonist eticlopride (0.1 mg/kg, i.p.), rats were divided into four treatment groups (n = 12/group): vehicle/vehicle, vehicle/morphine (10.0 mg/kg, i.p.), antagonist/vehicle, and antagonist/morphine. On the day before treatment began, all rats were injected with drug vehicle (deionized water; 1.0 ml/kg, i.p.) and tested to determine basal activity. The following day, all rats were tested with morphine (10.0 mg/kg, i.p.), which served as the pretest as well as the

first day of morphine treatment. On each of the following 12 days, rats were administered paired injections according to their assigned treatment group, with antagonist or vehicle injection preceding morphine or vehicle injection by 30 min. Rats were tested on days 2, 7 and 13 of the schedule to monitor progressive changes in the behavioral response. On the final day of treatment (day 14), all rats were again challenged with morphine (10.0 mg/kg) and tested for the presence of sensitized locomotion. The following day, all rats were challenged with vehicle (1.0 ml/kg) to test for possible conditioned increases in activity.

2.4. Electrophysiological recording

Rats used in electrophysiological experiments were pretreated with SCH 23390 (0.25 mg/kg per day) or vehicle for 12 days, then prepared for recording 24 h following the final treatment injection. Detailed procedures for in vivo extracellular single cell recordings of rat nucleus accumbens neurons have been published previously (Henry and White, 1991). Briefly, rats were anesthetized with chloral hydrate (400.0 mg/kg i.p.) and mounted in a standard stereotaxic apparatus. The scalp was incised and retracted and a burr hole was drilled in the skull overlying the nucleus accumbens. Five barrel micropipettes were pulled and broken back to a tip diameter of 3–8 μm. The center recording barrel was filled with 1% Fast Green dye in a 2 M saline solution. Three side barrels were filled with dopamine hydrochloride (Calbiochem; 0.1 M, pH 4.0), *R*(+)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride (SKF-38393, RBI; 0.01 M, pH 4.0, dissolved in 0.01 M saline), and monosodium *l*-glutamate (Sigma; 0.05 M, pH 8.0). The last side barrel was filled with 2 M saline for automatic current balancing. The in vitro impedance was measured and verified to fall between 1–6 MΩ for the center barrel and 20–100 MΩ for the side barrels. The electrode was stereotaxically placed above the nucleus accumbens (10.3–10.7 mm A, 1.0–1.4 mm L from lambda and 6.2–7.0 mm below the surface of the brain) according to published coordinates (Paxinos and Watson, 1986)

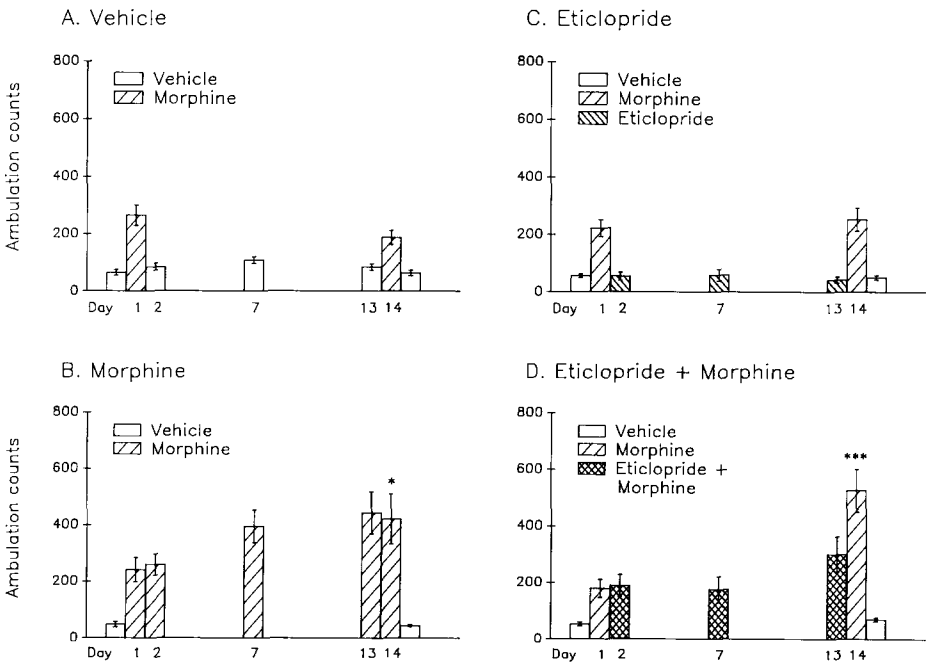

Fig. 1. Effects of eticlopride on morphine-induced ambulation and sensitization of that effect during repeated administration. All data are from 2 h tests and represent the means ± S.E.M. In each treatment group ($n = 12$/group, except for panel C where $n = 10$), the first bar represents locomotor activity produced by vehicle on the day prior to initiating drug treatments. The day 1 bar represents a morphine pre-test which allowed within-group comparisons following repeated drug treatments. Days 2, 7 and 13 represent tests conducted with paired injections on those days of the repeated treatment protocol (see legends within each panel) for each group. Day 14 represents the morphine challenge test when all groups received vehicle plus 10.0 mg/kg of morphine. The last bar represents a test for conditioned locomotion during which all groups received vehicle injections. Note that there was no evidence for conditioned locomotion in any of the four treatment groups. As shown in panel A, repeated vehicle administration failed to produce sensitization to morphine. In contrast, repeated morphine injections (panel B) caused a gradual enhancement of ambulation, resulting in a 170% increase on day 14 as compared to day 1. Panel C shows that eticlopride failed to alter normal ambulations or the response to morphine on day 14. Panel D shows that eticlopride completely prevented the expression of sensitized locomotion (day 2, 7 and 13) but failed to prevent the development of sensitization upon challenge with morphine alone (day 14). $^{*}P < 0.05$, $^{***}P < 0.001$ with respect to day 1 morphine values (within-group ANOVA).

and then lowered in approximate 4 μm increments with the use of a hydraulic microdrive. The analog signal from the electrode was amplified and filtered and was monitored on an oscilloscope and an audio amplifier. Individual action potentials ($\geq 3:1$ signal:noise ratio) were detected with a window discriminator. Rates of discharge were monitored on a polygraph recorder. Digital counts were also obtained for off-line analysis.

Because most nucleus accumbens neurons are quiescent in chloral hydrate-anesthetized rats, glutamate was iontophoretically pulsed in 30:30 s on:off cycles to stimulate firing. After a stable response to glutamate was established over a 7–10 min period, either dopamine or the dopamine D_1 receptor-selective agonist SKF 38393 was continually administered for a 10–12 min period, during which the iontophoretic current was doubled every 2 min. As a result, the response of the neuron to a given current of drug was determined over two 30 s epochs of glutamate stimulation. Following iontophoretic application of the drug, the neuron was allowed to return to a stable response to glutamate stimulation, and if the firing response was sufficiently stable, the response to a second drug was determined. The order of drug application was alternated from cell to cell. All nucleus accumbens neurons recorded in these experiments exhibited characteristics of type I (∓) nucleus accumbens neurons (White et al., 1987; White et al., 1993).

2.5. Statistical procedures

Statistical comparisons were made with analysis of variance (ANOVA) with repeated measures. Post-hoc comparisons were made with Newman-Keuls tests.

3. Results

3.1. Locomotor activity

3.1.1. Morphine sensitization

In all behavioral groups tested, the initial challenge with 10.0 mg/kg morphine elicited a significant increase in locomotor activity relative to control (Figs. 1 and 2). The effectiveness of 14 day treatment with morphine (10.0 mg/kg per day) in eliciting sensitization of its locomotor-stimulating effect is illustrated in Figs. 1B and 2B. Each morphine injection led to a progressive enhancement in activity, resulting in significantly elevated locomotion on day 14 compared to the first test day. In contrast, no enhancement was evident in rats administered vehicle injections during the course of treatment (Figs. 1A and 2A). Behavioral sensitiza-

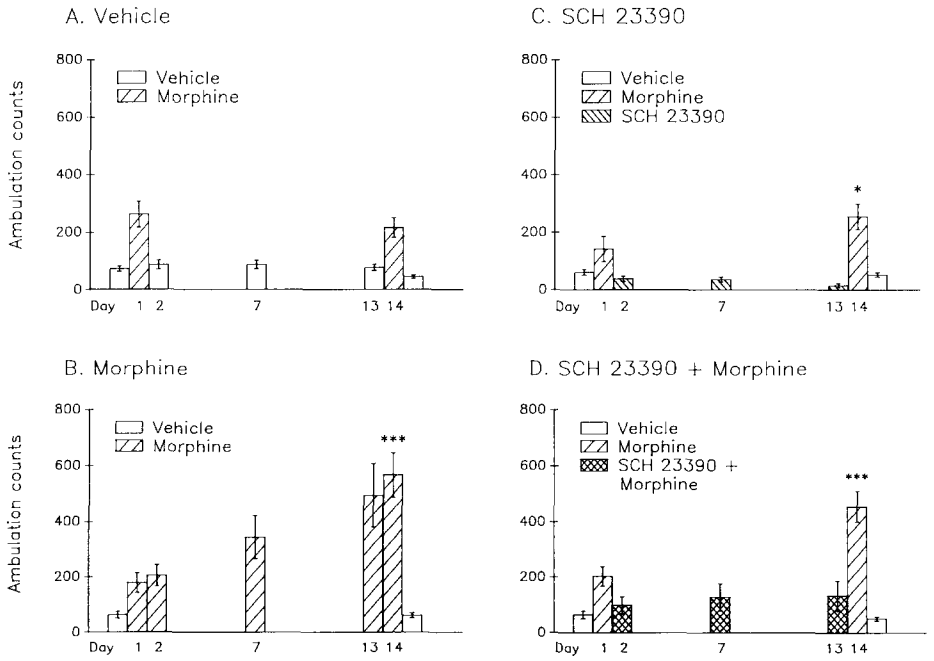

Fig. 2. Effects of SCH 23390 on morphine-induced ambulation and sensitization of that effect during repeated administration. Rats in each group ($n = 12$/group), were treated and tested in the manner described in Fig. 1, except that SCH 23390 injections (0.25 mg/kg) replaced eticlopride. Note that SCH 23390 prevented the expression of morphine sensitization (panel D, days 2, 7 and 13) as well as the acute locomotor-activating effects of morphine (panel D, compare day 1 to days 2, 7 and 13). $^{*}P < 0.05$, $^{***}P < 0.001$ with respect to day 1 morphine values (within-group ANOVA).

tion did not appear to result from conditioned pairings of morphine with the test environment, as vehicle challenge after the treatment period revealed no significant acquisition of environment-specific conditioned locomotion.

3.1.2. Eticlopride pretreatment

The effects of the dopamine D_2 receptor antagonist eticlopride on morphine-induced sensitization are depicted in Fig. 1D. When rats undergoing eticlopride + morphine treatment were monitored for locomotion on days 2, 7 and 13, activity levels were comparable to those produced by the initial morphine challenge, i.e. there were no significant differences between the ambulation counts during these three sessions. Nevertheless, this group exhibited significantly sensitized locomotion when challenged with morphine alone (day 14). In fact, the degree of enhancement was not significantly different from that seen in the morphine group (compare Figs. 1B and 1D). The effect of eticlopride itself upon locomotion was similar to that of vehicle (Figs. 1A and 1C), indicating that this dose was not behaviorally disruptive. Taken together, these results suggest that: (1) eticlopride did not prevent the acute locomotor-stimulating effects of morphine; (2) eticlopride did not prevent the *development* of morphine sensitization as evidenced by the sensitized response to morphine on day 14; and (3) eticlopride prevented the *expression* of morphine sensitization, which would normally be evident as a progressive increase in locomotion on days 2, 7 and 13 of treatment.

3.1.3. SCH 23390 pretreatment

In general, the dopamine D_1 receptor antagonist SCH 23390 produced a pattern of results similar to those obtained with the dopamine D_2 receptor antagonist eticlopride. Like eticlopride, SCH 23390 failed to prevent the *development* of sensitization as evidenced by the morphine challenge on day 14 (compare Figs. 2B and 2D). In addition, SCH 23390 prevented the progressive *expression* of increased locomotion on treatment days 2, 7 and 13 (compare Figs. 2B and 2D). One notable difference between eticlopride and SCH 23390 is that the D_1 antagonist not only prevented the sensitized component of morphine-induced locomotion on days 2, 7 and 13, but also reduced the acute effects of morphine on locomotor activity (compare day 1 and days 2, 7 and 13 in Fig. 2D). SCH 23390 also appeared to decrease ambulation when administered on its own (Fig. 2C, days 2, 7 and 13). However, this effect did not reach statistical significance (Fig. 2C).

3.2. Electrophysiological results

A potential interpretational problem with the SCH 23390 results is that repeated administration of SCH

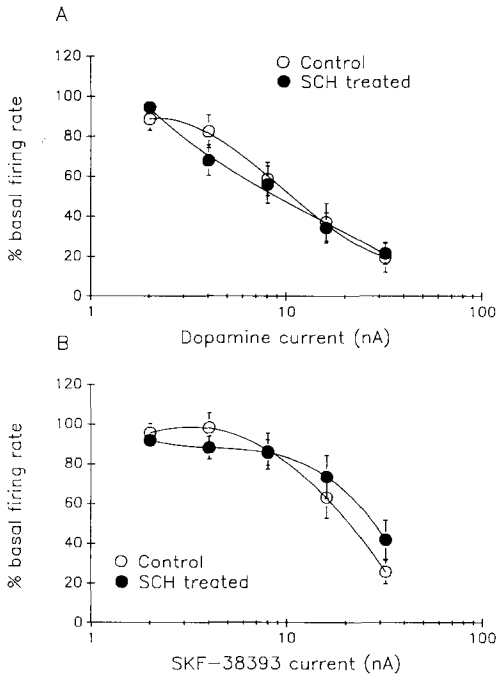

Fig. 3. Effects of chronic treatment with SCH 23390 on the sensitivity of nucleus accumbens neurons to the rate-suppressant effect caused by iontophoretic application of (A) dopamine (0.1 M, pH 4.0), or (B) SKF 38393 (0.01 M, pH 4.0). Rats were injected for 12 days with SCH 23390 (0.25 mg/kg per day) or drug vehicle, then tested on the day following the last injection. Points represent the means ± S.E.M. for 11–12 cells.

23390 can increase dopamine D_1 receptor density (Creese and Chen, 1985; Porceddu et al., 1985; McGonigle et al., 1989), as well as dopamine D_1 receptor-mediated neuronal responses within the nucleus accumbens and dorsal striatum (Hu and White, 1994). Since previous studies have demonstrated a close relationship between dopamine D_1 receptor sensitivity within the nucleus accumbens and the expression of sensitized locomotor activity induced by cocaine (Henry and White, 1991; White et al., 1995) and *d*-amphetamine (Wolf et al., 1994), it is possible that the sensitization observed in the SCH 23390 + morphine group on day 14 may have resulted from enhanced dopamine D_1 receptor sensitivity produced by repeated administration of SCH 23390. Indeed, it is noteworthy that the group treated with SCH 23390 alone exhibited a slight, but statistically significant, increase in morphine-induced activity on day 14 as compared to day 1 (Fig. 2C).

To test this possibility, an additional set of experiments investigated the electrophysiological response of neurons within the nucleus accumbens following repeated SCH 23390 administration. Thus, rats were prepared for single cell recording 24 h after the last of 12 SCH 23390 (0.25 mg/kg) injections. Fig. 3 depicts the response of nucleus accumbens cells in rats treated with SCH 23390 or vehicle to iontophoretic application

of either dopamine or the dopamine D_1 receptor-selective agonist SKF 38393. Cells recorded from the control group exhibited the expected pattern of current-dependent inhibition in response to dopamine, a pattern that was mimicked by iontophoretic application of SKF 38393. When tested 24–30 h after the last SCH 23390 injection of the treatment regimen, the response of nucleus accumbens neurons to either agonist was comparable to that in control rats, indicating that no alteration in nucleus accumbens dopamine D_1 receptor sensitivity had occurred at this time point after repeated administration of this dose of SCH 23390 (0.25 mg/kg).

4. Discussion

The present study has confirmed many previous reports regarding the ability of repeated systemic morphine administration to produce behavioral sensitization (see Kalivas and Stewart, 1991 for review). Additionally, we have demonstrated that selective antagonism of either the dopamine D_1 or D_2 receptor subfamilies prevented the *expression* of morphine-induced sensitization, but that neither treatment prevented the *development* of behavioral sensitization.

4.1. Dopamine antagonists and acute morphine responses

Although it was not the purpose of the present study to assess the abilities of dopamine receptor antagonists to prevent locomotor activation produced by *acute* morphine administration, it is worth noting that, at the doses tested, the dopamine D_1 receptor antagonist SCH 23390, but not the dopamine D_2 receptor antagonist eticlopride, attenuated this effect. The positive result with SCH 23390 is consistent with previous reports which used considerably lower doses of SCH 23390 (0.006–0.025 mg/kg, s.c.), and supports the conclusion that dopamine D_1 receptors play an essential role in morphine-induced hypermotility (Longoni et al., 1987). However, previous studies have provided disparate results with respect to the abilities of dopamine D_2 receptor antagonists to prevent morphine-induced locomotor activity. Some investigators have reported that classical dopamine receptor antagonists such as haloperidol (Eidelberg and Erspamer, 1975) and spiroperidol (Iwamoto, 1981), which are primarily dopamine D_2 receptor antagonists, can prevent morphine-induced hypermotility. However, other investigators failed to prevent locomotion produced by systemic morphine (or heroin) with either intra-nucleus accumbens injections of haloperidol (Teitelbaum et al., 1979), or systemic administration of the relatively non-selective dopamine D_1/D_2 receptor antagonist α-flupenthixol (Vaccarino et al., 1986).

In the present study, the dopamine D_2 receptor antagonist eticlopride failed to block morphine-induced ambulation, despite preventing the expression of enhanced locomotion induced by the repeated morphine treatment. It is possible that discrepancies in the literature regarding dopamine D_2 receptor antagonists are related to the combination of doses of the antagonists and morphine. Recent behavioral and electrophysiological studies have indicated that combinations of the dopamine D_2 receptor antagonist pimozide with certain doses of morphine resulted in the induction of apparent depolarization block of dopamine neurons (Rompré and Wise, 1989; Henry et al., 1992). If such an effect were to occur during behavioral tests with dopamine D_2 receptor antagonists and morphine, a reduction in morphine-induced locomotor activity would be likely due to the loss of impulse-dependent dopamine release.

4.2. Dopamine D_2 receptor antagonists and morphine sensitization

Previous studies regarding the effects of dopamine D_2 receptor antagonists on the development of opioid sensitization have provided inconsistent results. Pimozide, but not haloperidol, sulpiride or Ro 22-2586, prevented sensitization produced by repeated opioid microinjections into the ventral tegmental area (Kalivas, 1985; Vezina and Stewart, 1989). The present results with eticlopride add to the list of ineffective dopamine D_2 receptor antagonists and extend the findings to include sensitization produced by *systemic* morphine. Taken together, these results indicate that pimozide is peculiar among dopamine D_2 receptor antagonists, and suggest the likelihood that its ability to prevent sensitization might be due to its particularly long duration of action (Baldessarini, 1990) or to additional non-dopamine D_2 receptor actions. An obvious candidate is the high affinity of pimozide for voltage-sensitive Ca^{2+} channels (Snyder and Reynolds, 1985), particularly since Ca^{2+} channel antagonists have been demonstrated to prevent amphetamine-induced sensitization (Karler et al., 1991). However, given that similar treatment with pimozide failed to prevent *d*-amphetamine-induced sensitization (Vezina and Stewart, 1989), it seems more likely that the positive result with pimozide and intra-ventral tegmental area morphine resulted from a particular interaction between these two drugs. It was previously suggested (Vezina and Stewart, 1989) that such an interaction could involve the propensity of this combination to cause depolarization block of ventral tegmental area dopamine neurons (Rompré and Wise, 1989; Henry et al., 1992). However, acceptance of this conclusion would imply that the other dopamine D_2 receptor antagonists, when combined with intra-ventral tegmental area morphine,

would fail to produce depolarization block. Unfortunately, such evidence is not presently available. Whatever the cause of pimozide's peculiar effect, it would appear to be an inappropriate antagonist for studies of dopamine D_2 receptor involvement in sensitization.

4.3. Dopamine D_1 receptor antagonists and morphine sensitization

Studies regarding the effects of dopamine D_1 receptor antagonists on development of morphine sensitization, like those with dopamine D_2 receptor antagonists, have provided inconsistent results. Vezina and Stewart (1989) reported that SCH 23390 (0.04 and 0.2 mg/kg) failed to prevent sensitization induced by intra-ventral tegmental area morphine administrations. However, in their recent comprehensive review regarding mechanisms of behavioral sensitization, Kalivas and Stewart (1991) presented preliminary results showing that either systemic (1.0 mg/kg) or intra-ventral tegmental area administration of SCH 23390 (1.0 μg/side) prevented sensitization to systemic morphine (10.0 mg/kg), when the combinations were given every other day for 8 days. Their result with systemic SCH 23390 contrasts to the present finding that the dopamine D_1 receptor antagonist failed to prevent morphine sensitization. Although direct comparisons between the two experiments are made difficult by the lack of experimental detail provided in the earlier preliminary report, the different results may have resulted from different parameters used in the two studies, including the doses of SCH 23390 (1.0 mg/kg vs. the present 0.25 mg/kg), morphine treatment regimens (four injections, given every other day, vs. 12 daily injections), and the challenge doses of morphine (5.0 vs. 10.0 mg/kg).

With respect to doses of SCH 23390, it is possible that prevention of morphine sensitization might have been observed had higher doses been tested in the present studies. However, the 0.25 mg/kg dose of SCH 23390 used in the present investigation is considerably (5–10 times) higher than those required to completely block the behavioral effects of either dopamine D_1 receptor-selective agonists or non-selective dopamine receptor agonists (see Clark and White, 1987 for review). Indeed, 0.25 mg/kg is approximately 12–25-fold greater than the ED_{50} for displacement of ^3H antagonists from dopamine D_1 receptors by SCH 23390 in vivo (Andersen, 1988; McQuade et al., 1988a) and for effective prevention of morphine-induced hyperlocomotion in mice (Longoni et al., 1987). The 1.0 mg/kg dose used in the experiment reported by Kalivas and Stewart (1991) is close to the estimated ED_{50} (1.5 mg/kg) for binding of SCH 23390 to serotonin 5-HT_2 receptors in vivo (Bischoff et al., 1986; McQuade et al., 1988b). Thus, it is conceivable that this dose of SCH 23390 interfered with the process of sensitization via non-dopamine D_1 receptor mechanisms. It is also likely that disruption of sensitization by SCH 23390 was made more likely in the experiment reported by Kalivas and Stewart (1991) because the extent of sensitization was quite minimal, i.e. approximately a 35% increase in locomotor activity, as compared to the 170% increase in morphine-induced ambulation produced by our treatment regimen.

As mentioned above, Kalivas and Stewart (1991) also suggested dopamine D_1 receptor involvement in the development of morphine sensitization based upon an experiment demonstrating that intra-ventral tegmental area administration of SCH 23390 prevented sensitization to systemic morphine injections (Kalivas and Stewart, 1991). From our perspective, this report should be viewed with caution. Electrophysiological studies from in vitro slice preparations have shown that SCH 23390 concentrations of 30 μM can produce a long-lasting inactivation of midbrain dopamine neurons (Suppes and Pinnock, 1987), perhaps via local-anesthetic-like effects. Accordingly, it is possible that local microinjections of SCH 23390 (in mM concentrations) into the ventral tegmental area, which contains a very low density of dopamine D_1 receptors with which SCH 23390 might interact (Boyson et al., 1986; Dubois et al., 1986), result in a similar inactivation of dopamine neurons. Indeed, in a previous study regarding the ability of intra-ventral tegmental area SCH 23390 microinjections (0.5 and 1.0 μg/0.5 μl per side) to prevent the development of *d*-amphetamine sensitization, such treatments substantially reduced normal locomotor responses in saline-injected animals (Stewart and Vezina, 1989), suggesting a disruption of dopaminergic transmission by intra-ventral tegmental area SCH 23390. Since neither systemic nor iontophoretic administration of SCH 23390, at doses that are supra-effective at blocking dopamine D_1 receptors, influences the firing of ventral tegmental area dopamine neurons in vivo (White and Wang, 1984a; Wachtel et al., 1989), the disruption of dopaminergic transmission produced by intra-ventral tegmental area injections of SCH 23390 could have resulted from non-dopamine D_1 receptor actions of the drug occurring at high concentrations.

Although rats treated with the combination of SCH 23390 and morphine clearly exhibited sensitized behavior when challenged with morphine alone, a potential confound was introduced by repeated administration of the dopamine D_1 receptor antagonist. Such treatments are known to induce dopamine D_1 receptor supersensitivity (Creese and Chen, 1985; Porceddu et al., 1985; McGonigle et al., 1989; Hu and White, 1994). This possibility was underscored by the small but significant enhancement of morphine-induced activity in rats that received repeated injections of SCH 23390 plus vehicle. Given that supersensitivity of dopamine D_1 receptors

in the nucleus accumbens caused by repeated cocaine administration (Henry and White, 1991) is temporally correlated with the presence of behavioral sensitization (White et al., 1995), we conducted additional experiments to determine whether the apparent failure of SCH 23390 to prevent morphine sensitization could have resulted – not from morphine sensitization – but from dopamine D_1 receptor supersensitivity caused by repeated SCH 23390 treatment. When tested 24–30 h after the last of 12 daily SCH 23390 injections (0.25 mg/kg), the sensitivity of nucleus accumbens neurons to either dopamine or the dopamine D_1 receptor-selective agonist SKF 38393 was unaltered. This finding is consistent with previous reports indicating no increase in dopamine D_1 receptor density following short withdrawals from repeated SCH 23390 treatment (Lappalainen et al., 1990). While it is possible that dopamine D_1 receptor supersensitivity might have been detected at longer withdrawal times, we chose the 1 day withdrawal because it was also used in the behavioral studies of morphine sensitization. Thus, the failure of SCH 23390 to prevent morphine sensitization cannot be attributed to the induction of nucleus accumbens dopamine D_1 receptor supersensitivity by the SCH 23390 treatment. Of course, it is conceivable that alterations in dopamine D_1 receptor function in other brain regions might have been involved in enhanced morphine sensitivity in both the SCH 23390/vehicle and SCH 23390/morphine groups.

4.4. Development and expression of morphine sensitization

The present results support the notion that the events responsible for the *development* of morphine sensitization do not require dopamine receptor stimulation (Vezina and Stewart, 1989), and also indicate that the *expression* of sensitization *is* dependent upon dopamine receptor stimulation. The findings with eticlopride are particularly interesting in that they suggest that dopamine D_2 receptors are involved in the expression of sensitized, but not normal, morphine-induced locomotion. Given that repeated morphine administration has been reported to enhance morphine-induced release of dopamine (Kalivas and Stewart, 1991; Acquas and Di Chiara, 1992; Spanagel et al., 1993), it may be the case that enhanced synaptic levels of dopamine are more likely to stimulate dopamine D_2 receptors involved in the expression of locomotion, such that only the sensitized component of ambulation would be susceptible to dopamine D_2 receptor antagonism.

Taken together, the present findings with dopamine antagonists are consistent with a model in which the non-dopaminergic mechanisms responsible for the development of opioid sensitization must impact upon the normal functioning of ventral tegmental area dopamine neurons. Stimulation of μ-opioid receptors within the ventral tegmental area is necessary for the development of opioid sensitization as evidenced by the ability of opioid antagonists to prevent the effect (Kalivas and Duffy, 1987) and the ability of repeated intra-ventral tegmental area administration of μ-opioid receptor agonists to produce it (Kalivas and Stewart, 1991). Within the ventral tegmental area, μ-opioid receptors are expressed by non-dopaminergic (presumably GABAergic) neurons, but not by dopamine neurons (Dilts and Kalivas, 1989; Lacey et al., 1989). Thus, morphine excites ventral tegmental area dopamine neurons by inhibiting inhibitory interneurons (Gysling and Wang, 1983; Matthews and German, 1984; Lacey et al., 1989). Repeated administration of morphine, using the present dosing regimen, increases the basal firing rates of ventral tegmental area dopamine neurons as well as their sensitivity to morphine-induced excitation (Jeziorski and White, in preparation). Interestingly, this increase in basal activity of ventral tegmental area dopamine neurons represents the only known common effect of repeated treatments with morphine, amphetamine and cocaine on electrophysiological indices of mesoaccumbens dopamine activity (White and Wang, 1984b; Henry et al., 1989; Jeziorski and White, in preparation). Since cross sensitization between these various compounds has been reported (Kalivas and Stewart, 1991), it may be that the increase in basal firing rates of ventral tegmental area dopamine neurons following repeated administration is a common mechanism underlying the development of behavioral sensitization. Accordingly, any treatment that prevents the increase in basal dopamine neuronal activity would be expected to prevent sensitization.

Another manipulation which has been demonstrated to prevent morphine sensitization is a block of excitatory amino acid transmission through *N*-methyl-D-aspartate (NMDA) receptors. Thus, both the non-competitive NMDA receptor antagonist MK-801 and the competitive NMDA receptor antagonist CGS 19755 have been shown to prevent morphine sensitization (Wolf and Jeziorski, 1993; Jeziorski et al., 1994). According to the model outlined above, this effect is likely to occur by preventing the development of increased basal firing rates of ventral tegmental area dopamine neurons. There is now considerable electrophysiological evidence supporting the existence of tonic excitatory regulation of midbrain dopamine neurons via NMDA receptors (Svensson and Tung, 1989; Mereu et al., 1991; Johnson et al., 1992). Thus, prevention of this excitation by NMDA antagonists would be expected to dampen any excitatory influence resulting from opioid-induced disinhibition and might thereby prevent the increase in basal dopamine neuronal activity normally associated with repeated morphine administration. In fact, coadministration of MK-801 and mor-

phine significantly reduces the enhancement of basal dopamine neuron firing rates typically associated with repeated morphine treatment (Jeziorski and White, in preparation).

4.5. Conclusion

In conclusion, repeated coadministration of either dopamine D_1 or D_2 receptor-class antagonists with morphine did not prevent the *development* of behavioral sensitization, but did prevent the *expression* of the sensitized component of locomotor activity. When considered along with other evidence in the literature, the present results support the idea that the development of morphine sensitization involves alterations in the balance of inhibitory and excitatory inputs to ventral tegmental area dopamine neurons, resulting in enhanced basal activity of this neuronal population, but that dopamine receptor stimulation is not required for these initiating events.

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