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Effect of the 5-HT_6 serotonin antagonist MS-245 on the actions of (−)nicotine

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Abstract

The 5-HT_6 serotonin receptor antagonist MS-245 neither substitutes for nor antagonizes the discriminative stimulus effects of (−)nicotine. However, MS-245 was shown to enhance the potency of (−)nicotine in Sprague–Dawley rats trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle in a typical two-lever drug discrimination paradigm such that a combination of MS-245 (5.0 mg/kg) plus the ED_{50} dose of (−)nicotine caused the animals to respond as if they had received the training dose of (−)nicotine. MS-245 also potentiated the hypolocomotor actions, but not the antinociceptive effects, of (−)nicotine in mice. The results suggest possible involvement of serotonin-regulated signaling mechanisms in certain behavioral effects of nicotine.

Keywords: Nicotine; Serotonin; Dopamine; Drug discrimination; Locomotor activity; Antinociception; DARPP-32

1. Introduction

Certain drugs of abuse initiate a cascade of neuronal events that ultimately involves dopamine- and cAMP-regulated phosphoprotein (DARPP-32) — an integrator of intracellular signaling (Svenningsson et al., 2003, 2004). For example, stimulation of dopamine receptors alters cyclic adenosine 5′-monophosphate (cAMP) levels that subsequently regulate protein kinase A (PKA). Activation of PKA can further modulate the phosphorylation state of DARPP-32. That is, PKA-mediated phosphorylation of DARPP-32, depending upon where phosphorylation occurs, can either inhibit or disinhibit protein phosphatase-1 (Svenningsson et al., 2004). Phosphorylation of a particular DARPP-32 threonine residue (i.e., Thr^{34}) results in the inhibition of protein phosphatase-1 to enhance dopaminergic signaling. Phosphorylation of a different threonine residue (i.e., Thr^{75}) results in inhibition of Thr^{34} phosphorylation. Activation of D_1 receptors, – receptors that are positively coupled to adenylate cyclase – increases levels of phospho-Thr^{34}-DARPP-32 and decreases levels of phospho-Thr^{75}-DARPP-32. Activation of D_2 dopamine receptors, which are negatively coupled to adenylate cyclase, produces the opposite effect (Hamada et al., 2004; Svenningsson et al., 2004).

It has been demonstrated that DARPP-32 plays a role in nicotine-mediated behaviors. Nicotine, acting on α4β2 nicotinic acetylcholine receptors, stimulates release of striatal dopamine, and systemic administration of nicotine (presumably via its actions on nucleus accumbens) also depresses mouse spontaneous motor activity (Hamada et al., 2004, 2005; Marubio et al., 2003; Rao et al., 2003; Wonnacott et al., 2000; Zhu et al., 2005). Evidence suggests the actions might be related. Although the effect of nicotine on DARPP-32 phosphorylation may be dose- and time-dependent (Hamada et al., 2004, 2005), systemic injection of nicotine has been shown to increase phosphorylation of DARPP-32 both at Thr^{34} and Thr^{75} (Zhu et al., 2005). Evidence that DARPP-32 regulates some nicotine-induced behaviors is supported by the demonstration that DARPP-32 knock-out mice generally exhibit enhanced behavioral responses (e.g. motor effects) to nicotine (Hamada et al., 2004; Zhu et al., 2005) when compared to wild type mice. It has been postulated that because nicotine produces a more pronounced

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effect in DARPP-32 knock-out animals than in wild type mice, that DARPP-32 exerts an *inhibitory* effect over the behavioral actions of nicotine (Zhu et al., 2005).

Nicotine also stimulates the release of serotonin (5-HT) in the striatum (Reuben and Clarke, 2000). The neostriatum, nucleus accumbens, and prefrontal cortex receive moderately high serotonergic innervation, and 5-HT levels are further increased when dopamine levels in these regions are increased (Svenningsson et al., 2002). Certain behavioral and biochemical effects induced by enhanced serotonergic neurotransmission also appear to be regulated by DARPP-32 as evidenced by the action of 5-HT and other serotonergic agonists in DARPP-32 knock-out mice (Svenningsson et al., 2002). Furthermore, it has been shown that 5-HT can produce an increase in phospho-Thr^{34}-DARPP-32 and a decrease in phospho-Thr^{75}-DARPP-32. Although the actions of 5-HT on DARPP-32 seem to involve multiple phosphorylation sites, its effects on Thr^{34} and Thr^{75} can be accounted for, at least in part, by a 5-HT$_6$ receptor mechanism (Svenningsson et al., 2002). For example, the 5-HT$_6$ receptor antagonist Ro 04-6790 [4-amino-*N*-(2,6-bis-methylaminopyrimidin-4-yl)benzenesulfonamide] reduced the effects of 5-HT both on phospho-Thr^{34}- and phospho-Thr^{75}-DARPP-32 (Svenningsson et al., 2002).

If certain behavioral actions of nicotine are regulated by DARPP-32, and are enhanced in DARPP-32 knock-out mice, it might be possible to mimic these actions by direct antagonism of 5-HT$_6$ receptor pathways. That is, pretreatment of animals with a 5-HT$_6$ receptor antagonist might be reasonably expected to enhance certain actions of nicotine. We have identified MS-245 [5-methoxy-(N$_1$-benzenesulfonyl)-*N*,*N*-dimethyltryptamine) as one of the first examples of a 5-HT$_6$ receptor antagonist (Glennon et al., 2000; Tsai et al., 2000). In the present investigation this 5-HT$_6$ antagonist was utilized in attempts to modulate the actions of nicotine in three different behavioral assays: *a)* the discriminative stimulus effects of (−) nicotine-trained rats, *b)* the locomotor activity of (−)nicotine in mice, and *c)* antinociceptive actions of (−)nicotine in mice.

2. Materials and methods

2.1. Drug discrimination studies

Seven male Sprague–Dawley rats (Charles River Laboratories), weighing 250–300 g at the beginning of the study, were trained to discriminate (15-min pre-session injection interval) 0.6 mg/kg of (−)nicotine from saline vehicle (sterile 0.9% saline) under a variable interval 15-s schedule of reward (i.e., sweetened condensed milk) using standard two-lever Coulbourn Instruments operant equipment as previously described (Young and Glennon, 2002). Animal studies were conducted under an approved Institutional Animal Care and Use Committee protocol.

In brief, animals were food-restricted to maintain body weights at approximately 80% that of their free-feeding weight, but were allowed free access to water in their individual home cages. Daily training sessions were conducted with the training dose of (−)nicotine or saline. For approximately half the animals, the right lever was designated as the drug-appropriate lever, whereas the situation was reversed for the remainder of the animals. Learning was assessed every fifth day during an initial 2.5-min non-reinforced (extinction) session followed by a 12.5-min training session. Data collected during the extinction session included response rate (i.e., responses per minute) and number of responses on the drug-appropriate lever (expressed as a percent of total responses). Animals were not used in the subsequent stimulus generalization studies until they consistently made $\geq$80% of their responses on the drug-appropriate lever after administration of training drug and $\leq$20% of their responses on the same drug-appropriate lever after administration of saline. During the testing (i.e., stimulus generalization) phase of the study, maintenance of the training-drug/saline discrimination was insured by continuation of the training sessions on a daily basis (except on a generalization test day). On one of the two days before a generalization test, approximately half the animals would receive the training dose of training drug and the remainder would receive saline; after a 2.5-min extinction session, training was continued for 12.5 min. Animals not meeting the original training criteria during the extinction session were excluded from the subsequent generalization test session. During the investigations of stimulus generalization, test sessions were interposed among the training sessions. The animals were allowed 2.5 min to respond under non-reinforcement conditions. An odd number of training sessions (usually 5) separated any two generalization test sessions. In the combination tests, MS-245 was administered 30 min prior to (−)nicotine. Stimulus generalization was considered to have occurred when the animals, after a given dose of drug, or drug combination, made $\geq$80% of their responses (group mean) on the training drug-appropriate lever. Animals making fewer than 5 total responses during the 2.5-min extinction session were considered as being behaviorally disrupted. Percent drug-appropriate responding and response rate data refer only to animals making $\geq$5 responses during the extinction session (Young and Glennon, 1986). Where applicable, an ED$_{50}$ dose was calculated by the method of Finney (1952). These doses represent the drug dose where animals would be expected to make 50% of their responses on the drug-appropriate lever.

2.2. Locomotor studies

Male ICR mice (Harlan Sprague Dawley Inc.; Indianapolis, IN) were used, weighing 27 to 34 g at the time of testing. The animals were housed in groups of five in solid-bottom plastic cages (38×22×15 cm). Food and water were available ad lib. The mice were naïve to the test apparatus (Tru-Scan Activity System, Coulbourn Instruments Inc., Allentown, PA), which consisted of three plexiglas monitors (40 cm^3). At the start of the experiment, the mice were removed from the vivarium and brought to the test laboratory for a 45- to 60-min acclimation period. Tests were conducted between 0930 h and 1730 h. The animals were treated with either saline, MS-245 (5.0, 10, or 15 mg/kg), (−)nicotine (0.01, 0.03, 0.1, 0.3, 1.0, or 3.0 mg/kg) alone, or 0.3 mg/kg of nicotine in combination with 5.0, 10, or

15 mg/kg of MS-245 just prior to being placed in activity cages for 30 min. Mice were used only once and each dose of test agent (or combination of drugs) was studied in 8 mice ($n=8$/group). The behavioral analysis examined three measures of activity: movement time (s), movement distance (cm), and vertical entries (rearing). Data for each measure were analyzed statistically by an analysis of variance (ANOVA) followed by Dunnett's *t*-test (statistical significance set at $p \leq 0.05$) for post-hoc comparison tests.

2.3. Antinociception assay

Animals used in the study were male ICR mice (24 to 28 g) purchased from Harlan Laboratories (Indianapolis, IN). The animals were housed and fed as described for the locomotor studies (see above). Antinociception was assessed by the tail-flick method of D'Amour and Smith (1941) as modified by Dewey et al. (1970) using a standard Columbus Tail-Flick Analgesia Meter (Columbus Instruments Inc., Columbus, OH). A mouse's exposure to the heat source was limited to 10 s to prevent or minimize tissue damage to the animal's tail. A control response (2 to 4 s) was determined for each mouse before treatment, and a test latency was determined after drug administration. Specifically, 15 min prior to injection of drug, baseline tail-flick was determined for each mouse. The animal then was injected with MS-245 (1.0, 3.0, 10 or 30 mg/kg) or (−) nicotine (0.3, 1.0, or 3.0 mg/kg) 30 min or 5 min before the test, respectively. In the combination test, doses of MS-245 (10 or 30 mg/kg) were combined with doses of nicotine (0.3, 1.0 or 3.0 mg/kg). In this procedure, MS-245 was administered first and 25 min later a dose of nicotine was injected; after 5 min, the test started. An antinociceptive response was calculated as percent maximum possible effect (% MPE), where % MPE = [(test − control)/(10 − control)] × 100. Groups of 9 to 10 animals were used for each dose and for each combination of treatments. Data were analyzed statistically by analysis of variance (ANOVA) followed by Student's *t* (statistical significance set at $p \leq 0.05$) for post-hoc comparison tests.

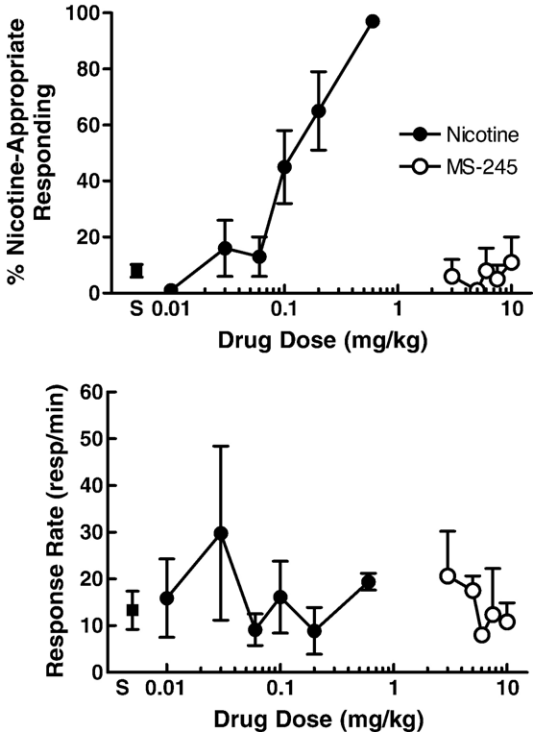

Fig. 1. Results (percent drug-appropriate responding ± S.E.M.) of stimulus generalization studies with (−)nicotine and MS-245 in rats ($n=7$) trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle (top panel). S = 0.9% saline. The animals' response rate at each dose is shown in the bottom panel.

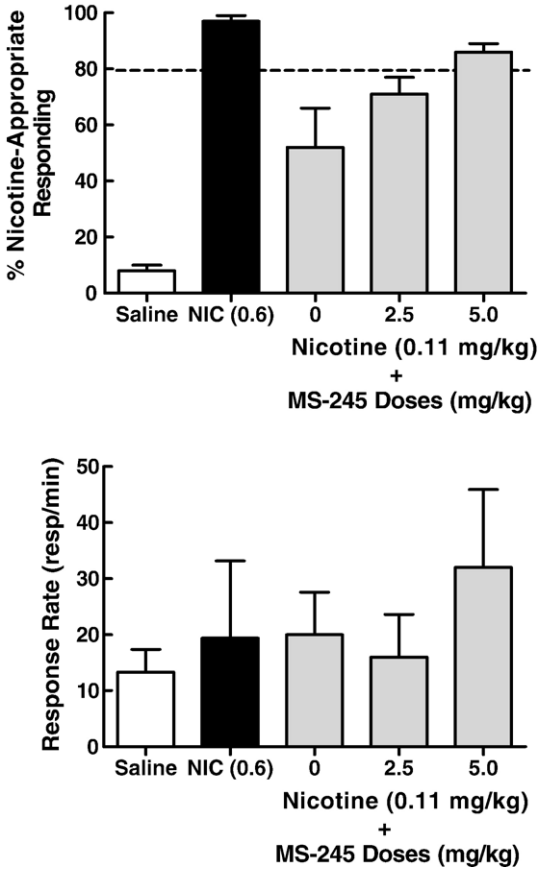

Fig. 2. MS-245 enhances the stimulus effect of (−)nicotine. Shown is the effect (percent (−)nicotine-appropriate responding ± S.E.M.) of the ED_{50} dose of (−) nicotine administered alone and in combination with doses of MS-245 to rats ($n=7$) trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle. The effect of administration of the (−)nicotine training dose (0.6 mg/kg) is shown for comparison; S = 0.9% saline (top panel). The animals' response rates are shown in the bottom panel.

2.3.1. Drugs

(−)Nicotine hydrogen tartrate was purchased from Sigma-Aldrich (St. Louis, MO). The 5-HT_6 antagonist 5-methoxy-(N_1-benzenesulfonyl)-*N*,*N*-dimethyltryptamine hydrogen oxalate (MS-245) was synthesized as previously described (Glennon et al., 2000). Doses of (−)nicotine refer to the weight of the base and doses of MS-245 refer to the weight of the salt. Solutions of both drugs were made fresh daily in 0.9% sterile saline. Both drugs were administered by the subcutaneous (s.c.) route of administration. Doses of nicotine were administered in a 1 ml/kg or 10 ml/kg injection volume in rats and mice, respectively.

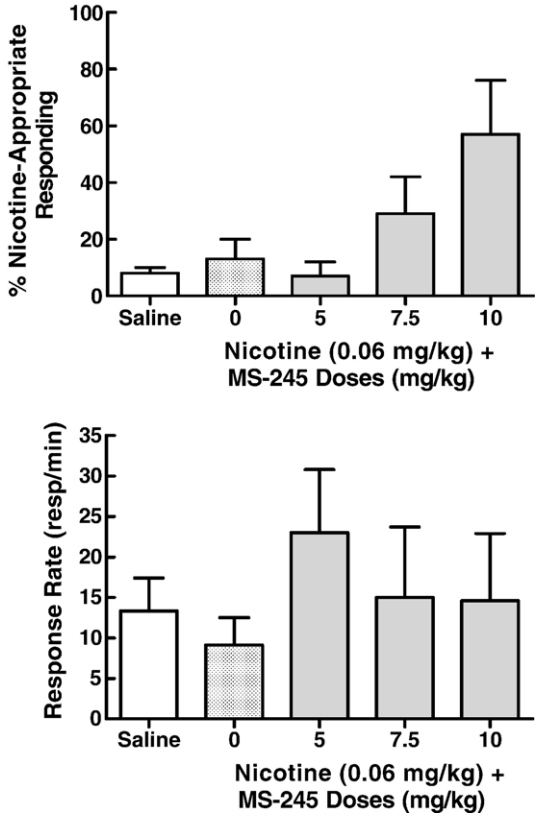

Fig. 3. MS-245 potentiates the stimulus effects of a saline-like dose of (−)nicotine. Shown is the effect (percent (−)nicotine-appropriate responding±S.E.M.) of 0.06 mg/kg of (−)nicotine administered alone and in combination with doses of MS-245 to rats ($n=7$) trained to discriminate 0.6 mg/kg of (−)nicotine from saline vehicle; S=0.9% saline (top panel). The animals' response rates are shown in the bottom panel.

Doses of MS-245 were administered in a 10 ml/kg injection volume in mice. In rats, doses of ≤5.0 mg/kg were administered in an injection volume of 1 ml/kg but solubility problems necessitated a 2 ml/kg injection volume for doses of MS-245>5.0 mg/kg. The pre-session injection interval(s) for each drug or drug combination in each test/species was based on previous studies (Dukat and Wesolowska, 2005; Young and Glennon, 2002). In drug discrimination substitution tests in rats, MS-245 was administered 45 min prior to tests. In combination tests with nicotine, MS-245 was administered 30 min prior to administration of nicotine; 15 min later, the test session started. In the locomotor activity test in mice, the administration of nicotine, MS-245, or the combination of both drugs, was immediately followed by placement into the activity arena for 30 min. In the tail-flick test, mice were administered MS-245 (alone) or nicotine (alone) at 30 min or 5 min prior to tests, respectively. In combination tests with nicotine, MS-245 was administered 25 min prior to administration of nicotine; 5 min later, a test session was begun.

3. Results

Rats were trained to discriminate 0.6 mg/kg (−)nicotine from saline vehicle (Fig. 1). The animals' response rates are also shown in Fig. 1. Administration of (−)nicotine doses lower than the training dose resulted in an orderly decrease in drug-appropriate responding. The ED_{50} dose calculated for (−)nicotine=0.11 (95% CL 0.05–0.24) mg/kg. Tests of stimulus generalization with MS-245 showed that doses of 3.0 mg/kg to 10 mg/kg produced a maximum of only 11% (−)nicotine-appropriate responding

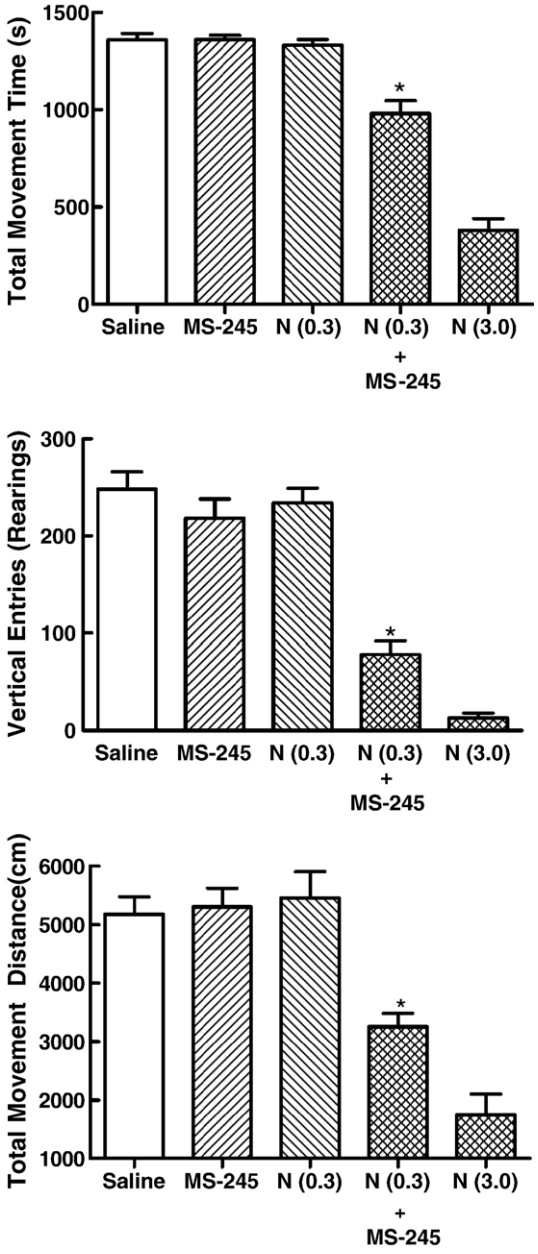

Fig. 4. MS-245 enhances the hypolocomotor actions of 0.3 mg/kg of (−)nicotine in mice. Shown is the effect (±S.E.M.) of saline, MS-245 (15 mg/kg), (−)nicotine (N; 0.3 and 3.0 mg/kg) alone, and 0.3 mg/kg of (−)nicotine in combination with MS-245 (15 mg/kg), on several measures of mouse locomotor activity ($n\geq 6$ animals per dose or dose combination). MS-245 doses (5, 10, and 15 mg/kg) had no effect on the actions of 3.0 mg/kg of (−)nicotine (data not shown). A combination of (−)nicotine (0.3 mg/kg) and MS-245 (15 mg/kg) produced a significant decrease in total movement time (upper panel; Dunnett's t *$p<0.0005$), total movement distance (center panel; Dunnett's t *$p<0.001$) and vertical entries (lower panel; Dunnett's t *$p<0.0001$) relative to 0.3 mg/kg of (−)nicotine administered alone (i.e., as control group for Dunnett's t test).

(Fig. 1). Administration of these doses did not substantially influence the animals' response rates (Fig. 1).

Administration of the calculated ED_{50} dose of (−)nicotine to the (−)nicotine-trained animals elicited 52% (−)nicotine-appropriate responding (Fig. 2). Administration of MS-245 in combination with the ED_{50} dose of (−)nicotine produced an increase in (−)nicotine-appropriate responding such that 0.11 mg/kg of (−)nicotine (i.e., the ED_{50} dose) together with 5.0 mg/kg of MS-245 resulted in stimulus generalization; that is, the combination produced 86% (−)nicotine-appropriate responding (Fig. 2). The animals' response rates were not substantially different from control response rates except that 5.0 mg/kg of MS-245 in combination with nicotine resulted in about a 50% increase in the animals' response rate (Fig. 2). Administered together with a low dose of (−)nicotine (i.e., 0.06 mg/kg) that elicited saline-appropriate responding, MS-245 produced a dose-related increase in drug-appropriate responding. A combination of 0.06 mg/kg of (−)nicotine (which, by itself, produced 13% nicotine-appropriate responding) with 10 mg/kg of MS-245 elicited nearly 60% (−)nicotine-appropriate responding (Fig. 3). The animals' response rates were fairly consistent under these conditions (Fig. 3).

Mice treated with (−)nicotine in the locomotor activity study displayed statistically significant effects in total movement time ($F(6,49)=66.24$, $p<0.0001$), total movement distance ($F(6,49)=9.44$, $p<0.0001$), and vertical entries ($F(6,49)=13.93$, $p<0.0001$). Dunnett's post-hoc comparison tests revealed that the effects of nicotine doses of 0.01, 0.03, 0.1, 0.3 and 1.0 mg/kg were not statistically different than those following administration of saline (data not shown, except see Fig. 4 for the effect of 0.3 mg/kg). In contrast, mice treated with 3.0 mg/kg of nicotine produced a statistically significant ($p<0.05$) decrease in all three measures: that is, 75% reduction in total movement time, 70% reduction in total movement distance, and 95% decrease in vertical entries (Fig. 4). Various doses (5.0, 10, and 15 mg/kg) of MS-245 produced effects in total movement time ($F(3,28)=0.37$, $p>0.05$), total movement distance ($F(3,28)=1.18$, $p>0.05$), and vertical entries ($F(3,28)=1.69$, $p>0.05$) that were not statistically different than those seen following administration of saline (see Fig. 4 for the effects of the 15 mg/kg dose).

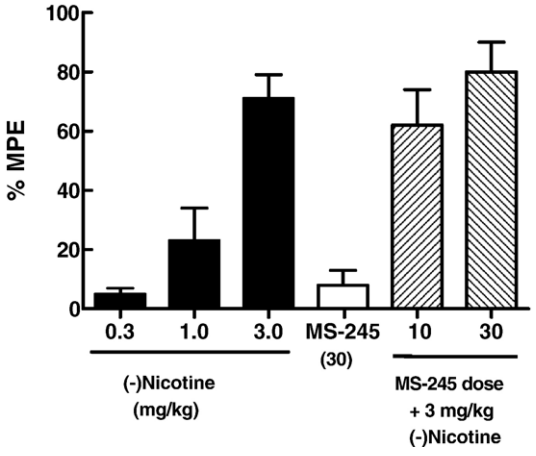

Fig. 5. MS-245 does not potentiate the antinociceptive effects of (−)nicotine in mice. Shown are the antinociceptive effect (% MPE, percent maximal possible effect, ±S.E.M.) of (−)nicotine doses and MS-245 (30 mg/kg) alone, and the effect (±S.E.M.) of 3.0 mg/kg of (−)nicotine in combination with 10 and 30 mg/kg of MS-245 ($n=9$–10 mice per dose or dose combination).

A combination of 0.3 mg/kg of (−)nicotine together with MS-245 doses of 5.0 and 10 mg/kg had little effect on locomotor activity (data not shown). However, 0.3 mg/kg of (−)nicotine plus 15 mg/kg of MS-245 resulted in significant suppression of total movement time ($p<0.0005$ relative to the control dose of 0.3 mg/kg of nicotine alone), total movement distance ($p<0.001$), and vertical entries ($p<0.0001$) (Fig. 4) such that they approached the effect of the higher (i.e., 3.0 mg/kg) dose of (−)nicotine.

(−)Nicotine (0.3–3.0 mg/kg) produced a dose-related ($ED_{50}=1.8$ mg/kg; 95%CL=0.9–3.6 mg/kg) and statistically significant ($F(3,35)=15.88$, $p<0.0001$) antinociceptive effect in mice using the tail-flick assay (Fig. 5). Student's t post-hoc comparison tests revealed that only 3.0 mg/kg, but not lower doses, of (−)nicotine produced a statistically significant ($p<0.05$) antinociceptive effect. MS-245 (i.e., 1.0, 3.0, 10 and 30 mg/kg) was found to lack statistically significant ($F(4,44)=0.84$, $p>0.05$) antinociceptive actions (the effect of the highest dose evaluated is shown in Fig. 5). Doses of 10 and 30 mg/kg of MS-245 were examined in combination with 0.3, 1.0, and 3.0 mg/kg of (−)nicotine. A statistical comparison of the results with 10 and 30 mg/kg of MS-245, in combination with nicotine versus 3.0 mg/kg of (−)nicotine alone, found no statistical significance ($F(2,26)=0.68$, $p>0.05$). These data are shown in Fig. 5. The combinations of MS-245 (30 mg/kg) with 0.3 and 1.0 mg/kg of (−)nicotine were not statistically significant (data not shown).

4. Discussion

Although the discriminative stimulus effects of nicotine are mediated primarily via nicotinic acetylcholinergic receptors (presumably by activation of α4β2 nACh receptors) (Rosecrans et al., 1978; Stolerman et al., 1983), it is generally agreed (but see Corrigall and Coen, 1994) that indirect dopamine receptor activation contributes to nicotine's stimulus actions (Desai et al., 2003; Gasior et al., 1999; Mansbach et al., 1998; Rosecrans et al., 1978; Schechter and Meehan, 1993 and references therein). Recent studies have suggested that 5-HT_6 serotonin receptors can modulate the actions of dopamine through phosphorylation of DARPP-32 at Thr^{34} and Thr^{75} (e.g. Svenningsson et al., 2002). In the present study, the 5-HT_6 antagonist MS-245 failed to produce (−)nicotine-like stimulus effects (Fig. 1). But, administered in combination with the ED_{50} dose of (−)nicotine, MS-245 enhanced the stimulus actions of (−)nicotine such that following a combination of 5.0 mg/kg of MS-245 plus the ED_{50} dose of (−)nicotine, the animals made >80% of their responses on the (−)nicotine-appropriate lever. That is, at this dose combination, the animals responded as if they had been administered the training dose of (−)nicotine. The potency enhancing action of MS-245 is not of a simple additive nature because a combination of a "saline-like" dose of MS-245 (10 mg/kg) together with an "inactive" dose of (−)nicotine (0.06 mg/kg; which, by itself, produced only 13% nicotine-appropriate responding), produced nearly 60% (−)nicotine-

appropriate responding (Fig. 3); solubility concerns with MS-245 precluded the evaluation of higher doses. Clearly, MS-245 is able to enhance the stimulus actions of (−)nicotine.

Nicotine produces a hypolocomotor effect in mice, and α4-containing (Marubio et al., 2003) and β2-containing (King et al., 2004) nACh receptors play an important role in this action as demonstrated by the attenuated responding seen in either α4 or β2 knock-out mice following administration of nicotine. Activation of nACh receptors results in, among other effects, the release of dopamine — particularly in the striatum (Rao et al., 2003, and references therein). In fact, nicotine can modulate dopaminergic neurotransmission by enhancing dopamine release in several brain regions (Ramos et al., 2004; Role and Berg, 1996; Wonnacott, 1997; Wonnacott et al., 2000). In the present study, low doses of (−)nicotine (0.01 to 1.0 mg/kg) produced an effect on mouse locomotor activity comparable to that seen following administration of saline. In contrast, 3.0 mg/kg of (−)nicotine decreased all measures of locomotor activity by >60% (Fig. 4). Co-administration of a MS-245 dose, which by itself had no effect on locomotor activity, together with nicotine, enhanced the effect of 0.3 mg/kg of (−)nicotine such that the combination produced effects approaching those seen following a 3.0 mg/kg dose of (−) nicotine administered alone (Fig. 4). Here too, then, MS-245 had a potentiating effect on the actions of (−)nicotine. A combination of MS-245 and a higher (i.e., 3.0 mg/kg) dose of (−)nicotine produced effects similar to that seen following 3.0 mg/kg of (−) nicotine alone. The lack of further or enhanced locomotor suppression of this high dose of nicotine by MS-245 might be simply due to the already low level (i.e., "flooring" or "bottoming out" effect) of locomotor activity observed at this high nicotine dose.

Because neither D_1 nor D_2 dopamine receptors seem to be involved in the antinociceptive effects of (−)nicotine in mice (Damaj and Martin, 1993), we also examined the effect of MS-245 alone, and in combination with (−)nicotine, in the mouse tail-flick assay for comparison. MS-245 displayed no antinociceptive action and failed to enhance the effect of (−)nicotine when given in combination. These results might reflect mechanistic differences and suggest that either DA-mediated DARPP-32 modulation is not involved in nicotine-induced antinociception, or that MS-245 behaves differently in this procedure.

Complicating the interpretation of results from mouse studies is that Hirst et al. (2003) have found lower levels of 5-HT_6 receptor mRNA in mouse brain than in rat brain. Additionally, despite the high degree of sequence homology between mouse and rat 5-HT_6 receptors, and even though 5-HT and certain other serotonergic agents display comparable affinities for the two species homologs, some agents bind with higher affinity at one over the other (Hirst et al., 2003). Hence, a specific role for 5-HT_6 receptors in the actions of MS-245 in mouse brain must be viewed cautiously.

Taken together, the data show that the 5-HT_6 antagonist MS-245, by itself, did not substitute for (−)nicotine in rats trained to discriminate (−)nicotine from vehicle, had no effect on mouse locomotor activity, and lacked measurable antinociceptive action in the mouse tail-flick assay. Yet, MS-245 enhanced the effects of (−)nicotine in the drug discrimination task and on measures of locomotor activity, but not on antinociception action. A common factor involving the actions of nicotine and 5-HT_6 antagonists is DARPP-32. Although involvement of DARPP-32 is one explanation for the observed effect of the MS-245/nicotine combinations, further work is obviously necessary. For example, nicotine can activate α7 nACh receptors which, too, can influence DARPP-32, but this occurs through a glutamatergic mechanism (Hamada et al., 2005). Also, dopamine released by nicotine can activate both D_1 and D_2 dopamine receptors and these receptor types have opposing actions on DARPP-32. In addition, MS-245 has been shown to bind at 5-HT_2 and D_3 receptors (Russell et al., 2001); although its affinity for these receptors is about 30- to 40-fold lower than its affinity for 5-HT_6 receptors, and although its function (as agonist or antagonist) at these receptor types is unknown, their involvement cannot be dismissed. In this regard, it might be noted that the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and/or the 5-HT_{2C} agonist Ro 60-0175 *depressed* the stimulus and locomotor effects of nicotine in rodents (Batman et al., 2005; Grottick et al., 2001), and that the 5-HT_2 antagonist ketanserin was without effect on the hypolocomotor actions of nicotine in mice (Damaj et al., 1994). It might be parenthetically noted, if there is evidence for 5-HT_2 receptor involvement, that 5-HT_2 receptor ligands can also modulate phosphorylation of DARPP-32 (Svenningsson et al., 2002). Although more studies are required to assess the role of D_3 dopamine autoreceptors in mediating the stimulus effects of nicotine, it would seem their role is minimal (Gasior et al., 1999). For example, neither a D_3 partial agonist nor D_3 antagonist had an effect on nicotine discriminative performance or nicotine-induced motor activity of rats (Le Foll et al., 2005). Nevertheless, DARPP-32 has been implicated in certain behavioral (e.g. locomotor) actions of nicotine, and 5-HT_6 antagonists have been shown to modulate the actions of DARPP-32 (e.g. Hamada et al., 2004; Svenningsson et al., 2002).

We have recently found that MS-245 enhances the stimulus properties of (+)amphetamine in rats trained to discriminate (+) amphetamine from saline vehicle in a two-lever operant procedure (Pullagurla et al., 2004). Furthermore, Frantz et al. (2002) have shown that (+)amphetamine produces enhanced locomotor effects following administration of the 5-HT_6 antagonist SB-258510A, and that SB-258510A also altered self-administration of (+)amphetamine in a manner indicative of enhanced reinforcing properties (Frantz et al., 2002). In both studies, a role for DARPP-32 might be suspected. Alternatively, it could be that 5-HT_6 receptor antagonists simply increase levels of dopamine by an as yet to be elucidated mechanism. Indeed, there is some evidence for the latter possibility. Administered alone, 5-HT_6 antagonists generally have little to no effect on the basal levels of dopamine, serotonin, or norepinephrine (Dawson et al., 2000, 2001, 2003; Frantz et al., 2002; Lacroix et al., 2004) in various brain regions. The pretreatment of rats with SB-258510A, however, potentiated amphetamine-induced increases in dopamine levels in rat frontal cortex and nucleus accumbens (Frantz et al., 2002), and administration of the 5-HT_6 antagonist SB-271046 (at a dose that had no effect by itself) to rats in combination with (+)

amphetamine, elevated striatal levels of DA and 5-HT beyond that produced by (+)amphetamine alone (Dawson et al., 2003). The mechanism whereby this occurs is not understood and may or may not be related to the effects observed for nicotine in this investigation. Nonetheless, the present results do demonstrate, for the first time, that MS-245 can enhance the stimulus effects of (−)nicotine in the drug discrimination paradigm with rats trained to discriminate (−)nicotine from vehicle, and the hypolocomotor actions of (−)nicotine in mice. Moreover, the results of the locomotor studies are not inconsistent with what has been reported with DARPP-32 knock-out mice. The most parsimonious explanation for the observed results is that blockade of 5-HT_6 serotonin receptors might interfere with down-stream signaling of dopamine receptor activation.

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