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Locomotor stimulant effects of nornicotine: role of dopamine

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Abstract

Nornicotine (NORNIC) is a tobacco alkaloid and behaviorally active nicotine metabolite in vivo. Previous behavioral research has shown that NORNIC has locomotor stimulant and reinforcing effects in rats similar to that of nicotine. Results from the current study showed that a bilateral lesion of the nucleus accumbens decreased the locomotor stimulant effect of NORNIC across repeated injections. Pretreatment with the dopamine (DA) D1 receptor antagonist SCH23390 did not block the locomotor stimulant effect of NORNIC or the initiation of sensitization following repeated NORNIC administration. The D2 receptor antagonist eticlopride, however, blocked both the stimulant effect and the initiation of sensitization following repeated NORNIC. Additionally, NORNIC was found to increase synthesis and metabolism of DA, with a greater effect in the mesolimbic pathway compared to the nigrostriatal pathway. Taken together, these results suggest that expression of NORNIC-induced locomotor activity is dependent upon ascending dopaminergic mesolimbic projections, providing additional evidence that NORNIC plays a contributory role in tobacco dependence.

Keywords: Nicotine metabolite; Locomotion; Behavior; Activity; Sensitization; DOPAC; Synthesis; Metabolism; Tolerance; Tobacco alkaloid

1. Introduction

The behavioral and neurochemical effects of nicotine (NIC) involve the mesolimbic dopaminergic system (Balfour and Benwell, 1993). Specifically, pharmacological antagonism of dopaminergic receptors, or lesions of the mesolimbic dopamine (DA) system, block the locomotor stimulant and reinforcing effects of NIC (Clarke et al., 1988; Corrigall and Coen, 1991; Corrigall et al., 1992; O'Neill et al., 1991; Damaj and Martin, 1993). NIC also increases dihydroxyphenylalanine (DOPA) accumulation following inhibition of DOPA decarboxylase activity and increases dihydroxyphenylacetic acid (DOPAC) in dopaminergic terminal brain regions (Grenhoff and Svensson, 1988; Mitchell et al., 1989; Brazell et al., 1990; Vezina et al., 1992). However, investigations of the effect of NIC metabolites on dopaminergic systems have been limited until recently.

The *N*-demethylated NIC metabolite and tobacco alkaloid, nornicotine (NORNIC), has a longer half-life than NIC in plasma and brain (Kyerematen et al., 1990; Crooks et al., 1995, 1997; Ghosheh et al., 1999). Upon acute NIC administration, NORNIC reaches a three-fold higher concentration in brain than plasma and accumulates in brain following repeated NIC administration (Ghosheh et al., 2001). Based on several lines of evidence, NORNIC has been suggested to play a significant role in the neuropharmacological effects of tobacco smoking (Crooks and Dwoskin, 1997). Both NORNIC enantiomers displace [^{3}H]NIC from its high affinity binding sites in brain (Reavill et al., 1988; Copeland et al., 1991; Zhang and Nordberg, 1993). Nicotinic receptors are located on dopaminergic terminals (Clarke and Pert, 1985) and stimulation of these receptors by NORNIC releases DA, as indicated by the observation that NORNIC evokes [^{3}H]DA overflow from superfused striatal (Dwoskin et al., 1993, 1995; Teng et al., 1997) and nucleus accumbens (NAcc) slices (Green et al., 2001).

In behavioral studies, NORNIC generalizes fully to the NIC discriminative cue in squirrel monkeys (Takada et al., 1989) and rats (Goldberg et al., 1989). More recent work has shown that NORNIC maintains intravenous self-administration in rats (Bardo et al., 1999), providing evidence that it has a reinforcing effect and may play a role in tobacco dependence. Locomotor activity studies have shown that

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NORNIC, like NIC, produces an initial decrease in locomotor activity (Stolerman et al., 1995; Dwoskin et al., 1999). With repeated administration, tolerance develops to the hypoactivity and, during the latter part of the session, hyperactivity is observed (Dwoskin et al., 1999), indicative of a psychostimulant profile.

To determine the potential role of DA in the psychostimulant effect of NORNIC, the present study assessed the effect of pharmacological blockade of DA receptors and a 6-hydroxydopamine (6-OHDA) lesion of NAcc DA on the locomotor stimulant effect of repeated NORNIC administration. The effects of NORNIC on DOPA accumulation and DOPAC levels in dopaminergic terminal regions were also determined.

2. Methods and results

2.1. Animals

Male Sprague–Dawley rats (200–250 g) were obtained from Harlan Laboratories (Indianapolis, IN) and were housed individually with free access to food and water in polycarbonate cages. Experimental protocols involving the animals were in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky. Rats were maintained on a 12/12-h light cycle with lights on at 0700 h. Subjects were tested daily at approximately 1200 h.

2.2. Apparatus

The apparatus for locomotor activity experiments consisted of a rectangular activity chamber (24.5 × 28 cm) with white walls and pine bedding under a wire grid floor. Two photobeams, located 3 cm above the floor, were oriented at 90° angles, dividing the chamber into four equal quadrants. Infrared detectors were linked to a personal computer and photobeam interruptions were recorded in 10-min bins with overhead fluorescent illumination in the test room. Rats were not habituated to the locomotor chambers before testing.

2.3. Drugs

(±)-NORNIC (ICN, Costa Mesa, CA) was prepared as the free base in 0.9% NaCl and adjusted to pH 7. Ketamine hydrochloride was obtained from Fort Dodge Animal Health (Fort Dodge, IA) and diazepam was obtained from Steris (Phoenix, AZ). SCH23390 hydrochloride, eticlopride hydrochloride, desipramine hydrochloride, *m*-hydroxybenzylhydrazine dihydrochloride (NSD 1015), 6-OHDA hydrobromide and pargyline hydrochloride were obtained from Research Biochemicals (Natick, MA) and were prepared as their respective salts. Standards for high-pressure liquid chromotography (HPLC) analysis and chemicals used in the mobile phase were obtained from Sigma-Aldrich (St. Louis, MO).

2.4. Experiment 1: effect of 6-OHDA lesion in NAcc on locomotor effect of NORNIC

2.4.1. Surgery

A 6-OHDA lesion was made using the general procedures described previously (Pierce et al., 1990). Briefly, rats were first anesthetized with 80 mg/kg ketamine and 5 mg/kg diazepam (ip) and then were given 25 mg/kg desipramine (a norepinephrine uptake inhibitor) to protect noradrenergic neurons and 50 mg/kg pargyline (a monoamine oxidase inhibitor) to inhibit metabolism of 6-OHDA. Rats were then placed in a Kopf stereotaxic apparatus and injected bilaterally with 6-OHDA (8 μg/2 μl; 2 min infusion) into the NAcc using the following coordinates relative to bregma: AP=+1.9, L=±1.5, V=−7 mm, with the incisor bar at −3.3 mm (Paxinos and Watson, 1997). Sham control animals underwent the same surgical procedure, but 6-OHDA was not injected. Rats recovered for 7 days prior to behavioral testing.

2.4.2. Behavioral testing

Lesion ($n=25$) and sham control ($n=18$) rats were assigned randomly to one of two treatment groups: NORNIC (5 mg/kg sc) or saline. The 5 mg/kg dose was chosen based on a previous report suggesting that this dose was behaviorally active (Dwoskin et al., 1999). Rats were placed immediately into the activity chamber after injection. Activity was recorded for 60 min in 10-min bins. Sensitization and tolerance were assessed by measuring NORNIC-induced activity every other day for a total of seven tests. Half of the rats were tested each day beginning at 1200 h to minimize circadian effects.

2.4.3. DA assay procedure

After completion of behavioral testing, rats were killed by rapid decapitation and the NAcc tissue was dissected and analyzed for DA concentration using HPLC with electrochemical detection (BAS, West Lafayette, IN). The HPLC system consisted of Applied Biosystems autosampler (model 878A), a BAS ODS phase II silica column (3.2 × 100 mm), BAS unijet electrochemical detector (set at 0.7 V) and BAS dual-piston pump (PM-80). The mobile phase (flow rate = 0.7 ml/min) consisted of 124 mM citric acid, 50 mM sodium phosphate, 10 mM sodium chloride, 0.1 mM ethylenediamine tetra-acetic acid, 5% methanol and 0.2% sodium octyl sulfate, adjusted to pH 3.0 and degassed. Retention times and peak heights were recorded (BAS, RYT chart recorder) and were compared to external standards.

2.4.4. Data analyses

Only 6-OHDA-treated rats that had greater than 50% depletion of DA in the NAcc compared to the mean DA concentration in sham rats were used in the statistical

analyses of the locomotor data. Nine of the 25 lesioned rats were excluded according to this criterion. Data were analyzed with mixed-factor ANOVAs. Lesion and drug factors were between-subject variables, while time block and session factors were within-subject variables. Since previous work has shown that NORNIC produces a biphasic effect on locomotor activity across a 1-h session, the temporal pattern of effect for NORNIC was evaluated using a priori pair-wise comparisons between groups at each time block. Results were significant at $P < .05$ using two-tailed tests.

2.4.5. Results

2.4.5.1. Effect of 6-OHDA lesion on locomotor activity following acute and repeated NORNIC treatment (Sessions 1 and 7). Analysis of NAcc DA content revealed a mean (±S.E.M.) depletion of 65±3% in rats given 6-OHDA treatment compared to controls (data not shown). Fig. 1 (Panels a and b) shows the locomotor pattern of 6-OHDA-lesioned and sham control rats injected acutely with NORNIC (5 mg/kg) or saline. A three-factor ANOVA (Lesion × NORNIC Treatment × Time Block) revealed a two-way interaction of NORNIC Treatment × Time Block [$F(5,145) = 15.4$, $P < .001$]; however, no main effect or interaction of the lesion factor was found. Rats injected acutely with NORNIC showed a transient decrease in locomotor activity, followed by a rebound period of hyperactivity relative to saline. A priori pair-wise comparisons between NORNIC and saline groups at each time interval revealed that sham control rats displayed significant hyperactivity at the 50-min time block, while lesioned rats did not show significant hyperactivity at any time block. Analysis of the data from Session 7 illustrated in Fig. 1 (Panels c and d) revealed a similar pattern of results, with a significant NORNIC Treatment × Time Block interaction [$F(5,145) = 8.60$, $P < .05$]. A priori pair-wise comparisons showed robust hyperactivity in response to NORNIC for the 40–60-min time blocks of the session for sham control rats, but not for 6-OHDA-lesioned rats. Thus, acute hyperactivity and sensitization were observed following NORNIC in sham control rats, whereas 6-OHDA-lesioned rats did not show these effects.

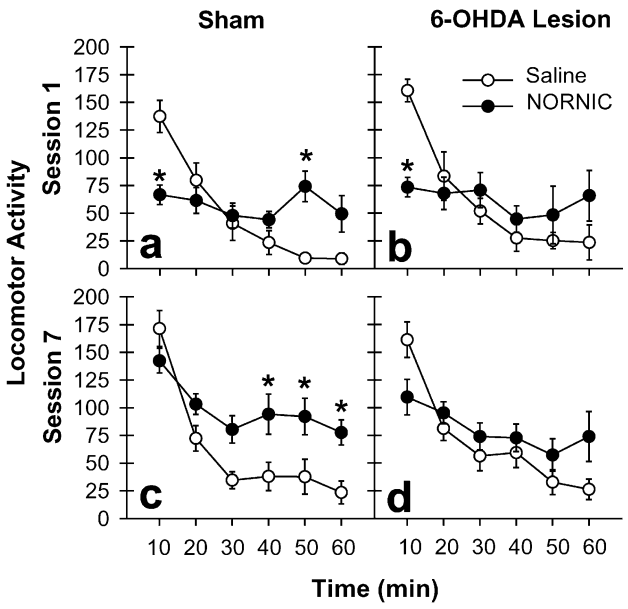

Fig. 1. Effect of acute (Panels a and b) and repeated (Panels c and d) NORNIC on locomotor activity in sham (Panels a and c) or lesion (Panels b and d) animals. Rats were treated with saline or NORNIC (5 mg/kg) immediately prior to session. Data are expressed as mean±S.E.M. photocell interrupts during 10-min time blocks across 60-min sessions. Asterisk (*) denotes statistical difference from saline control. $N = 7–9$ per group.

2.5. Experiment 2: effect of SCH23390 or eticlopride on locomotor effect of NORNIC

2.5.1. Behavioral testing

Rats were assigned randomly to one of six groups making up a 3 × 2 factorial design ($n = 8$ per group). Rats were pretreated with either SCH23390 (0.005 mg/kg sc), eticlopride (0.02 mg/kg sc) or saline in the home cage, followed 15 min later by an injection of either NORNIC (5 mg/kg sc) or saline. The SCH23390 and eticlopride doses were chosen based on previous unpublished results from our laboratory showing that these doses did not significantly alter activity, whereas higher doses decreased activity. Immediately after the second injection, rats were placed into the activity chamber and data were collected for 60 min in 10-min bins. The injection and test regimen was repeated every 48 h for a total of seven tests. In order to determine if SCH23390 or eticlopride altered the initiation of sensitization, a final test (Session 8) was given in which all rats were pretreated with saline prior to an eighth injection of NORNIC or saline.

2.5.2. Data analyses

SCH23390- and eticlopride-pretreated rats were compared to saline-pretreated rats in separate mixed-factors ANOVAs. Pretreatment (SCH23390, eticlopride or saline) and treatment (NORNIC or saline) factors were between-subject variables. Two repeated-measures factors were included in the design: a time block factor for within-test analysis of activity (10-min blocks) and a test factor for between-test analysis of tolerance and sensitization (seven tests). As described previously, the temporal pattern of effect for NORNIC was evaluated using a priori pair-wise comparisons between groups at each time block. For data from Test 8, two separate three-factor mixed ANOVAs were conducted. Results were significant at $P < .05$ using a two-tailed test.

2.5.3. Results

2.5.3.1. Effect of SCH23390 or eticlopride on locomotor activity following acute NORNIC (Session 1). Fig. 2 (Panels a and b) illustrates the effect of SCH23390 (0.005

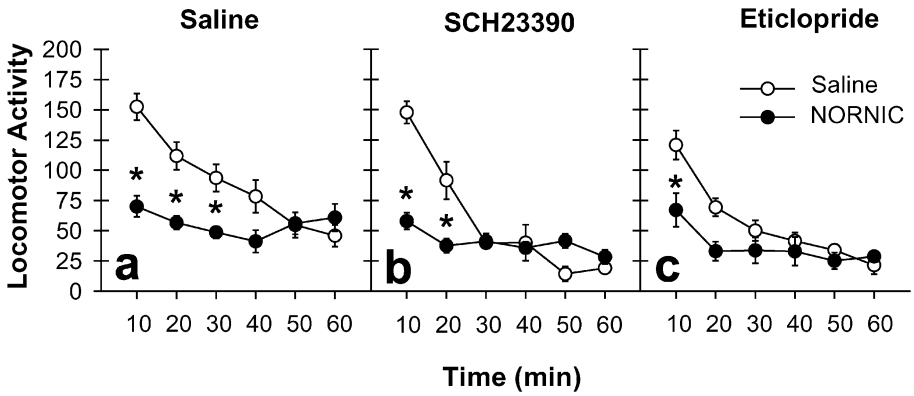

Fig. 2. Effect of acute NORNIC on locomotor activity in rats pretreated with (a) saline, (b) SCH23390 (0.005 mg/kg) or (c) eticlopride (0.02 mg/kg). Rats were pretreated in the home cage 15 min prior to administration of saline or NORNIC (5 mg/kg). Activity was measured for 60 min immediately following NORNIC injection. Data are expressed as the mean ± S.E.M. photocell interrupts during 10-min time blocks across 60-min sessions. Asterisk (*) denotes statistical difference from saline control. $N = 8$ per group.

mg/kg) on locomotor activity following acute NORNIC (Session 1). A three-factor ANOVA revealed significant main effects of time block [$F(5,140) = 47.4$, $P < .001$], SCH23390 pretreatment [$F(1,28) = 14.3$, $P < .001$] and NORNIC treatment [$F(1,28) = 18.4$, $P < .001$]. The SCH23390 and NORNIC main effects indicated that rats receiving either of these drugs showed significantly less activity than the corresponding saline control rats across the duration of the session. Although the SCH23390 × NORNIC interaction was not significant, the Time Block - × NORNIC interaction was significant [$F(5,140) = 26.8$, $P < .001$]. A priori pair-wise comparisons at each time block revealed significant decreases in activity in response to NORNIC treatment during the 10–30-min time blocks for rats pretreated with saline, and the 10- and 20-min time blocks for rats pretreated with SCH23390.

Fig. 2 (Panels a and c) also depicts the effect of eticlopride (0.02 mg/kg) on locomotor activity following acute NORNIC (Session 1). A three-factor ANOVA of these data revealed main effects of time block [$F(5,140) = 40.2$, $P < .001$], NORNIC [$F(1,28) = 16.2$, $P < .001$] and eticlopride pretreatment [$F(1,28) = 15.6$, $P < .001$]. Injections of either NORNIC or eticlopride decreased locomotor activity. In addition to the main effects, the Time Block × NORNIC interaction was significant [$F(5,140) = 14.8$, $P < .001$], revealing that NORNIC decreased locomotor activity only during the early part of the session.

2.5.3.2. Effect of SCH23390 or eticlopride pretreatment on the locomotor response to repeated NORNIC. A four-factor ANOVA for SCH23390 and saline control data from Sessions 1 through 7 revealed main effects of session [$F(6,168) = 4.2$, $P < .001$], time block [$F(5,140) = 187.7$, $P < .001$] and SCH23390 pretreatment [$F(1,28) = 56.0$, $P < .001$]. Significant interactions included Time Block × NORNIC [$F(5,140) = 55.0$, $P < .001$], Session × NORNIC × SCH23390 Pretreatment [$F(6,168) = 2.3$, $P < .05$] and a four-way interaction [$F(30,840) = 1.8$, $P < .01$]. To aid in understanding the four-way interaction, activity was collapsed across the first and last 30 min of the sessions (Fig. 3, top and bottom panels, respectively). During the first 30 min of the session, NORNIC decreased activity on Sessions 1–3, but not on Sessions 4–7, indicating the development of tolerance to NORNIC-induced hypoactivity. During the latter 30 min of the session, NORNIC did not significantly alter activity on Sessions 1–4, but increased activity on Sessions 5–6, indicating the development of NORNIC-induced sensitization. Regardless of NORNIC treatment, SCH23390 (0.005 mg/kg) produced an overall decrease in activity across the 60-min session. However, SCH23390 did not block NORNIC-induced hypoactivity during the first 30 min or hyperactivity during the last 30 min (Fig. 3).

A four-factor ANOVA for eticlopride and control data from Sessions 1 through 7 revealed main effects of time block [$F(5,140) = 156.0$, $P < .001$], as well as eticlopride pretreatment [$F(1,28) = 100.3$, $P < .001$]. Significant interactions included the three-way Session × NORNIC × Eticlopride Pretreatment interaction [$F(6,168) = 2.712$, $P < .05$] and the three-way Time Block × NORNIC × Eticlopride interaction [$F(5,140) = 10.3$, $P < .001$]. The four-way interaction, however, was not significant [$F(30,840) = 1.04$, $P = .40$]. Eticlopride (0.02 mg/kg) produced an overall decrease in locomotor activity compared to saline control. More important, eticlopride did not alter the NORNIC-induced hypoactivity during the first 30 min, but did block completely the NORNIC-induced sensitization that developed during the last 30 min across sessions (Fig. 3).

2.5.3.3. Effect of repeated SCH23390 and eticlopride on NORNIC-induced locomotor activity (Session 8). To determine if repeated pretreatment (Sessions 1–7) with either SCH23390 or eticlopride altered subsequent NORNIC-induced hyperactivity, data from the second 30 min of Session 8, in which none of the rats received SCH23390 or eticlopride, were analyzed. Analysis revealed a main effect of pretreatment [$F(2,42) = 11.8$, $P < .001$].

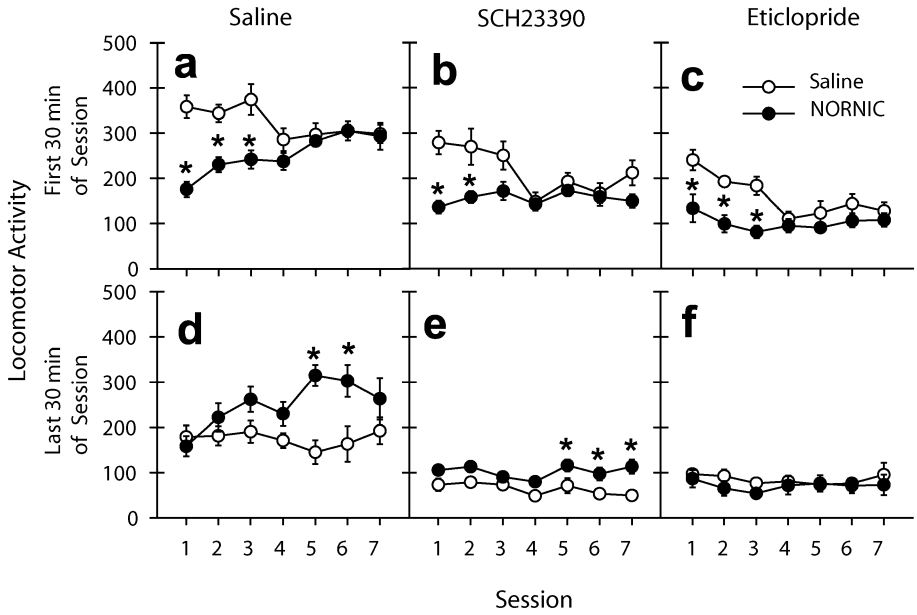

Fig. 3. Locomotor activity following pretreatment with saline (Panels a and d), SCH23390 (0.005 mg/kg; Panels b and e) or eticlopride (0.02 mg/kg; Panels c and f), followed by NORNIC on Sessions 1–7. Top panels illustrate activity during the first 30 min (Panels a–c) and bottom panels during the last 30 min (Panels d–f). Pretreatment was administered in the home cage 15 min prior to session. Saline or NORNIC (5 mg/kg) was injected immediately prior to of the session. Data are expressed as mean ± S.E.M. photocell interruptions during the respective 30-min period. Asterisk (*) denotes statistical difference from saline treatment control. $N = 8$ per group.

Regardless of NORNIC treatment, rats previously pretreated with SCH23390 exhibited reduced activity compared to saline-pretreated animals. Furthermore, there was a main effect of NORNIC treatment [$F(1,42) = 5.4$, $P < .05$], indicating NORNIC-induced sensitization was still apparent. A priori comparisons revealed that rats given saline or SCH23390 displayed significant sensitization following NORNIC, whereas eticlopride-pretreated animals did not (Fig. 4).

2.6. Experiment 3: effect of NORNIC on DOPAC levels and DOPA accumulation

2.6.1. Drug treatment

To determine the effect of NORNIC on DOPAC concentrations, rats were injected subcutaneously with either NORNIC (5 or 10 mg/kg; $n = 10$ per group) or saline ($n = 10$). The high dose was added in the event that the neurochemistry procedures were less sensitive to NORNIC than the behavioral procedures. Thirty minutes after injection, rats were killed by rapid decapitation and brain regions were dissected for HPLC analysis.

To determine the effect of NORNIC on accumulation of DOPA following inhibition of DOPA decarboxylase activity, rats were injected IP with NSD-1015 (100 mg/kg) and, 15 min later, were injected subcutaneously with either NORNIC (5 or 10 mg/kg; $n = 7$ and 8, respectively) or saline ($n = 7$). Thirty minutes after injection, rats were killed by rapid decapitation and brain regions were dissected for HPLC analysis.

2.6.2. DA, DOPAC and DOPA assay procedure

Medial prefrontal cortex (PFC) and striatum (STR) were stored in 10 volumes of 0.1 N perchloric acid at −70 °C until assay. Olfactory tubercles (OT) and NAcc were stored

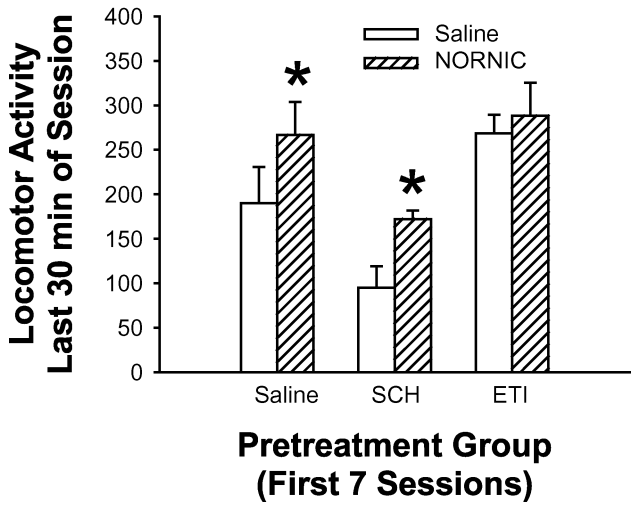

Fig. 4. Effect of previous pretreatment with saline, SCH23390 (SCH) or eticlopride (ETI) on NORNIC-induced hyperactivity. Saline, SCH and ETI groups refer to previous pretreatments on Sessions 1–7. All rats received saline pretreatment on Session 8 and either saline or NORNIC (5 mg/kg) injection immediately prior to Session 8. Data are expressed as mean ± S.E.M. photocell interruptions during the last 30 min of the session. Asterisk (*) denotes significant difference from saline control. $N = 8$ per group.

in 20 volumes of 0.1 N perchloric acid at −70 °C until assay. Samples were thawed on ice, sonicated and centrifuged at 30,000 × *g* for 15 min at 4 °C. For each sample, 20 μl of the supernatant was assayed using the HPLC system described previously.

2.6.3. Data analysis

Data were analyzed with separate ANOVAs for the effects of NORNIC on DOPAC levels and DOPA accumulation. Based on the results of previous studies with NIC (Grenhoff and Svensson, 1988; Mitchell et al., 1989; Brazell et al., 1990; Vezina et al., 1992), directional hypotheses were tested with NORNIC; results were significant at $P < .05$ using one-tailed tests.

2.6.4. Results

2.6.4.1. Effect of acute NORNIC on DOPAC levels. Acute NORNIC produced a dose-dependent increase in DOPAC concentrations in the NAcc [$F(2,30) = 4.05$, $P < .05$], STR [$F(2,30) = 4.32$, $P < .05$], OT [$F(2,30) = 21.33$, $P < .001$] and PFC [$F(2,30) = 2.72$, $P < .05$]. The high dose of NORNIC (10 mg/kg) significantly increased DOPAC levels in all areas analyzed, while the lower dose of NORNIC (5 mg/kg) increased DOPAC levels only in the mesolimbic terminal regions of the NAcc and the OT (Fig. 5).

2.6.4.2. Effect of acute NORNIC on DOPA accumulation following NSD-1015. There was a significant main effect of NORNIC treatment on DOPA accumulation in the NAcc [$F(2,22) = 2.86$, $P < .05$] and OT [$F(2,22) = 3.19$, $P < .05$]. The low dose of NORNIC (5 mg/kg) significantly increased DOPA accumulation in the OT and NAcc (Fig. 6). Interestingly, no increase in DOPA was evident following the high dose (10 mg/kg).

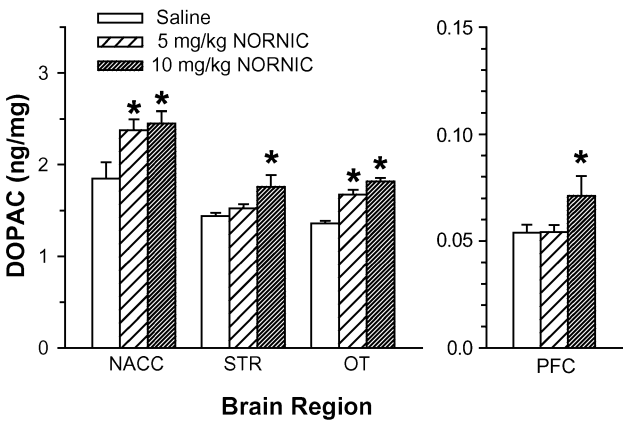

Fig. 5. Effect of NORNIC on DOPAC concentration in the OT, NAcc, STR and PFC. Drug-naive rats were injected subcutaneously with saline or NORNIC (5 or 10 mg/kg) and then were killed 30 min later. Data are expressed as mean ± S.E.M. ng/mg wet tissue weight. Asterisk (*) denotes significant difference compared to saline control rats. $N = 10$ per group.

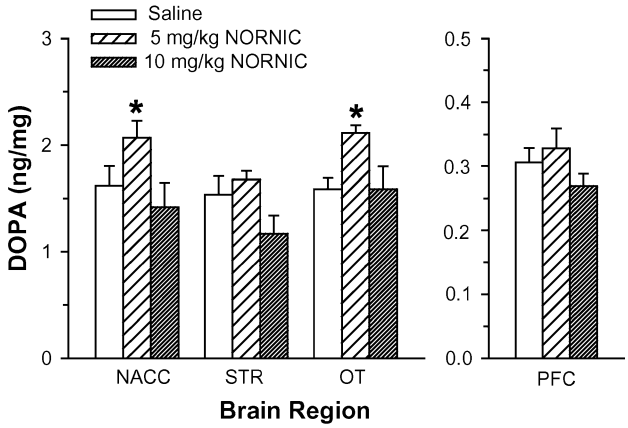

Fig. 6. Effect of NORNIC on DOPA accumulation in the OT, NAcc, STR and PFC. Drug-naive rats were injected subcutaneously with saline or NORNIC (5 or 10 mg/kg) 15 min before being treated with the DOPA decarboxylase inhibitor NSD-1015. Thirty minutes after NSD-1015, rats were killed and tissue dissected. Data are expressed as mean ± S.E.M. ng/mg wet tissue weight. Asterisk (*) denotes significant difference compared to saline control rats. $N = 7–8$ per group.

3. Discussion

As expected from previous work (Dwoskin et al., 1999), acute NORNIC produced an initial period of hypoactivity, followed by a period of hyperactivity. With repeated injections, tolerance developed to the hypoactivity and sensitization developed to the hyperactivity. The current study showed that a 6-OHDA lesion of the NAcc did not alter NORNIC-induced hypoactivity, but blocked the hyperactivity following acute administration, as well as sensitization following repeated administration. These results indicate that the NORNIC-induced hyperactivity, but not the hypoactivity, involves a mesolimbic DA mechanism. Furthermore, NORNIC induced hypoactivity was not altered by pretreatment with either SCH23390 or eticlopride. Sensitization to repeated NORNIC was blocked by eticlopride, but not SCH23390. Moreover, when tested in the absence of DA receptor antagonists, rats that previously received SCH23390 displayed NORNIC-induced hyperactivity, whereas rats previously receiving eticlopride did not, suggesting a D2 DA receptor-mediated mechanism in the development of NORNIC-induced sensitization. Acute NORNIC increased DA metabolism in both mesolimbic and nigrostriatal systems, but increased DA synthesis only in the mesolimbic system. Taken together, these results suggest that the locomotor stimulant effect of NORNIC is dependent upon activation of D2 DA receptors in the mesolimbic system.

When comparing the locomotor results of the present study with the previous results by Dwoskin et al. (1999), it

should be noted that the current study utilized (±)-NORNIC, whereas the previous work was done with the pure enantiomers. In vitro studies have shown that both enantiomers had similar affinity for the high-affinity nicotinic receptor (Reavill et al., 1988; Copeland et al., 1991; Zhang and Nordberg, 1993). However, the *R*(+)-enantiomer was more potent than the *S*(−)-enantiomer in an in vitro DA release assay using NAcc slices (Green et al., 2001), whereas the *S*(−)-enantiomer was more potent in STR slices (Teng et al., 1997). The current results with (±)-NORNIC do not address the relative contribution of the enantiomers to activate D2 DA receptors in the mesolimbic system.

Clarke et al. (1988) showed that 89% depletion of NAcc DA by a bilateral 6-OHDA lesion abolished the locomotor stimulant effect of NIC. In the present study, a 65% depletion of NAcc DA similarly abolished the locomotor stimulant effect of NORNIC. Thus, although the lesion was not extensive in the current study, evidence for a diminished NORNIC-induced hyperactivity was observed.

Previous research has shown that SCH23390 or the D2 receptor antagonist raclopride blocked the acute locomotor stimulant effect of NIC (O'Neil et al., 1991). In the present study, eticlopride, but not SCH23390, blocked the locomotor stimulant effect of acute and repeated NORNIC, as well as blocking the initiation of sensitization tested in the absence of eticlopride (Session 8). Taken together, these results suggest that while D2 DA receptors mediate the stimulant effects of both NIC and NORNIC, D1 DA receptors appear to play a role in the effect of NIC only.

With respect to the initial NORNIC-induced hypoactivity, neither the 6-OHDA lesion nor pharmacological antagonism of DA receptors attenuated the decrease in activity upon acute presentation of NORNIC. These findings are consistent with the NIC literature showing that SCH23390 and the D2 antagonist sulpiride failed to block NIC-induced hypoactivity (Damaj and Martin, 1993). Therefore, it appears that the hypoactivity induced by NORNIC involves a nonmesolimbic mechanism. This hypoactivity is likely due to effects on the interpeduncular nucleus of the ventral midbrain, as has been shown with nicotine (Hentall and Gollapudi, 1995).

The current locomotor studies did not employ a habituation phase prior to testing with NORNIC. Thus, one cannot rule out lesion and/or drug effects on novelty as a factor mediating the present results. However, more experiments would be needed to determine the potential interactions of novelty with NORNIC-induced locomotor activity.

Acute NIC increases DOPAC concentrations in DA rich terminal regions (Grenhoff and Svensson, 1988; Brazell et al., 1990; Vezina et al., 1992), presumably due to the NIC-induced increase in firing rate in ventral tegmental area and substantia nigra neurons (Grenhoff et al., 1986). NORNIC also increased DA metabolism in the present report; however, the effect of NORNIC has not been determined in electrophysiological studies. The NORNIC-induced increase in DOPAC was evident at the low dose (5 mg/kg) only in the mesolimbic terminal regions of the NAcc and OT, suggesting that the mesolimbic DA system is more sensitive to NORNIC compared with the mesocortical or nigrostriatal systems. These results parallel studies showing that NIC also has preferential effects on the mesolimbic DA system (Imperato et al., 1986; Pontieri et al., 1996).

DOPA accumulation in the presence of the DOPA decarboxylase inhibitor NSD-1015 reflects tyrosine hydroxylase activity. An increase in DOPA accumulation in the NAcc and OT was observed following an acute dose of NORNIC (5 mg/kg) shown to produce hyperactivity, consistent with an increase in impulse flow in the mesolimbic DA system after administration of NORNIC. Interestingly, the high NORNIC dose (10 mg/kg) did not increase DOPA accumulation, suggesting either inhibition of tyrosine hydroxylase directly or an indirect action on non-DA systems that regulate mesolimbic DA neuronal activity.

In conclusion, the current study indicates that the psychostimulant effect of NORNIC is mediated by D2 DA receptors in the mesolimbic system, consistent with the notion that these receptors play a vital role in the relapse to various drugs of abuse (Self and Nestler, 1998). Combined with other converging lines of evidence from behavioral studies (Goldberg et al., 1989; Bardo et al., 1999; Dwoskin et al., 1999) and pharmacological studies (Reavill et al., 1988; Copeland et al., 1991; Zhang and Nordberg, 1993; Crooks and Dwoskin, 1997; Green et al., 2001), the current results suggest further that NORNIC may contribute to tobacco dependence and relapse.

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