Stimulation of Serotonin$_{2C}$ Receptors Blocks the Hyperactivation of Midbrain Dopamine Neurons Induced by Nicotine Administration

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ABSTRACT

In vivo electrophysiological techniques were used to study the effect of nicotine on the basal activity of dopamine (DA)-containing neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) of chloral hydrate-anesthetized rats. Acute i.v. injections of nicotine (25–400 μg/kg) caused a dose-dependent increase of the firing rate and the bursting activity of DA neurons both in the SNc and the VTA. Repeated daily injection of nicotine (1 mg/kg i.p.) for 10 consecutive days did not cause any significant change in the basal activity of DA neurons in the SNc and the VTA. Acute challenge with nicotine (25–400 μg/kg i.v.) in animals treated repeatedly with this drug caused a dose-related excitation of DA neurons in both areas. To test the hypothesis that stimulation of 5-hydroxytryptamine (5-HT; serotonin)$_{2C}$ receptors could affect nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective 5-HT$_{2C}$ receptor agonist RO 60-0175 was used. Pretreatment with 100 μg/kg i.v. (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$ (RO 60-0175) prevented the enhancement of nicotine-induced stimulation of DA neuronal activity, the selective

Nicotine, the major psychoactive agent present in tobacco, acts as a potent addictive drug both in humans and laboratory animals whose locomotor activity is also stimulated (Reavill and Stolerman, 1990; Corrigall and Coen, 1991; Rose and Corrigall, 1997; Olausson et al., 1999; Laviolette and van der Kooy, 2003). A large body of evidence indicates that the locomotor activation and the reinforcing effects of nicotine may be related to its stimulatory effects on the mesolimbic dopaminergic function (Clarke et al., 1988; Reavill and Stolerman, 1990; Corrigall et al., 1992, 1994; Louis and Clarke, 1998; Olausson et al., 1999; Dani and De Biasi, 2001). Thus, it is now well established that nicotine can increase in vivo DA outflow in the nucleus accumbens (Di Chiara and Imperato, 1988; Dansma et al., 1989; Brazell et al., 1990; Nisell et al., 1994b; Pontieri et al., 1996; Maisonneuve et al., 1997; Marshall et al., 1997; Balfour et al., 1998; Schilström et al., 1998) and the corpus striatum (Imperato et al., 1986; Brazell et al., 1990; Toth et al., 1992; Marshall et al., 1997). The stimulatory effect of nicotine on DA release most probably results from its ability to excite neuronal firing rate and to increase bursting activity of DA neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) (Lichtensteiger et al., 1982; Clarke et al., 1985; Grendhoff et al., 1986; Mereu et al., 1987; Calabresi et al., 1989; Pidoplichko et al., 1997; Sorensen et al., 1998; Schilström et al., 2003) and from its stimulatory action on DA terminals in the corpus striatum and the nucleus accumbens (Mifsud et al., 1989; Rowell and Hillebrand, 1994; Marshall et al., 1997; Ferrari et al., 2002). The neurochemical data are consistent with neuroanatomical findings showing the presence of nicotinic acetylcholine receptors in the SNc, the VTA, and in projection areas of the central dopaminergic system such as the corpus striatum and the nucleus accumbens (Clarke and Pert, 1985; Clarke et al., 1985).

Several lines of evidence indicate that the reinforcing properties of drug of abuse, including nicotine, can be affected by several transmitter systems that may act by modulating central dopaminergic function. Among these, particularly important

ABBREVIATIONS: DA, dopamine; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; 5-HT, 5-hydroxytryptamine, serotonin; RO 60-0175, (S)-2-(chloro-5-fluoro-indo-l-yl)-l-methylethlyamine 1:1 C$_4$H$_4$O$_4$; SB 242084, 6-chloro-5-methyl-l-[2-(2-methylpyridiyl-3-oxy)-pyrid-5-yl carbomoyl] indoline; ANOVA, analysis of variance.
portant seems to be the role played by 5-hydroxytryptamine (5-HT, serotonin), which may prevalently act through the 5-HT$_{2C}$ receptor subtypes. In fact, the selective 5-HT$_{2C}$ receptor agonist (S)-2-(chloro-5-fluoro-indo-1-yl)-1-methyltryptamine 1:1 CH$_2$O (RO 60-0175) (Martin et al., 1998) was found to reduce the basal firing rate of DA neurons in the VTA (Di Matteo et al., 1999, 2000; Gobert et al., 2000), whereas 6-chloro-5-methyl-[2-(2-methylpyridylidin-3-oxo)-pyrid-5-yl carbomoyl] indoline (SB 242084), the most potent and selective 5-HT$_{2C}$ receptor antagonist available (Kennett et al., 1997), enhanced it (Di Matteo et al., 1999; Millan et al., 1998; Di Giovanni et al., 1999; Gobert et al., 2000). SB 242084 was also found to potentiate the phencyclidine-induced increase in accumbal DA release (Hutson et al., 2000) and stress-stimulated DA outflow in the rat prefrontal cortex (Pozzi et al., 2002), whereas stimulation of 5-HT$_{2C}$ receptors by RO 60-0175 in the VTA suppressed it (Pozzi et al., 2002), suggesting a role of these receptors in controlling evoked DA release also. Consistent with these findings, stimulation of central 5-HT$_{2C}$ receptors has been shown to inhibit morphine-induced increase in DA release in the nucleus accumbens of freely moving rats (Willins and Meltzer, 1998). In addition, it was found that RO 60-0175 reduced cocaine-reinforced behavior by stimulating 5-HT$_{2C}$ receptors (Grottick et al., 2000). Moreover, these studies showed that RO 60-0175 reduced ethanol- and nicotine-induced self-administration and hyperactivity (Grottick et al., 2001; Tomkins et al., 2002). Interestingly, RO 60-0175 not only reduced the operant responding for nicotine and the nicotine-induced hyperlocomotion in sensitized rats but also was capable of blocking the sensitization to nicotine, which occurs after repeated treatment with this drug (Grottick et al., 2001).

On the basis of the above-mentioned considerations, the effects of acute and repeated (10-day) administration of nicotine on the firing rate of DA-containing neurons was investigated by using single-cell extracellular recordings of neurochemically identified DA neurons in the SNc and the VTA of chloral hydrate-anesthetized rats. The effect of pretreatment with RO 60-0175 on nicotine-induced DA hyperactivity was also evaluated to test the possible modulation by 5-HT$_{2C}$ receptors of this action of nicotine. The present investigation was designed as an electrophysiological counterpart of Grottick’s et al. (2001) study, with the aim of elucidating the possible involvement of DA neuronal activity in the behavioral effects of RO 60-0175 against nicotine-induced hyperlocomotion and reward.

**Materials and Methods**

**Animals.** Male Sprague-Dawley rats (Consorzio Mario Negri Sud, Chieti, Italy), weighing 300 to 350 g, were used. Animals were kept at constant room temperature (21 ± 2°C) and relative humidity (60%) with a 12-h light/dark cycle (dark from 8:00 PM) and had free access to water and food. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, Feb. 18, 1992) and international laws and policies [EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; National Institutes of Health Guide for the Care and Use of Laboratory Animals, NIH Publication 85-23, 1985; and Guidelines for the Use of Animals in Biomedical Research, Thromb. Hemost. (1987) vol 58, pp 1078–1084]. All efforts were made to minimize animal suffering and to reduce the number of animals used.

**Single-Cell Recording Procedures.** Rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and mounted on a stereotaxic instrument (SR-6; Narishige, Tokyo, Japan). Supplemental doses of anesthetic were administered via a lateral tail vein cannula. Throughout the experiment, the animal’s body temperature was maintained at 36–37°C by a thermostatically regulated heating pad. The coordinates, relatively to the interaural line, for placement of the recording electrode in the areas studied were anterior, 2.7 to 3.4 mm; lateral, 1.8 to 2.2 mm; and ventral, 6.5 to 7.5 mm for the SNc; and anterior, 2.7 to 3.4 mm; lateral, 0.1 to 0.5 mm; and 7 to 8 mm ventral to the level of exposed tissue for the VTA (Paxinos and Watson, 1986). Extracellular recordings were performed using single-barrel micropipettes (4–7 MΩ resistance containing 2% pontamine sky blue dye in 2 M NaCl). DA neurons were identified by their location, waveform, firing rate, and pattern (Bunney et al., 1973; Grace and Bunney, 1980; Wang, 1981). Electrical signals of spike activity were passed through a high-impedance amplifier whose output was led into an analog oscilloscope, audio monitor, and window discrimina-

Unit activity was then converted to an integrated histogram by a rate-averaging computer and displayed as spikes per 10-s intervals.

After each experiment, the recording site was marked by the ejection of pontamine sky blue dye from the electrode using a ~20 μA current for 10 min. Brains were removed and placed in 10% buffered formalin for 2 days before histological examination. Frozen sections were cut at 40-μm intervals and stained with neutral red. Microscopic examination of the sections was carried out to verify that the electrode tip was in the SNc or the VTA.

**Drug Administration Protocols.** Nicotine and apomorphine were freshly diluted in physiological saline (0.9% NaCl). RO 60-0175 was dissolved in 200 μl of 10% acetic acid and made up to almost required volume with 0.9% saline brought to pH 6 with 2.5% NaOH. Nicotine (25–400 μg/kg) was administered i.v. (via a lateral tail vein) every 2 min in exponentially increasing doses, and the effect on the activity of DA neurons was recorded. Each dose of nicotine was dissolved in 100 μl of saline. The doses of nicotine were chosen on the basis of preliminary experiments carried out in our laboratory, which were also based on data published in the literature (Mereu et al., 1987). Control rats were treated with repeated injections (100 μl each) of saline every 2 min. The doses of RO 60-0175 (100 and 300 μg/kg i.v.) were chosen on the basis of previous studies carried out in our laboratory (Di Matteo et al., 1999, 2000). In chronic experiments, nicotine (1 mg/kg i.p.) was administered once daily for 10 consecutive days. The day after the last nicotine injection (i.e., 10th day), rats were given an acute challenge of nicotine (25–400 μg/kg i.v.), which was administered with the same regimen used for drug-naive aniamls. Both in acute and chronic experiments, some groups of rats were treated with RO 60-0175 (100 and 300 μg/kg i.v.) 5 to 10 min before acute nicotine or saline injections. In several instances, apomorphine (10–30 μg/kg i.v.) was given at the end of the experiment to confirm the dopaminergic identity of the neuron recorded. Only one cell per animal was studied.

**Data Analysis.** Data acquisition and analysis were accomplished using an 83386-based PC and an integrated software package for electrophysiology (RISI; Symbolic Logic, Dallas, TX). Dose-response curves were constructed by comparing the mean firing rate of 300 to 500 consecutive spikes, starting immediately after the injection of each drug, with the basal firing rate. Burst analysis of dopaminergic neurons was performed by using the RISI program running on a PC computer. A total of 300 to 500 consecutive spikes were recorded for each neuron before and at the peak of drug effect. Burst firing, when present, was detected using an algorithm similar to that previously described by Grace and Bunney (1984). The absolute change in the percentage of spikes occurring in bursts (i.e., the difference (Δ) between the percentage of spikes fired within bursts during the baseline period from the percentage of spikes fired within bursts after drug administration) was used as a measure of drug-induced changes in bursting. Inasmuch as burst firing values did not show a
normal distribution, they were analyzed by the nonparametric Mann-Whitney U test. Drug-induced changes in neuronal firing rate were analyzed by one- or two-way analysis of variance (ANOVA) with repeated measures followed by post hoc Tukey-Kramer tests, where appropriate. Differences between basal DA neuronal activity in rats treated acutely or repeatedly with nicotine were analyzed by Student's t test. All statistical analyses were performed with StatView version 5.0.1 (SAS Institute, Cary, NC).

Drugs. (-)-Nicotine hydrogen tartrate salt (nicotine) and apomorphine were from Sigma-Aldrich (St. Louis, MO). RO 60-0175 was kindly donated by Dr. Eva-Maria Gutknecht (F. Hoffmann-La Roche, Basel, Switzerland). All drugs dosages refer to the weight of the salt.

Results

Effect of RO 60-0175 on Acute Nicotine-Induced Excitation of DA-Containing Neurons in the SNc. Acute intravenous administration of nicotine (25–400 μg/kg) caused a dose-dependent increase in the firing rate (Fig. 1) and the bursting activity (Table 1) of DA-containing neurons in the SNc. As shown by the dose-response curve reported in Fig. 1, nicotine reached its maximal effect (+103 ± 21%, above baseline) at the cumulative dose of 775 μg/kg. Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; \( F_{1,10} = 16.94; p < 0.01; n = 7 \)) compared with the group treated with the vehicle of RO 60-0175 + saline (\( n = 5 \)). Pretreatment with RO 60-0175 (100 μg/kg i.v.), which did not cause any significant effect by itself (one-way ANOVA; \( F_{1,9} = 1.25; p = 0.29; n = 6 \)), completely prevented nicotine-induced increase in DA firing rate (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; \( F_{5,105} = 7.13; p < 0.01; n = 7 \) (Fig. 1). Thus, activation of 5-HT\(_{2C}\) receptors by RO 60-0175 was capable to counteract the activation of nigral DA neurons caused by acute administration of nicotine.

Effect of RO 60-0175 on Acute Nicotine-Induced Excitation of DA-Containing Neurons in the VTA. Acute

Fig. 1. Effect of nicotine (25–400 μg/kg i.v.) administration on the firing rate of DA-containing neurons in the SNc of drug-naive rats. Two groups of rats were treated with RO 60-0175 (100 μg/kg i.v.) 5 to 10 min before nicotine or saline injection. The control group was given the vehicle of RO 60-0175 + saline i.v. The control group was given the vehicle of RO 60-0175 + saline i.v. The left column shows representative rate histograms of the effects of i.v. nicotine (25, 50, 100, 200, and 400 μg/kg, at arrows), i.v. saline (100 μl, at each arrow), and pretreatment with i.v. RO 60-0175 (RO, 100 μg/kg, at arrow) on the basal firing rate of single DA neurons. APO, apomorphine administration (10 μg/kg i.v., at arrow). Top right reports the cumulative dose-response curve showing the mean percentage change (±S.E.M.) in firing rate after nicotine, saline, and RO 60-0175 + nicotine. Pretreatment indicates values recorded 2 to 3 min before either nicotine or saline administration. Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; \( F_{1,10} = 16.94; p < 0.01; n = 7 \)) compared with the group treated with the vehicle of RO 60-0175 + saline (\( n = 5 \)). Pretreatment with RO 60-0175 (100 μg/kg i.v.), which did not cause any significant effect by itself (one-way ANOVA; \( F_{1,10} = 1.25; p = 0.29; n = 6 \)), completely prevented nicotine-induced increase in DA firing rate (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; \( F_{5,105} = 7.13; p < 0.01 \) by Tukey-Kramer post hoc test).
treatment with nicotine (25–400 µg/kg i.v.) induced a drug-related increase in the firing activity of DA neurons in the VTA (Fig. 2). Also in the VTA, the maximal increase of the firing rate was reached at the cumulative dose of 775 µg/kg, which enhanced the activity of DA neurons by 70 ± 10%

TABLE 1

Effects of nicotine and RO 60-0175 on the firing pattern of SNc and VTA dopaminergic neurons of drug-naive rats

<table>
<thead>
<tr>
<th></th>
<th>SNc</th>
<th>VTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + saline</td>
<td>0.38 ± 0.23</td>
<td>2.97 ± 2.50</td>
</tr>
<tr>
<td>Vehicle + nicotine</td>
<td>17.14 ± 6.31*</td>
<td>22.95 ± 7.73*</td>
</tr>
<tr>
<td>RO 60-0175 100 µg/kg + saline</td>
<td>0.48 ± 1.03</td>
<td>–2.80 ± 2.09</td>
</tr>
<tr>
<td>RO 60-0175 100 µg/kg + nicotine</td>
<td>13.55 ± 6.79</td>
<td>8.64 ± 3.35</td>
</tr>
<tr>
<td>RO 60-0175 300 µg/kg + saline</td>
<td>2.04 ± 6.04</td>
<td></td>
</tr>
<tr>
<td>RO 60-0175 300 µg/kg + nicotine</td>
<td>–5.07 ± 5.69</td>
<td></td>
</tr>
</tbody>
</table>

*p < 0.05 compared with vehicle + saline group (Mann-Whitney U test).

(above baseline) (Fig. 2). Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; F_{1,10} = 20.96; p < 0.01; n = 8) compared with the group treated with the vehicle of RO 60-0175 + saline (n = 4). Pretreatment with 100 µg/kg i.v. RO 60-0175 did not cause any significant effect by itself (one-way ANOVA; F_{1,9} = 3.98; p = 0.07; n = 7) and did not significantly affect nicotine-induced increase in DA firing rate (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; F_{5,120} = 1.46; p = 0.20; n = 9) (Fig. 2). However, RO 60-0175 at the dose of 300 µg/kg i.v., which did not cause any significant effect by itself (one-way ANOVA; F_{1,8} = 1.22; p = 0.30; n = 5), significantly reduced nicotine-induced increase in DA firing rate (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; F_{5,100} = 5.40; p < 0.01; n = 6) (Fig. 3). Moreover, nicotine significantly increased the percentage of spikes occurring in bursts of VTA DA neurons (Table 1). Pretreatment with RO 60-0175 (100 and 300 µg/kg i.v.) reduced the nicotine effect on bursting activity (Table 1).

![Figure 2](image-url)
Effect of RO 60-0175 on Nicotine-Induced Excitation of SNc DA Neurons in Rats Treated Repeatedly with Nicotine.

Repeated treatment with nicotine for 10 consecutive days (1 mg/kg i.p.) did not cause any significant change in the basal firing rate of SNc DA neurons (data not shown). Acute challenge with nicotine (25–400 μg/kg i.v.) after repeated nicotine administration caused a dose-dependent increase in the firing rate of DA neurons in the SNc, which reached a peak of 47 ± 5% (above baseline) at the cumulative dose of 775 μg/kg (Fig. 4). The effect of acute nicotine challenge was evident and statistically significant (one-way ANOVA; F_{1,8} = 22.48; p < 0.01; n = 8) compared with the group treated with the vehicle of RO 60-0175 + saline (n = 5). Pretreatment with 300 μg/kg i.v. RO 60-0175, which did not cause any significant effect by itself (one-way ANOVA; F_{1,8} = 1.22; p = 0.30; n = 5), significantly reduced nicotine-induced increase in DA firing rate (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; F_{5,100} = 5.40; n = 6; *, p < 0.05; **, p < 0.01 by Tukey-Kramer post hoc test).

Effect of RO 60-0175 on Nicotine-Induced Excitation of VTA DA Neurons in Rats Treated Repeatedly with Nicotine. Repeated treatment with nicotine for 10 consecutive days (1 mg/kg i.p.) did not cause any significant change in the basal firing rate of SNc DA neurons (data not shown). Acute challenge with nicotine (25–400 μg/kg i.v.) after repeated nicotine administration caused a dose-dependent increase in the firing rate of DA neurons in the SNc, which reached a peak of 47 ± 5% (above baseline) at the cumulative dose of 775 μg/kg (Fig. 4). The effect of acute nicotine challenge was evident and statistically significant (one-way ANOVA; F_{1,8} = 11.38; p < 0.01; n = 5) compared with the group treated with the vehicle of RO 60-0175 + saline (n = 10). However, the peak excitatory effect on SNc DA neurons of rats treated repeatedly with this alkaloid was lower compared with that observed in drug naive animals (47 ± 5 versus 103 ± 20%). Statistical analysis of the dose-response curves revealed a significant difference between acute and chronic effects of nicotine (one-way ANOVA; F_{1,10} = 4.86; p < 0.05) (Fig. 4). Interestingly, acute nicotine challenge was unable to increase the bursting activity in animals treated repeatedly with nicotine (Table 2).

To test the effect of 5-HT_{2c} receptor activation in rats treated repeatedly with nicotine, RO 60-0175 (100 μg/kg i.v.) was given 5 to 10 min before the acute challenge with nicotine (25–400 μg/kg i.v.). Pretreatment with RO 60-0175, which did not alter basal DA firing rate by itself (one-way ANOVA; F_{1,21} = 0.29; p = 0.61; n = 10), partially but significantly attenuated the stimulatory effect of nicotine (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; F_{5,100} = 4.11; p < 0.01; n = 5) (Fig. 4).
Acute challenge with nicotine (25–400 μg/kg i.v.) after repeated administration caused a dose-dependent increase in the firing rate of DA neurons in the VTA, which reached a peak of 52% above baseline at the cumulative dose of 775 μg/kg (Fig. 5). The effect of acute nicotine challenge was evident and statistically significant (one-way ANOVA; \( F_{1,13} = 11.38; p < 0.01; n = 5 \)) compared with the group treated with the vehicle of RO 60-0175 saline (\( n = 5 \)). Pretreatment with RO 60-0175, which did not alter basal DA firing rate by itself (one-way ANOVA; \( F_{1,8} = 0.03; p = 0.85; n = 5 \)), did not significantly affect the stimulatory effect of nicotine (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; \( F_{5,105} = 4.11; n = 5 \); *, \( p < 0.05 \) by Tukey-Kramer post hoc test).

TABLE 2
Effects of nicotine and RO 60-0175 on the firing pattern of SNc and VTA dopaminergic neurons of rats treated repeatedly with nicotine for 10 days

<table>
<thead>
<tr>
<th>SNc</th>
<th>VTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle + saline</td>
<td>0.30 ± 2.40</td>
</tr>
<tr>
<td>Vehicle + nicotine</td>
<td>1.78 ± 1.43</td>
</tr>
<tr>
<td>RO 60-0175 100 μg/kg + saline</td>
<td>6.60 ± 2.68</td>
</tr>
<tr>
<td>RO 60-0175 100 μg/kg + nicotine</td>
<td>13.76 ± 8.35</td>
</tr>
<tr>
<td>RO 60-0175 300 μg/kg + saline</td>
<td>0.26 ± 1.79</td>
</tr>
<tr>
<td>RO 60-0175 300 μg/kg + nicotine</td>
<td>0.63 ± 1.79</td>
</tr>
</tbody>
</table>

*\( p < 0.05 \) compared with vehicle + saline group (Mann-Whitney \( U \) test).

To test the effect of 5-HT\(_{2A}\) receptor activation in rats treated repeatedly with nicotine, two doses of RO 60-0175 (100 and 300 μg/kg i.v.) were given 5 to 10 min before the acute challenge with nicotine (25–400 μg/kg i.v.). Pretreatment with 100 μg/kg i.v. RO 60-0175, which did not cause any significant change in the basal DA firing rate by itself (one-way ANOVA; \( F_{1,8} = 0.03; p = 0.85; n = 5 \)), did not significantly affect the excitatory effect of nicotine (25–400 μg/kg i.v.) administration on the firing rate of DA-containing neurons in the SNc of rats treated for 10 consecutive days with nicotine (1 mg/kg i.p.). The experiments were carried out 24 h after the last nicotine administration (day 11). Two groups of rats were treated with RO 60-0175 5 to 10 min before nicotine or saline injection. The control group was given the vehicle of RO 60-0175 + saline i.v. The left column shows representative rate histograms of the effects of i.v. nicotine (25, 50, 100, 200, and 400 μg/kg, at arrows), i.v. saline (100 μl, at each arrow), and pretreatment with i.v. RO 60-0175 (RO, 100 μg/kg, at arrow) on the basal firing rate of single DA neurons. APO, apomorphine administration (10 μg/kg i.v., at arrows). Top right reports the cumulative dose-response curve showing the mean percentage change (±S.E.M.) in firing rate after nicotine, saline, and RO 60-0175 nicotine. Predrug indicates values recorded 2 to 3 min before either nicotine or saline administration. Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; \( F_{1,8} = 11.38; p < 0.01; n = 5 \)) compared with the group treated with the vehicle of RO 60-0175 saline (\( n = 5 \)). Pretreatment with RO 60-0175, which did not alter basal DA firing rate by itself (one-way ANOVA; \( F_{1,13} = 0.29; p = 0.59; n = 10 \)), partially but significantly attenuated the stimulatory effect of nicotine (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; \( F_{5,105} = 4.11; n = 5 \); *, \( p < 0.05 \) by Tukey-Kramer post hoc test).
nicotine (two-way ANOVA; interaction RO 60-0175/nicotine dose; $F_{5.115} = 1.33; p = 0.25; n = 7$) (Fig. 5). On the other hand, RO 60-0175 at the dose of 300 $\mu$g/kg i.v., which did not modify DA neuronal basal activity by itself (one-way ANOVA; $F_{1.8} = 0.002; p = 0.97; n = 5$), significantly reduced the excitatory effect of nicotine (two-way ANOVA; interaction RO 60-0175/nicotine dose; $F_{5.128} = 7.70; p < 0.01; n = 6$) (Fig. 6). Acute nicotine challenge was also capable of significantly increasing the bursting activity of VTA DA neurons in rats treated repeatedly with nicotine (Table 2). Pretreatment with RO 60-0175 (100 and 300 $\mu$g/kg i.v.) counteracted the stimulatory effect of nicotine on bursting activity (Table 2).

**Discussion**

The present study shows that acute intravenous nicotine administration enhances the basal firing rate of DA-containing neurons in the SNc and the VTA of chloral hydrate-anesthetized rats. These findings are consistent with previous data reported in a number of studies showing that nicotine increases DA neuronal activity in both brain areas (Lichtensteiger et al., 1982; Clarke et al., 1985; Grenhoff et al., 1986; Mereu et al., 1987; Calabresi et al., 1989; Pidoplichko et al., 1997; Sorenson et al., 1998; Schilstrom et al., 2003). Moreover, nicotine significantly increased the bursting activity of DA neurons in the VTA and the SNc, as already reported in previous studies (Grenhoff et al., 1986; Schilstrom et al., 2003).

Nicotine can increase the firing rate and the bursting activity of SNc and VTA DA-containing neurons by several mechanisms, including a direct depolarizing effect mediated by the activation of somatodendritic nicotinic receptors (Calabresi et al., 1989; Pidoplichko et al., 1997; Sorenson et al., 1998; Schilstrom et al., 2003).
Moreover, nicotine can indirectly increase DA activity by eliciting the release of glutamate from nerve terminals synapsing on DA neurons and by depressing the inhibitory GABAergic input to these neurons (Mansvelder and McGehee, 2000, 2002; Mansvelder et al., 2002). In addition to its stimulatory effects on neuronal DA firing rate, nicotine has been shown to elicit DA release from DA terminals in the corpus striatum and the nucleus accumbens (Mifsud et al., 1989; Grady et al., 1994; Nisell et al., 1994a,b; Marshall et al., 1997; Ferrari et al., 2002). It seems, however, that the action of nicotine on terminal areas of the mesolimbic DA system is less relevant compared with the effect exerted by this drug on the VTA (Nisell et al., 1994a,b; Ferrari et al., 2002). Thus, it has been shown that intra-VTA infusion of nicotine causes a more robust and prolonged increase of accumbal DA release compared with intra-accumbens nicotine administration (Nisell et al., 1994b; Ferrari et al., 2002). These neurochemical findings are consistent with behavioral data showing that nicotine-stimulated locomotor activity and self-administration are mediated by a direct action of this drug on DA neurons in the VTA (Reavill and Stolerman, 1990; Corrigall et al., 1994).

Repeated administration of nicotine for 10 consecutive days, a procedure previously used for behavioral studies (Grottick et al., 2001), did not cause any significant changes in the basal electrical activity of DA neurons either in the SNc or the VTA but significantly reduced the excitatory effect of an acute challenge administration of nicotine in the SNc but not in the VTA. In addition, acute challenge with nicotine in rats treated repeatedly with this drug was still capable of significantly increasing bursting activity of DA neurons in the SNc. However, 10 consecutive days of nicotine administration reduced the excitatory effect of nicotine challenge in the SNc (Reavill and Stolerman, 1990; Corrigall et al., 1994).

**Fig. 6.** Effect of nicotine (25–400 μg/kg i.v.) administration on the firing rate of DA-containing neurons in the VTA of rats treated for 10 consecutive days with nicotine (1 mg/kg i.p.). The experiments were carried out 24 h after the last nicotine administration (day 11). Two groups of rats were treated with RO 60-0175 5 to 10 min before nicotine or saline injection. The control group was given the vehicle of RO 60-0175 + saline i.v. The left column shows representative rate histograms of the effects of i.v. nicotine (25, 50, 100, 200, and 400 μg/kg, at arrows), i.v. saline (100 μl, at each arrow), and pretreatment with i.v. RO 60-0175 (RO, 300 μg/kg, at arrow) on the basal firing rate of single DA neurons. APO, apomorphine administration (10 μg/kg i.v., at arrows). Top right reports the cumulative dose-response curve showing the mean percentage change (±S.E.M.) in firing rate after nicotine, saline, and RO 60-0175 + nicotine. Predrug indicates values recorded 2 to 3 min before either nicotine or saline administration. Statistical analysis revealed a significant effect of nicotine (one-way ANOVA; $F_{1,15} = 18.47; p < 0.01; n = 12$) compared with the group treated with the vehicle of RO 60-0175 + saline ($n = 5$). Pretreatment with 300 μg/kg i.v. RO 60-0175, which did not modify DA neuronal basal activity by itself (one-way ANOVA; $F_{1,6} = 0.002; p = 0.97; n = 5$), significantly reduced the excitatory effect of nicotine (two-way ANOVA; interaction RO 60-0175 × nicotine × dose; $F_{5,120} = 7.70; n = 6$; **, $p < 0.01$ by Tukey-Kramer post hoc test).
the VTA but not in the SNc. These data are consistent with the hypothesis that tolerance to the stimulatory effect of nicotine occurs in the nigrostriatal but not in the mesolimbic DAergic system after repeated nicotine administration. Indeed, the mesolimbic DA system undergoes sensitization after repeated nicotine exposure (Benwell and Balfour, 1992; Olausson et al., 1999; Grottick et al., 2001; Shim et al., 2001; Schoffelmeer et al., 2002), a phenomenon that is common to psychostimulant drugs (White and Kalivas, 1998; Vandenschuren et al., 1999; Robinson and Berridge, 2001; Schoffelmeer et al., 2002), and is opposite to that of tolerance. The major aim of the present study was to demonstrate the possible modulation of nicotine-induced excitation of midbrain DA neurons by RO 60-0175, a selective agonist of 5-HT2c receptors (Martin et al., 1998). The choice of using a 5-HT2c receptor agonist was based on a series of studies conducted in our laboratory showing that 5-HT2c receptors have a prominent role in the control of central DA function (Prisco et al., 1999; Di Giovanni et al., 1999; Di Matteo et al., 2001). As reported under Results, the dose of 100 µg/kg RO 60-0175 was capable of preventing the increase in DA neuronal firing rate induced by nicotine administration (both in drug naive and chronically treated rats) in the SNc but not in the VTA. On the other hand, 300 µg/kg RO 60-0175 prevented the enhancement of VTA DA neuronal activity induced by nicotine administration both in drug naive and chronically treated rats. Moreover, RO 60-0175 prevented nicotine-induced increase in bursting activity in the VTA. Because there is evidence that the effects of RO 60-0175, at the doses used in the present study, are completely blocked by SB 242084 (Di Matteo et al., 2000), it is possible to conclude that selective activation of 5-HT2c receptors can counteract the stimulation of DA neuronal activity elicited by nicotine in the SNc and the VTA. It is thus conceivable that the blockade by RO 60-0175 of the hyperlocomotive and rewarding effects of nicotine (Grottick et al., 2001) are most probably mediated by its ability to inhibit mesolimbic DA function. However, the higher sensitivity of the nigrostriatal DA system to the inhibitory effect of 5-HT2c receptor stimulation seems to contradict previous data obtained in our laboratory showing a preferential effect of RO 60-0175 on the basal function of the mesolimbic DA system (Di Matteo et al., 1999, 2001). Nevertheless, it is important to point out that in the present study both the nigrostriatal and mesolimbic DA systems were activated by nicotine, and it is conceivable that the modulation of the activity of these systems by 5-HT2c receptors might be state-dependent.

The effects of RO 60-0175 on nicotine-induced DA increase in midbrain DA neuronal activity are consistent with previous data showing that the unselective 5-HT2 receptor agonist (+)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane can reduce nicotine-stimulated locomotor activity and accumbal DA release in nicotine-sensitized rats (Olausson et al., 2001, 2002). Moreover, repeated administration of the selective serotonin reuptake inhibitor citalopram counteracts the expression of nicotine-induced locomotor sensitization (Olausson et al., 1999). It thus seems that activation of the serotonergic system can oppose both the development and expression of sensitization of mesolimbic DA system after repeated nicotine exposure. This action can ultimately lead to the extinction of nicotine-induced reward, an effect that can be eventually exploited for therapeutical purposes. In this respect, it is interesting to note that bupropion, which is an effective drug in the treatment of nicotine dependence (Jorenby et al., 1999), causes a sustained increase in the basal firing activity of 5-HT neurons in the dorsal raphe nucleus (Dong and Blier, 2001). That blockade or reduction of nicotine-induced hyperfunction of mesolimbic DA system might be a useful approach in reducing nicotine reward and eventually helping in smoking cessation in human subjects is further indicated by the evidence that ibogaine, an alkaloid found in Tabernanthe iboga, which is claimed to decrease smoking, attenuates the increase in DA release elicited by nicotine administration in the nucleus accumbens of rats (Maisonneuve et al., 1997). Moreover, there is evidence that γ-vinyl GABA, which increases endogenous GABA, reduces nicotine-stimulated DA overflow in the nucleus accumbens and blocks the acquisition and expression of place preference induced by nicotine (Dewey et al., 1999). Consistent with these data, it also has been found that muscimol and baclofen, two GABA agonists, significantly reduce nicotine self-administration when infused into the VTA (Corrigall et al., 2000). It is thus tempting to speculate that the inhibitory action of RO 60-0175 on nicotine-induced DA increase in neuronal activity might be mediated, at least in part, by stimulation of GABAergic neurons impinging on DA-containing neurons in the SN pars reticulata and in the VTA, an effect that was previously associated with activation of 5-HT2c receptors (Di Matteo et al., 2001).

In conclusion, selective activation of 5-HT2c receptors by RO 60-0175 significantly attenuates nicotine-induced stimulation of DA neuronal activity in the SNc and the VTA. The inhibitory effect of RO 60-0175 on nicotine-stimulated hyperfunction of mesolimbic DA system may explain its reported antiaddictive effects toward nicotine and other drugs of abuse and suggests that it might be useful as a smoking cessation aid.

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