Pharmacological Characterization of Nicotine’s Interaction with Cocaine and Cocaine Analogs

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ABSTRACT

Cocaine and a number of 3β-phenyltropane cocaine analogs were investigated for their potential to block various pharmacological effects of nicotine in animals. They blocked the antinociceptive effect of nicotine in the tail-flick test after systemic administration in a dose-dependent manner. Similarly, cocaine was also able to block nicotine-induced motor impairment in mice. Furthermore, cocaine blocked nicotine-induced seizures at a lower potency than for antinociception, but failed to block nicotine’s effect on body temperature and drug discrimination. The antagonistic potencies of the 3β-phenyltropane cocaine analogs were not correlated with their affinity for monoamine transporters. Additionally, bupropion, nomifensin, GBR 12909, and nisoxetine, but not methylphenidate and fluoxetine, blocked nicotine-induced antinociception; however, their antagonistic potencies were unrelated to their affinities for the transporters. Taken together, these results suggest that the mechanism of cocaine’s antagonistic activity is not related to its binding and uptake of inhibition on monoamine neurotransmitters. The failure of lidocaine and procaine to antagonize nicotine’s effects in the tail-flick assay rules out local anesthetic effects. In addition, cocaine blocked differentially the response of nicotine in the oocyte receptor expression system for the α4β2 and α5β2 subtypes in a dose-dependent manner. Our results suggest that cocaine is a noncompetitive nicotinic antagonist with some selectivity for neuronal nicotinic receptor subtypes. Our studies also demonstrate that 3β-phenyltropane analogs constitute a new class of nicotinic antagonists. Elicitation of the mechanism of action of this new class of antagonists may provide an explanation for the effectiveness of agents such as bupropion for the treatment of smoking cessation.

Nicotine produces a myriad of behavioral effects and is unquestionably one of the most abused reinforcing agents. This agent acts at the neuromuscular junction, at autonomic ganglia, and in the brain. A large body of evidence implicates nicotine’s action on the central nervous system as the primary determinant for tobacco addiction. Although a large number of drug abusers smoke tobacco, potential interactions between nicotine and other drugs of abuse, such as cocaine, remains mostly unknown. Epidemiological studies suggest that smoking increases the intake of cocaine and the vice versa, cocaine users consume more cigarettes than nonusers (Higgins et al., 1994). However, little is known about mechanisms that would support such interactions. Major pharmacological actions of cocaine include inhibition of neuronal synaptic reuptake of dopamine, serotonin, and norepinephrine, as well as local anesthetic actions (Kuhar et al., 1991). The site associated with dopamine neuronal transporter has been implicated most frequently in causing the reinforcing properties of cocaine (Kuhar et al., 1991). Recent reports suggested that a synergistic action of nicotine and cocaine on the neuronal mesolimbic dopamine system may explain the enhancement between the two drugs (Horger et al., 1992; Zernig et al., 1997). In contrast to possible synergistic effects of cocaine and nicotine, Lerner-Marmarosh et al. (1995) observed that a number of synthetic cocaine analogs were effective in blocking nicotine-induced seizures in mice and that a good correlation was observed between pharmacological potencies and [3H]mecamylamine binding to brain membranes. Thus, it was concluded that cocaine and cocaine analogs are neuronal nicotinic antagonists acting on a similar site to that of mecamylamine, a noncompetitive nicotinic antagonist. Cocaine is structurally similar to other noncompetitive antagonists of muscle nicotinic receptors including local anesthetics such as procaine and QX-222 (Leonard et al., 1995). In addition, cocaine has been also shown to inhibit the ion flux through nicotinic receptors on the neuromuscular junction and sympathetic ganglia (Swanson and Albuquerque, 1987; Lu and Bieger, 1996). Neuronal nicotinic receptors are of particular interest because they are critical sites at which acetylcholine must act to excite the brain. These receptors are of particular interest with respect to memory.

ABBREVIATIONS: AD50, antagonist dose 50%; DHβE, dihydro-β-erythroidine.

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(Court et al., 1992) and are likely sites at which nicotine exerts its psychoactive and addictive effects. Therefore, the interaction between nicotine and cocaine deserves serious consideration with respect to the physiological and pharmacological importance of nicotinic receptors and the possibility of providing a conceptual basis for the development of new nicotinic antagonists. Moreover, it would be important to establish what role, if any, neurotransporters might play in the actions of nicotine.

In the present study, we examined the mechanisms of the cocaine-nicotine interaction using various in vitro and in vivo assays. For that, the blockade potency of a number of cocaine and 3β-phenyltropane cocaine analogs on various pharmacological effects of nicotine (antinociception, hypothermia, seizures, drug discrimination, and motor impairment) in animals was examined and correlated with their affinity to different neurotransmitter transporters. Such a wide range of nicotinic effects is important to consider, because it is believed that various nicotinic receptor subtypes mediate different pharmacological effects of nicotine. In addition, a number of neurotransporter blockers, central nervous system stimulants, and local anesthetics were evaluated as potential antagonists of nicotine-induced antinociception. Using the oocyte expression system, the effects of cocaine on the activity of α3β2- and α3β2-expressed receptors, neuronal nicotinic receptor subtypes, were also studied. Finally, the effect of nicotinic antagonists on cocaine’s pharmacological effect was also investigated.

Materials and Methods

Animals

Male ICR mice (20–25 g) and male Sprague-Dawley rats (175–225 g) obtained from Harlan Laboratories (Indianapolis, IN) were used throughout the study. The mice were housed in groups of six and had free access to food and water. The rats were housed individually and had restricted access to food as described later.

Drugs

(−)-Nicotine was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI) and converted to the ditartrate salt as described by Aceto et al. (1979). Dihydro-β-erythroidine, fluoxetine, nomifensine, GBR 12909, lidocaine, amphetamine, caffeine, and nisoxetine were purchased from Research Biochemicals Inc. (Natick, MA). Mecamylamine hydrochloride was a gift from Merck, Sharp and Dohme & Co. (West Point, PA). Procaine was purchased from Sigma Chemical Co. (St. Louis, MO). Cocaine HCl, cocaine methiodide, methamphetamine, and methyleneblue were supplied by the National Institute on Drug Abuse (Washington, DC). The cocaine analogs used in the present study were various carboxylic acid esters of substituted phenyltropanes (Carroll et al., 1991, 1992; Lewin et al., 1992). All drugs were dissolved in physiological saline (0.9% sodium chloride) and given in a total volume of 0.2 ml/100 g b.wt. in rats and 1 ml/100 g b.wt. in mice for s.c. and i.p. injections. Cocaine HCl and cocaine methiodide were administered i.p. to animals. All doses are expressed as the free base of the drug.

Behavioral and Pharmacological Assays in Mice

Locomotor Activity. Mice were placed into individual Omnitech photocell activity cages (28 × 16.5 cm) 10 min after i.p. administration of either 0.9% saline or cocaine. Interruptions of the photocell beams (two banks of eight cells each) were then recorded for the next 30 min. Data were expressed as number of photocell interruptions. For antagonism studies, the mice were pretreated s.c. with either saline, dihydro-β-erythroidine, or mecamylamine 10 min before cocaine.

Antinociception. The tail-flick method of D’Amour and Smith (1941) as modified by Dewey et al. (1970) was used. A control response (2–4 s) was determined for each animal before treatment, and a test latency was determined after drug administration. To minimize tissue damage, a maximum latency of 10 s was imposed. Antinociceptive response was calculated as percent maximum possible effect (% MPE), where %MPE = [(test-control)/(10-control)] × 100]. Groups of 8 to 12 animals were used for each dose and for each treatment. Mice were tested 5 min after nicotine administration for the dose-response evaluation. Antagonism studies were carried out by pretreating the mice s.c. with either saline or various drugs at different times before nicotine. The animals were tested 5 min after administration of nicotine.

Body Temperature. Rectal temperature was measured by a thermost probe (inserted 24 mm) and digital thermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Readings were taken just before and at 30 min after the s.c. injection of nicotine. For antagonism studies, mice were pretreated with either saline or various drugs 10 min before nicotine. The difference in rectal temperature before and after treatment was calculated for each mouse. The ambient temperature of the laboratory varied from 21 to 24°C from day to day.

Motor Coordination. To measure motor coordination, a wooden rod 6 cm in diameter was partitioned into three compartments by circular metal discs (28 cm in diameter) at 16-cm intervals. The rod was attached to a motor and rotated at a rate of 4 rpm. Naive mice were trained until they could remain on the rotarod for 3 min. Animals that failed to meet this criterion within 5 trials were discarded. This training took place no longer than 15 min before the s.c. administration of nicotine. Twenty minutes after the injection, mice were placed on the rotarod for 5 min. The amount of time the animals remained on the rotarod was recorded and percent impairment was calculated as % Impairment = [(1-test time in s/300)] × 100]. An impairment value of 0% corresponds to the subjects that remained on the rotarod for 5 min (300 s), whereas 100% impairment corresponds to subjects that fell off the rotarod immediately.

Seizure Activity. Following s.c. injection of nicotine at a dose of 9 mg/kg, each animal was placed in a 30 cm × 30 cm Plexiglas cage and observed for 5 min. Whether a clonic seizure occurred within a 5-min time period was noted for each animal after s.c. administration of different drugs. This amount of time was chosen because seizures occur very quickly after nicotine administration. Results are expressed as percentage seizure. Antagonism studies were carried out by pretreating the mice i.p. with either saline or cocaine 5 min before nicotine.

Nicotine Drug Discrimination in Rats

Rats were individually housed in a temperature-controlled environment and were maintained on a diet (Agway Rodent Chow) that restricted their body weight to approximately 85% of their free feeding weight. Water was available ad libitum in the home cages. A two-lever operant drug-discrimination paradigm (VI 15) was carried out in eight operant chambers (4 Lafayette model 80001 and 4 BRS/LVE model s 002). Reinforcement was a Bioserv 45-mg precision dustless pellet. Data were collected automatically by two Commodore 64 microcomputers.

Rats were trained to respond on one lever after a s.c. injection of (−)-nicotine (0.4 mg/kg) and another lever after a s.c. injection of saline. Rats were placed in an operant chamber 5 min after injections. The specific procedure for training rats to discriminate between nicotine and saline has been described previously (Rosecrans, 1989). Animals were required to meet a criterion of three successive days of 80% or greater correct-lever responding before testing was initiated. Injections were given 5 min before placing the animal in the operant chamber. The schedule of injections was determined using a Latin Square design. Dose-response curves were determined
for nicotine 5 min after s.c. injections. For antagonism testing, animals were assessed for the behavioral effects of cocaine in conjunction with the training dose of nicotine. Cocaine was administered 10 min before the injection of (∼)-nicotine.

### Oocyte Expression Studies

**Oocyte Preparation.** Oocyte preparation was performed according to the method of Mirshahi and Woodward (1995) with minor modifications. Briefly, oocytes were isolated from female adult oocyte-positive *Xenopus laevis* frogs. Frogs were anesthetized in a 0.2% 3-aminobenzoic acid ethyl ester solution (Sigma Chemical Co.) for 30 min and a fraction of the ovarian lobes were removed. The eggs were rinsed in Ca²⁺-free ND96 solution, treated with collagenase type IA (Sigma Chemical Co.) for 1 h to remove the follicle layer, and then rinsed again. Healthy stage V-VI oocytes were selected and maintained for up to 14 days after surgery in 0.5× L-15 media.

**mRNA Preparation and Microinjection.** α₄, α₃, and β₂ rat subunit cDNA contained within a pcDNAIneo vector were kindly supplied by Dr. James Patrick (Baylor College of Medicine, Houston, TX). The template was linearized downstream of the coding sequence and mRNA was synthesized using an in vitro transcription kit from Ambion (Austin, TX). The quantity and quality of message were determined via optical density (spectrophotometer; Beckman Instruments Inc., Schaumburg, IL) and denaturing formaldehyde gel analysis. Oocytes were injected with either 51 ng (41 nl) of α₄ and β₂ and α₃ mRNA mixed in a 1:1 ratio using a Variable Nanject (Drummond Scientific Co., Broomall, PA). Oocytes were incubated in 0.5× L-15 media IA (Sigma Chemical Co.) supplemented with penicillin, streptomycin, and gentamicin for 4 to 6 days at 19°C before recording.

**Electrophysiological Recordings.** Oocytes were placed within a Plexiglas chamber (total volume 0.2 ml) and continually perfused (10 ml/min) with buffer consisting of 115 mM NaCl, 1.8 mM CaCl₂, 2.5 mM KCl, 1.0 μM atropine, and 10.0 mM HEPES at pH 7.2. Oocytes were impaled with two microelectrodes containing 3 M KCl (0.3–3 MΩ) and voltage-clamped at −70 mV using an Axon Geneclamp amplifier (Axon Instruments Inc., Foster City, CA). Oocytes were stimulated for 10 s with various concentrations of acetylcholine and nicotine using a six-port injection valve. Except where noted, applications were separated by 5-min periods of washout. Currents were filtered at 10 Hz and collected by a Macintosh Centris 650 with a 16-bit analog digital interface board, and data were analyzed using Pulse Control voltage-clamp software running under the Igor Pro graphic platform (Wavemetrics, Lake Oswego, OR). Drugs were applied at different concentrations and concentration-response curves were normalized to the current induced by 1 μM (α₄β₂ receptors) or 10 μM (α₃β₂ receptors) of acetylcholine. The normalizing concentration of acetylcholine was applied before and after drug application to each oocyte to check for desensitization. Data were rejected if responses to the normalizing dose fell below 75% of the original response.

### Statistical Analysis

Data were analyzed statistically by an analysis of variance followed by the Fisher’s P least-significant difference multiple comparison test. The null hypothesis was rejected at the 0.05 level. ED₅₀, EC₅₀, and AD₅₀ (antagonist dose 50%) values with 95% CIs were calculated by unweighted least-squares linear regression as described by Tallarida and Murray (1987).

### Results

**Effect of Cocaine Analogs on Nicotine-Induced Antinociception in Mice.** Cocaine and its derivatives, the structures of which are described in Fig. 1, were evaluated for their ability to antagonize a 2.5-mg/kg dose of nicotine in the tail-flick procedure. Cocaine as well as all of its analogs, with the exception of RTI-70, produced dose-dependent inhibition of nicotine’s antinociceptive effect. Their antagonistic potencies are presented in Table 1, and dose-response curves of cocaine and selected analogs are shown in Fig. 2. The latter demonstrates that the antinociceptive effects of nicotine can be completely blocked by these agents. By themselves, these analogs did not produce significant effects on tail-flick latencies at any of the doses tested.

In regard to the structure activity relationship of the 3-phenyltropane analogs, the effects of substitution on the aromatic ring, of changes in the 2β-substituent, and of removal of the N-methyl group were investigated. Because compounds RTI-29, -32, -51, -96, -111, and -112 differ only in their aromatic substituents, a comparison of the results from these compounds reveals the effect of these substituents. The 4-bromo analog (RTI-51) and the 3,4-dichloro analog (RTI-111) were approximately 3-fold and 2.5-fold, respectively, more potent than cocaine in blocking nicotine’s antinociceptive effect. The 4-methyl analog (RTI-32) was slightly more potent than cocaine, the 3-methyl-4-chloro analog (RTI-112) had approximately the same activity as cocaine, and the 4-aminoo analog (RTI-29) was one-half as potent as cocaine. RTI-120, which differs from RTI-32 by having a phenyl ester substituent in the 2-position, is only one-half as potent as RTI-32. The 2β-phenyl ester (RTI-113), which also has a 4-chloro substituent, was even less potent. In contrast, RTI-121, which is a 2β-isopropyl ester possessing a 4-iodo substituent, was about one-half as potent as cocaine. The 2β-pyrrolidinoamide analog (RTI-147), which has a 4-chloro substituent, was about one-half as potent as cocaine, whereas the 2β-pyrrolidinoamide (RTI-229), which has a 4-iodo substituent, possessed about the same potency as cocaine. The nortropane analog (RTI-110) was 3-fold more potent than cocaine. The 2-carboxy analog (RTI-70) and the 2α analog (RTI-258) were both much less potent than cocaine. WIN
35,065-2, which differs structurally from cocaine by having the aromatic ring connected directly to the 3-position of the tropane ring, was 2.5-times less potent than cocaine. However, the addition of substituents to the aromatic ring of WIN 35,065-2 led to compounds with increased potency. A comparison of the potency of WIN 35,065-2 to those of RTI-29, -31, -32, -51, -55, -111, and -112, which differ only in their aromatic substituents, reveals the effect of these substituents. The 4-iodo analog (RTI-55), the 4-bromo analog (RTI-51), the 3,4-dichloro analog (RTI-111), and the 4-methyl analog (RTI-32) were approximately 9- to 4-fold more potent than the unsubstituted analog WIN 35,065-2 in blocking nicotine’s antinociceptive effect. The 4-amino analog (RTI-29) had approximately the same activity as WIN 35,065-2.

As mentioned above, cocaine dose-dependently blocked nicotine-induced antinociception with an AD$_{50}$ of 3.2 µmol/kg (1 mg/kg). In addition, the dose-response curve of nicotine-induced antinociception was shifted to the right by cocaine (5 mg/kg) (Fig. 3), and the ED$_{50}$ value of nicotine was increased from 1.5 mg/kg (0.8–2.6) to 7.4 mg/kg (4.7–12.0).

To determine whether these cocaine analogs could be blocking nicotine’s effects through actions on neurotransmitters, their potency to inhibit dopamine, norepinephrine, or serotonin transporters was correlated with their antagonistic potency (Fig. 4). The rank-order analysis did not show any significant correlation between the potency of 3-b-phenyltropane cocaine analogs in blocking nicotine’s action and their affinity to the different transporters.

Pharmacological Interaction of Nicotine and Cocaine. To further characterize cocaine/nicotine interactions, additional experiments were conducted to determine whether cocaine would attenuate several of nicotine’s effects in a dose-responsive manner. Pretreatment with cocaine blocked the effect of a dose of 2.5 mg/kg of nicotine on the rotarod test in a dose-dependent manner (Fig. 5) with an AD$_{50}$ of 2 µmol/kg (0.7 mg/kg). By itself, cocaine did not significantly alter performance on the rotarod test. Cocaine was moderately effective in antagonizing nicotine-induced seizures in mice with an estimated AD$_{50}$ of 50 µmol/kg (Table 2, Seizure activity). However, cocaine failed to significantly

Table 1: Comparison of pharmacological potencies of tropane analogs in blocking nicotine-induced antinociception (tail-flick test) and hypothermia after systemic administration to their binding affinities to [3H]monoamine transporters in brain

<table>
<thead>
<tr>
<th>Analog</th>
<th>AD$_{50}$ Tail-Flick</th>
<th>AD$_{50}$ Hypothermia</th>
<th>Dopamine IC$_{50}$</th>
<th>Serotonin IC$_{50}$</th>
<th>Norepinephrine IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/kg</td>
<td>µmol/kg</td>
<td>mM</td>
<td>mM</td>
<td>mM</td>
</tr>
<tr>
<td>Cocaine</td>
<td>3.2</td>
<td>20% @ 32</td>
<td>102</td>
<td>1060</td>
<td>3830</td>
</tr>
<tr>
<td>RTI-29</td>
<td>6.8</td>
<td>NT</td>
<td>9.8</td>
<td>5110</td>
<td>151</td>
</tr>
<tr>
<td>RTI-31</td>
<td>7.2</td>
<td>1.1</td>
<td>1.12</td>
<td>44.5</td>
<td>37</td>
</tr>
<tr>
<td>RTI-32</td>
<td>2.0</td>
<td>31.7</td>
<td>1.7</td>
<td>240</td>
<td>60</td>
</tr>
<tr>
<td>RTI-51</td>
<td>1.0</td>
<td>NT</td>
<td>1.69</td>
<td>10.6</td>
<td>37.4</td>
</tr>
<tr>
<td>RTI-55</td>
<td>0.9</td>
<td>1.2</td>
<td>1.26</td>
<td>4.2</td>
<td>63</td>
</tr>
<tr>
<td>RTI-70</td>
<td>0% @ 90</td>
<td>40% @ 90</td>
<td>2070</td>
<td>59,500</td>
<td>&gt;200,000</td>
</tr>
<tr>
<td>RTI-110</td>
<td>1.0</td>
<td>NT</td>
<td>0.62</td>
<td>4.1</td>
<td>5.42</td>
</tr>
<tr>
<td>RTI-111</td>
<td>1.3</td>
<td>NT</td>
<td>0.79</td>
<td>3.1</td>
<td>17.9</td>
</tr>
<tr>
<td>RTI-112</td>
<td>3.5</td>
<td>1.5</td>
<td>0.8</td>
<td>10.5</td>
<td>36.2</td>
</tr>
<tr>
<td>RTI-113</td>
<td>5.6</td>
<td>NT</td>
<td>1.98</td>
<td>2340</td>
<td>2926</td>
</tr>
<tr>
<td>RTI-114</td>
<td>5.3</td>
<td>9</td>
<td>1.4</td>
<td>1404</td>
<td>778</td>
</tr>
<tr>
<td>RTI-120</td>
<td>4.0</td>
<td>NT</td>
<td>3.26</td>
<td>24,500</td>
<td>5830</td>
</tr>
<tr>
<td>RTI-121</td>
<td>0.3</td>
<td>49</td>
<td>0.4</td>
<td>66.8</td>
<td>285</td>
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<tr>
<td>RTI-147</td>
<td>5.7</td>
<td>NT</td>
<td>1.38</td>
<td>12,400</td>
<td>3950</td>
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<tr>
<td>RTI-229</td>
<td>3.6</td>
<td>NT</td>
<td>0.37</td>
<td>1730</td>
<td>990</td>
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<td>RTI-258</td>
<td>19.1</td>
<td>NT</td>
<td>22.7</td>
<td>68.3</td>
<td>760</td>
</tr>
<tr>
<td>WIN 35,065-2</td>
<td>8.0</td>
<td>6.7</td>
<td>23</td>
<td>1962</td>
<td>920</td>
</tr>
</tbody>
</table>

Fig. 2. Comparison of cocaine and its analogs for blockade of antinociception induced by nicotine. Antagonists were administered i.p. 10 min before nicotine (2.5 mg/kg) was administered s.c., and mice were tested 5 min after nicotine injection in tail-flick test. Each point represents mean ± S.E. of 8 to 12 mice.

Fig. 3. Dose-response relationship of nicotine-induced antinociception and its antagonism by cocaine. Cocaine (5 mg/kg) was administered i.p. 10 min before nicotine, and mice were tested 5 min after nicotine injection in tail-flick test. Each point represents mean ± S.E. of 8 to 12 mice.

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b NT, not tested.
block the discriminative stimulus effect of nicotine in rats (Table 2, Drug discrimination). Cocaine and a selected number of analogs were also evaluated for potential blockade of nicotine-induced hypothermia. Cocaine produced little antagonism of nicotine’s hypothermic effects at doses that were 10-fold greater than those effective for antinociceptive blockade (Table 2, Body temperature). Among the cocaine analogs tested, RTI-31, -32, -55, -112, -121, and WIN 35,065-2 significantly blocked nicotine-induced hypothermia in mice, with RTI-31 being the most potent blocker (AD50 of 1.1 μmol/kg) (Table 1). Interestingly, RTI-31 was 6.5-fold more potent in blocking nicotine hypothermia than antinociception, whereas RTI-32 and RTI-121 were 15-fold and more than a 100-fold less potent, respectively.

To assess a bidirectional cross-reactivity between cocaine and nicotine, dihydro-β-erythroidine (DHβE) and mecamylamine were evaluated for their ability to influence cocaine-induced hyperactivity in mice. Indeed, pretreatment with DHβE and mecamylamine at 1 mg/kg administered s.c. 10 min before the injection of cocaine (15 mg/kg, i.p.) did not significantly reduce the hypermotility induced by cocaine (Fig. 6). Higher doses of DHβE and mecamylamine could not be tested because they significantly decreased mouse spontaneous activity.

Mechanisms of Antagonistic Effect of Cocaine in Tail-Flick Test. To ascertain that the cocaine/nicotine interaction was taking place centrally, cocaine methiodide was evaluated as a potential nicotinic antagonist. As seen in Fig.
7, cocaine methiodide given at doses 10 and 25 times higher than the AD$_{50}$ dose of cocaine (1.1 mg/kg) failed to significantly block nicotine-induced antinociception in mice.

The most prominent central nervous system effects of cocaine are thought to be mediated through blockade of neurotransmitter transporters. Nomifensine, GBR 12909, and bupropion, which are dopamine uptake inhibitors with different affinity and selectivity to the transporter, dose-dependently blocked nicotine's antinociceptive effect in mice (Table 3). However, their potency of blockade did not correlate well with dopamine uptake inhibition. Although nomifensine and GBR 12909 inhibit dopamine uptake with similar affinity, nomifensine was five times more potent than GBR 12909 as a blocker. In addition, bupropion, a nonselective weak dopamine uptake inhibitor, failed to block nicotine's action. In contrast, methyphenidate, a nonselective monoamine uptake inhibitor, failed to block nicotine's effect. Furthermore, dopaminergic and nondopaminergic central stimulants, such as amphetamine and caffeine, blocked nicotine-induced antinociception (Table 3).

Fluoxetine, a selective serotonin uptake inhibitor, failed to significantly block (Table 3) or enhance the effects of nicotine in the tail-flick test. However, nisoxetine, a selective inhibitor of the norepinephrine transporter, antagonized nicotine-induced antinociception in a dose-related manner, with an AD$_{50}$ value of 7.4 μmol/kg (2.3 mg/kg).

Finally, because cocaine is known to possess local anesthetic properties, lidocaine and procaine, two local anesthetics, were evaluated as nicotinic antagonists. However, they failed to significantly block (Table 3) or enhance the effects of nicotine, when injected at high doses (up to 75 μmol/kg) into mice.

**α$_4$β$_2$ and α$_3$β$_2$ Expressed Receptor in Oocytes.** Cocaine at 100 μM elicited little current when applied for 10 s to oocytes expressing the α$_4$β$_2$ or α$_3$β$_2$ subunit combination. Although it did not activate α$_4$β$_2$- and α$_3$β$_2$-expressed receptors, cocaine antagonized the effects of nicotine in a concentration-related manner. Indeed, the current induced by nicotine was blocked by coapplication of cocaine at different concentrations (Fig. 8). The concentration of cocaine that blocked 50% of the nicotinic current was determined to be 5.5 μM (range, 4.4–6.9) and 30.5 μM (range, 22–42.3) for α$_4$β$_2$ and α$_3$β$_2$ receptors, respectively.

### Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>AD$_{50}$ μmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupropion</td>
<td>8</td>
</tr>
<tr>
<td>GBR 12909</td>
<td>8.4</td>
</tr>
<tr>
<td>Nomifensine</td>
<td>1.7</td>
</tr>
<tr>
<td>Amphetamine</td>
<td>36.9</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>5% @ 74</td>
</tr>
<tr>
<td>Caffeine</td>
<td>32.4</td>
</tr>
<tr>
<td>Nisoxetine</td>
<td>7.4</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>15% at 86.7</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>7% @ 74</td>
</tr>
<tr>
<td>Procaine</td>
<td>5% @ 75</td>
</tr>
</tbody>
</table>

### Discussion

The observation by Lerner-Marmarosh and colleagues (1995) that cocaine was capable of blocking the seizure activ-

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**Fig. 7.** Lack of blockade of nicotine-induced antinociception by cocaine-methiodide after i.p. administration in mice using tail-flick test. Cocaine-methiodide (at 10 and 25 mg/kg) was administered 10 min before nicotine and mice were tested 5 min after nicotine (2.5 mg/kg) injection. Each point represents mean ± S.E. of 8 to 12 mice. Coc-I, cocaine-methiodide; Nic, nicotine.

**Fig. 8.** Effect of different concentrations of cocaine on current activated by 1 μM nicotine (A) applied in α$_4$β$_2$-expressing oocytes and 10 μM nicotine (B) applied in α$_3$β$_2$-expressing oocytes. Nicotine or cocaine was applied as a 10-s pulse and changes in current from baseline values was measured for a total of 1 min. Oocytes were held at −70 mV.
ity of nicotine was indeed intriguing, particularly in the light of the fact that cocaine and several of its derivatives were capable of competing with the binding of the nicotinic antagonist [3H]mecamylamine. That study raised several questions, the most prominent being whether cocaine could block nonneurotoxic effects of nicotine. Indeed, cocaine was found to block nicotine’s antinociceptive and motor effects, but failed to alter the hypothemic or discriminative-stimulus cue in the present investigation. The low doses of cocaine that were effective in blocking nicotine’s antinociception suggest a specific action. This notion was supported by the structure-activity relationship studies of β3-phenyltropane cocaine analogs. Several rather subtle changes in cocaine’s structure produced dramatic changes in antagonistic potency. It was equally important to establish that cocaine was acting centrally, as shown by cocaine methiodide’s failure to antagonize nicotine.

Whereas cocaine binding to the [3H]mecamylamine site was considered as putative mechanism for cocaine/nicotine interaction, a lack of understanding of the physiological role of this site limits our ability to postulate a mechanism of cocaine’s action. Interestingly, the potency of blocking nicotine-induced antinociception for some cocaine analogs was equal and even greater than that reported for mecamylamine and DHβE, two classical nicotinic antagonists. Indeed, RTI-31, -51, -55, -110, and -111 had similar potencies (Table 1) to that of DHβE (a competitive nicotinic antagonist) in the tail-flick test (1.6 μmol/kg) (Damaj et al., 1995). Of particular interest was the cocaine analog RTI-121, which was equipotent with mecamylamine (AD50 = 0.27 μmol/kg) in blocking nicotine’s effect (Damaj et al., 1995). RTI-121 was recently reported to be a potent dopamine uptake inhibitor (at least 50 times more potent than cocaine in inhibiting dopamine uptake) and to induce long-lasting increases in locomotor activity in mice (Fleckenstein et al., 1996).

It is reasonable to speculate that cocaine’s antagonistic effects are mediated through its actions on neurotransmitters. Indeed, cocaine is thought to exert its behavioral effects, at least in part, by binding to the dopamine transporter, blocking synaptic dopamine reuptake and thereby potentiat- ing dopaminergic neurotransmission (for review see Kuhar et al., 1991). Because nicotine was also reported to enhance dopamine release in the brain (Grady et al., 1992), it was conceivable that the antagonistic effects of cocaine could be related to the dopamine system. However, a poor correlation between dopamine transporter binding potencies of cocaine analogs and their antagonistic potency in the tail-flick test (Fig. 4) was observed. Furthermore, we previously reported that various dopamine agonists and antagonists failed to block nicotine-induced antinociception in mice (Damaj and Martin, 1993). In addition, when several classical dopamine uptake inhibitors were tested as potential nicotinic antagonists, no relationship was found between their potency in inhibiting dopamine uptake and blocking nicotine’s analgesic effect. Although nomifensine and GBR 12909 inhibit dopamine uptake with similar affinity (Richelson and Pfennning, 1984), nomifensine was five times more potent than GBR 12909 as a nicotine blocker. Furthermore, bupropion, a non-selective weak dopamine uptake inhibitor (micromolar range; Ascher et al., 1995) was equipotent as GBR 12909 in blocking nicotine’s action. Moreover, cocaine, which inhibits the dopamine transporter with a roughly similar affinity to that of amphetamine (Azzaro et al., 1974), was 12 times more potent in blocking nicotine’s effect. Finally, methylphenidate, a dopamine uptake inhibitor (Richelson and Pfennning, 1984), failed to block nicotine’s effect. Taken together, these results rule out a role for the dopamine transporter in nicotine’s effects. It is also interesting to note that nomifensine and bupropion are also used as antidepressant agents, with the latter being recently used for smoking cessation (Hurt et al., 1997). A more in-depth investigation of these agents could reveal an as yet unidentified neurochemical property that explains their usefulness in the treatment of nicotine dependence.

The failure of fluoxetine in blocking nicotine’s effect and the poor correlation between serotonin transporter binding potencies of cocaine analogs and their antagonistic potency in the tail-flick test does not suggest the involvement of serotonin transporter in cocaine’s antagonistic effects. Although nisoxetine, a selective norepinephrine uptake inhibitor (Wong and Bymaster, 1976), was able to block nicotine’s analgesic action, our correlation results do not support the involvement of norepinephrine transporter in cocaine’s blocking effects. Furthermore, nisoxetine was reported to enhance morphine analgesia in rats (Izenwasser and Kornetsky, 1988). Nisoxetine itself could be acting as a noncompetitive nicotinic antagonist. However, other norepinephrine uptake inhibitors were not tested. Finally, the local anesthetic property of cocaine does not seem to be involved in its antagonistic effect, because lidocaine and procaine, two local anesthetics, failed to block nicotine-induced antinociception in mice.

Our results and the above arguments suggest that cocaine is a nicotinic antagonist with a mechanism of blockade not involving the “classical” reported neurochemical effects of cocaine. The blocking action of cocaine on neuromuscular transmission (Swanson and Albuquerque, 1987), is not involved in its antagonistic effect because cocaine-methiodide, a potent peripheral cocaine analog, failed to block nicotine-induced antinociception in mice. Such failure to modify nicotine’s action suggests the involvement of central receptors or “sites” in cocaine’s antagonistic action. Particularly intriguing was the competitive-like nature of the antagonism observed with cocaine. The actions of cocaine and cocaine analogs were not only dose dependent but the dose-response curve for nicotine-induced antinociception was shifted in a parallel fashion to the right by cocaine pretreatment. The question arises as to whether the actions of cocaine reflect a direct interaction with neuronal nicotine receptors. However, a direct interaction at the nicotine binding site would appear to be an unlikely possibility. Binding studies show that cocaine has no affinity at central nicotine receptors, namely [3H]nicotine and 125I-α-bungarotoxin binding sites (Marks and Collins, 1982; Lerner-Marmarosh et al., 1995). An “indirect” or noncompetitive blockade is a possible mechanism by which cocaine interaction with central nicotinic receptors occurs. Noncompetitive binding sites on neuronal nicotinic receptors are also reported with other drugs, such as dihydropyridine calcium channel antagonists (Donnelly-Roberts et al., 1995), steroids, and various tachykinines (Ke and Lukas, 1996; Lukas and Eisenhour, 1996).

The lower potency of cocaine in blocking nicotine-induced seizures and its failure in blocking nicotine’s effect on body temperature and drug discrimination, suggest that cocaine possesses some selectivity for neuronal nicotinic receptors.
The αβ2 nicotinic receptor subtype is a possible target for cocaine’s actions, because cocaine and cocaine analogs blocked nicotine-induced anticonvulsion in the tail-flick test. Indeed, the antinociceptive response of nicotine in this test appears to involve the αβ2 nicotinic receptor subtype (Damaj et al., 1998). In addition, our results with the αβ2-expressed nicotinic receptor suggest that cocaine is a blocker of nicotinic receptor subtype containing α4 and β2 subunits. However, the lack of affinity for the [3H]nicotine binding site suggests that cocaine and its analogs (Lerner-Marmarosh et al., 1995) are noncompetitive nicotinic antagonists. Other nicotinic receptor subtypes are likely involved in cocaine’s action, namely αG-containing receptors. Indeed, our results showed that cocaine blocked nicotinic currents in the αβ2-expressed nicotinic receptor, with a lower potency (5.5-fold difference) than that determined for αβ2 receptors. Moreover, the fact that cocaine was able to block nicotine-induced seizures, a response that is known to involve αβ subunits (Miner et al., 1985; Miner and Collins, 1989), supports an interaction between cocaine and α-containing receptors. The involvement of other receptor subunits as a target for cocaine’s action is also possible. In addition, our in vivo and in vitro results showed that cocaine blocks several nicotinic receptor subtypes with different potencies. Such difference suggest that cocaine possesses some selectivity for neuronal nicotinic receptors. In summary, we demonstrated in the present investigation that cocaine appears to be a noncompetitive nicotinic antagonist with some selectivity for nicotinic pharmacological effects. It would appear that the mechanisms for cocaine’s antagonistic action is not related to its effects on monoamine transporters and its local anesthetic effect. Our studies also demonstrate that β-phenyltropanes analogs constitute a new class of nicotinic antagonists. Further studies are needed to determine the in vitro and in vivo selectivity profile of these analogs.

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References

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