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The Effect of Cotinine on Nicotine- and Cocaine-Induced Dopamine Release in the Nucleus Accumbens*

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Cotinine is the major metabolite of nicotine. Nicotine is rapidly metabolized and has a short half-life, but cotinine is metabolized and eliminated at a much lower rate. Because of the resulting increase with time in the cotinine to nicotine ratio in the body, including in the brain, it is of interest to examine the effect of cotinine on nicotine-induced changes. In studies on conscious, freely-moving rats, intravenous administration of either nicotine or cocaine induced the release of dopamine in the nucleus accumbens, as assayed by microdialysis. Prior intravenous administration of a high dose of cotinine (500 μg/kg) inhibited this nicotine- or cocaine-induced dopamine release. The action of cotinine does not seem to occur through its effect on the metabolism of nicotine or on its binding at the receptor site, because cotinine, unlike nicotine, does not affect the binding of the nicotinic ligand cytisine. The findings suggest that cotinine affects a putative component of the reward mechanism, and as such could have therapeutic value.

KEY WORDS: Cotinine; nicotine; dopamine release; nicotine binding.

INTRODUCTION

Several studies, including some recent extensive ones, have established that cotinine is the major metabolite of nicotine (1–8). Although the liver seems to be the major site of the conversion of nicotine to cotinine, this conversion has also been found in brain preparations in vitro (1). The rate and the extent of this conversion are variable; in man, an average of about 72% of the administered nicotine is converted to cotinine (2). The formation of cotinine in the liver is primarily by monooxygenase enzymes such as cytochrome P450 and aldehyde oxidase, and it can depend, among other things, on the level of enzymes, or on prior administration to the subject of drugs such as barbiturates (3). In human in vitro liver preparations, cytochrome P450 plays a major role, with large individual variations (4).

Most studies have indicated that whereas the metabolic conversion of nicotine to cotinine is rapid, further metabolism of cotinine is slow, with corresponding differences in their rates of elimination. In rats, after a single intravenous nicotine dose, the half-life of nicotine was 1 hr and that of cotinine was 6.4 hr (5). In a study using an intravenous nicotine bolus in rat, the half-life of nicotine in the plasma was 20 min, in brain 50 min. Nicotine and cotinine concentrations became equal in the plasma at 30 min, and then nicotine levels rapidly decreased while cotinine levels remained constant (6). In humans, the half-life of cotinine administered intravenously was about 12 hr (7).

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Because of its longer half-life and lower metabolic rate, cotinine has been proposed as a measure of nicotine intake. It could be used as the indicator of daily nicotine intake when measured around the time of the last cigarette of the day (8). Nicotine is more efficient than cotinine in passing through the blood-brain barrier in rats (9). The cotinine that is able to enter the brain is not biotransformed in this organ (10).

The above studies indicate that whereas after its administration nicotine is rapidly distributed, metabolized, and eliminated, the level of cotinine remains elevated above that of nicotine in many organs, including the brain. Because cotinine is thus accumulated in the brain, it is of interest to examine its possible modulation of nicotine effects.

Cotinine does not seem to affect nicotine metabolism or nicotine pharmacokinetics (11), and unlike nicotine does not alter dopamine level in the striatum (12). A number of laboratories reported that nicotine or cocaine administration increases the level of dopamine in the nucleus accumbens and suggested this increase as an important part of the reward mechanism (28,31). The central role of accumbens dopamine increase in the reward mechanism has recently been questioned (32,33). In a previous study we reported that dopamine release in nucleus accumbens induced by the intravenous administration of nicotine in conscious rats can be inhibited by various nicotinic, muscarinic, dopaminergic, and glutamatergic antagonists. A few of these also inhibit cocaine-induced dopamine increase in the accumbens (13). Here we report that the intravenous administration of a bolus of cotinine inhibits the nicotine- and cocaine-induced increase of dopamine in nucleus accumbens of freely-moving conscious rats. High cerebral levels of cotinine thus antagonize in vivo an important effect of nicotine or cocaine in the brain. Since nicotine- or cocaine-induced dopamine increase was shown to play an important role in supporting their use, the cotinine effect may have pharmacological significance.

EXPERIMENTAL PROCEDURE

In this continuation of our previous studies with nicotine, we used as before 8-week-old male Sprague-Dawley rats (300–350 g) bred in our animal facility, thus making it possible to compare our present results with those of earlier assays of nicotine and cocaine effects in the absence of cotinine (13). The use of the animals had the approval of the Institutional Animal Care Committee. NIH guidelines for the care of experimental animals were followed. The rats were cannulated in the jugular vein on the day before the experiment, and nicotine and cotinine were administered by intravenous bolus injection. Brain microdialysis, assay of dopamine in the dialysate, and verification of the placement of the microdialysis probe were performed as described previously (13). Briefly, the guide cannula was stereotactically implanted one day before the experiment into the nucleus accumbens; the stereotaxic coordinates with respect to the bregma were: AP: + 1.6, ML: −0.85, DV: −5.7 (34). The microdialysis probe (0.5 mm diameter) was lowered 2 mm below the end of the guide cannula, and was perfused at 1 μl/min with Ringer's solution. After completion of the experiment the rats were killed with an overdose of sodium pentobarbital, and the brain was removed and kept in 10% formaldehyde. The probe placement was verified histologically and by a stereo microscope. The dialysate samples (15 μl) were measured in a BAS 200 A HPLC system with a microbore column (BAS 5 μ C18 150 × 1 mm column with a 6 mm BAS Unijet glassy carbon electrode) and reverse-phase liquid chromatography with electrochemical detection. The mobile phase contained 25 mM sodium phosphate, 50 mM sodium citrate, 27 mM EDTA, 10 mM diethylamine, 2.2 mM octanesulfonic acid, and 10 mM sodium chloride with 30 ml methanol and 22 ml dimethylacetamide per liter, pH = 3.5, at a 100 μl/min flow rate. The potential was set at 500 mV and the detection limit was 300 fg of dopamine at 1 nA gain. We found that nicotine-induced dopamine release in the nucleus accumbens can be inhibited by several receptor subtype-specific antagonists (13).

Nicotine ditartrate, cotinine, and cytisine were purchased from Sigma Chemical Co. (St. Louis, MO), and drugs used for the binding competition study were from RBI (Natick, MA). Cotinine, nicotine (ditartrate), cocaine (hydrochloride), and amphetamine (sulfate) were dissolved in 0.9% saline. The drugs were administered by bolus injection (in a volume of 100 μl) through the intravenous catheter tubing washed in with an equal volume of saline. The doses given in the figures represent the free base form of the drugs. The small amount injected did not affect blood pH.

For the determination of nicotine and cotinine in brain and in plasma the gas chromatographic assay of Davis (14) was used. The preparation and extraction of the tissue were done as in our previous nicotine assay (13). The compounds were extracted from the tissue, and were separated by gas chromatography using a Carbowax DB megabore 30-meter column at increasing temperature (80–260°C) with the Nitrogen Phosphorus detector run in the nitrogen mode. The extraction recovery was over 95%, intra- and interassey RSP% < 6%.

Binding Assays. Membrane fractions were prepared using the modified procedure of Pabreza et al. (15). The brains from control Sprague-Dawley rats (300–350 g) after decapitation under anesthesia were rapidly removed, and cortical tissue was dissected and homogenized in 25 ml of 50 mM Tris-HCL buffer (pH 7.4) using a Brinkman Polytron (Brinkman Instruments, Westbury, NY) at the setting 6 for 6 seconds. The homogenate was centrifuged at 40,000 *g* for 10 min, and the pellet was resuspended in fresh buffer and centrifuged a second time. The final pellet was resuspended in 25 ml of fresh buffer, and triplicate aliquots of homogenate equivalent to approximately 1.2 mg of protein were added to test tubes containing 5 nM [^{3}H]cytisine. We used cytisine because it has high affinity for the receptor, and unlike nicotine, exhibits a low nonspecific binding component (16). After adding buffer to a final volume of 1 ml, membranes were incubated for 75 min at room temperature in the presence and absence of 100 nM or 1000 nM unlabeled nicotine, cotinine, cocaine, dopamine, carbachol, and D-tubocurarine. Nonspecific binding was determined in tissues incubated in the presence of 1000 nM cytisine. The incubation was terminated by rapid vacuum filtration through Whatman GF/B filter paper. To reduce nonspecific filter

binding, the filters were presoaked in ice-cold 0.5% polyethylenamine just before sample filtration and then were rapidly rinsed with 10 ml of ice-cold Tris-HCL buffer. The radioactivity retained by the filters was counted in 4 ml of Liquiscint (National Diagnostics, Atlanta, GA). Protein values were determined by the method of Lowry et al. (17) using bovine serum albumin as a standard.

RESULTS

Nicotine-Induced Dopamine Release. As in numerous previous studies, the intravenous administration of nicotine resulted in an increase of dopamine in the extracellular fluid, as assayed by microdialysis. The increase occurred soon after nicotine administration, and dopamine levels returned to baseline values rapidly. A second nicotine administration similarly resulted in a transient increase in dopamine levels in the nucleus accumbens (Fig. 1).

Effects of Cotinine on Dopamine Release. Previous studies (28,31), confirmed in our recent experiments (13), showed that nicotine or cocaine administration induces the release of dopamine in the shell of the nucleus accumbens. In one of our studies, under in vivo conditions in rats in which nicotine administered via microdialysis induced dopamine release, cotinine when added to the microdialysis buffer at 100 nM did not have such an effect (12). In the present experiments, administration of a bolus of cotinine (500 μg/kg) inhibited dopamine release by subsequently administered nicotine (50 μg/kg nicotine at 120 min, and 100 μg/kg at 210 min) (Fig. 2). As before (13), at the end of the experiments we tested for the presence of releasable dopamine pools by adding a large dose of amphetamine. Amphetamine induced dopamine release in the accumbens after the administration of nicotine, and cotinine. The effect of amphetamine was much greater than that of nicotine or cocaine under our experimental circumstances. Amphetamine effects were not inhibited by cotinine in these experiments.

Prior cotinine administration inhibited the dopamine release induced by cocaine as well, with a possible minor cocaine effect remaining under the experimental conditions. Amphetamine in these experiments induced a large increase of dopamine in the extracellular fluid, which was not inhibited by cotinine (Fig. 3). The effect of cotinine was dose dependent, since at lower cotinine levels (100 μg/kg administered) inhibition of nicotine-induced dopamine release was negligible (Fig. 4). These results show that while in vivo cotinine by itself does not induce dopamine release (there are usually baseline fluctuations), if the cotinine level is sufficiently high, it inhibits dopamine release induced by nicotine or cocaine. A recent paper (35) reported that cotinine evoked ^{3}H-overflow from superfused rat striatal slices preloaded with [^{3}H]dopamine without inhibiting [^{3}H]dopamine uptake.

Cotinine Concentrations in the Brain. The lowest dose of nicotine used in this study (50 μg/kg) is somewhat higher than that received by smoking a cigarette; the average yield of nicotine from a cigarette, according

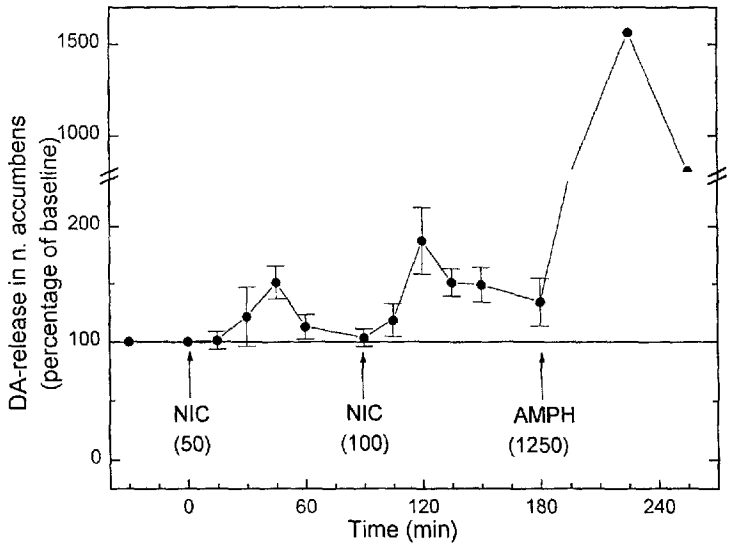

Fig. 1. Effects of repeated administration of nicotine on extracellular dopamine levels in the nucleus accumbens. Numbers in parenthesis indicate the dose (μg/kg) of nicotine (NIC) and amphetamine (AMPH). Averages of three experiments ± SEM are shown.

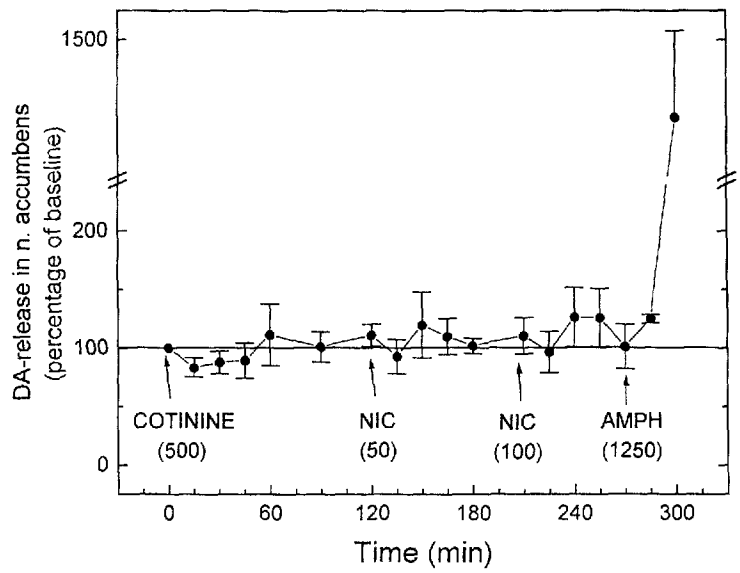

Fig. 2. Effects of a high dose of cotinine on the nicotine-induced increase of extracellular dopamine in the nucleus accumbens. Numbers in parenthesis indicate the dose (μg/kg) of cotinine, nicotine, and amphetamine. Averages of four experiments ± SEM are shown.

to the Federal Trade Commission, is about 1 mg. For an 80-kg subject this would be a dose of 12.5 μg/kg taken over a period of time (average 3–6 min). We (12) and others (18) found that to reliably detect and measure dopamine release the use of somewhat higher doses of nicotine is necessary. Thus, under the present experimental conditions, brain levels of nicotine and cotinine would be expected to be higher than those reached by smokers. The level of cotinine in the various experiments is shown in Table I. We administered intravenous cotinine and measured its level 135 min later; nicotine was administered 120 minutes after the cotinine; and the effect on the dopamine level was measured 15 min later. At the 500 μg/kg body weight

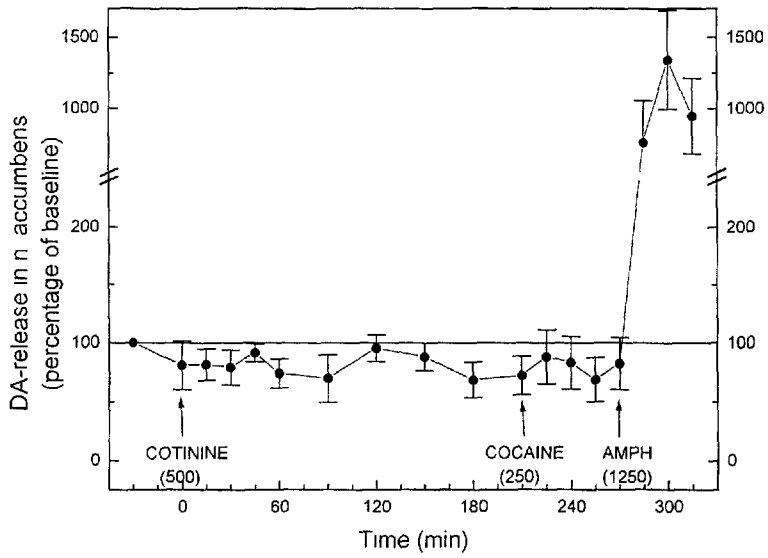

Fig. 3. Effects of cotinine on the cocaine-induced increase of extracellular dopamine in the nucleus accumbens. Numbers in parenthesis indicate the dose (μg/kg) of cotinine, cocaine, and amphetamine. Averages of four experiments ± SEM are shown.

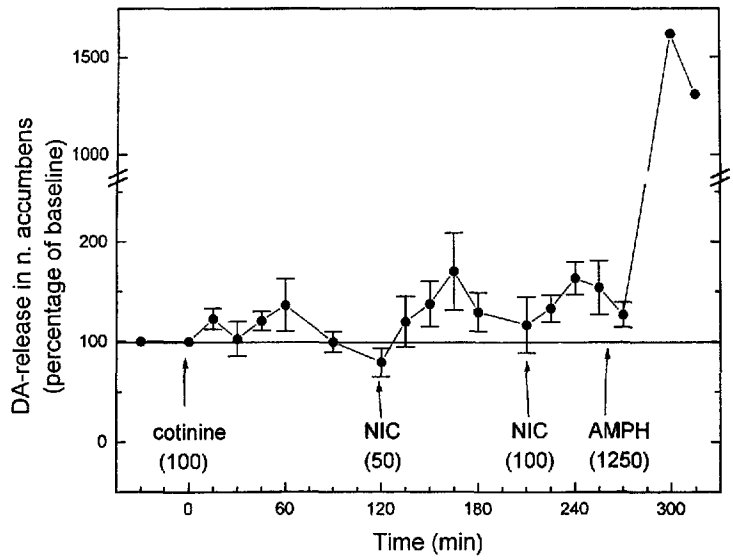

Fig. 4. Effects of a lower dose of cotinine on the nicotine-induced increase of extracellular dopamine in the nucleus accumbens. Numbers in parenthesis indicate the dose (μg/kg) of cotinine, nicotine, and amphetamine. Averages of three experiments ± SEM are shown.

dose, the cotinine level in the brain reached 300 μg/kg brain; at the 100 μg/kg body weight dose, the brain level was proportionate to the dose ~60 μg/kg brain. This lower level was still about 10-fold higher than that measuring cotinine levels 15 min after nicotine administration alone. After the second administration of nicotine at a dose of 100 μg, brain cotinine levels increased further (Table I).

Effect of Cotinine on Nicotinic Binding. We used the nicotinic ligand cytisine for binding assays, because the nonspecific binding of nicotine is high. Cytisine binding was inhibited by nicotine, but it was not significantly inhibited by cotinine even at high concentrations. Thus, it is unlikely that cotinine acts by displacing nicotine from its receptor site. Because of partial nonspecific binding of cytisine, its binding was not inhibited more than 75%; the nicotinic cholinergic compounds carbachol and turbocurarine inhibited cytisine binding partially under our experimental conditions (Table II).

DISCUSSION

Our study did not reveal a mechanism for the inhibition of nicotine- or cocaine-reduced dopamine release by cotinine. We did not find direct competition at nicotinic sites, and others did not find any effect on

Table I. Nicotine and Cotinine Levels Under Our Experimental Conditions

		Levels (μg/kg)			
		Cotinine		Nicotine	
Experiment	Time Min	Brain	Blood	Brain	Blood
Cotinine 500 μg/kg	135	300 ± 33	313 ± 40		
Cotinine 500 μg/kg	225	246 ± 38	303 ± 39		
Cotinine 100 μg/kg	135	60 ± 3.3	75 ± 9.5		
Nicotine 50 μg/kg	15	5.8 ± 0.5	4.5 ± 0.5	70 ± 9	16 ± 3
Nicotine 50 μg/kg at 0 100 μg/kg at 90 min	15	21 ± 1.7	18 ± 2.5	169 ± 12	43 ± 9.6

Averages of four experiments ± SEM are given.

Table II. Competition by Drugs for [^{3}H]Cytisine Binding Sites in Rat Cerebral Cortex

Drugs	% inhibition	
	100 nM	1000 nM
Cytisine	75.4 ± 12.2	85.5 ± 9.4
Nicotine	65.0 ± 15.2	71.3 ± 7.1
Cotinine	10.3 ± 3.5	8.3 ± 0.87
Cocaine	9.6 ± 3.2	4.6 ± 1.2
Dopamine	9.6 ± 3.8	5.0 ± 1.0
Carbachol	16.0 ± 4.9	32.3 ± 8.1
D-Tubocurarine	14.0 ± 6.0	21.0 ± 3.3

Homogenates of cortex were incubated in the presence of 100 nM or 1000 nM of each drug and 5 nM [3H] cytisine. Values are the mean ± standard error from three experiments each done in triplicate.

nicotine pharmacokinetics (11). A previous study of nicotinic receptor binding found that whereas cytisine and nicotine had high affinity to the nicotinic binding site, cotinine's affinity was very low, making it unlikely that cotinine could antagonize nicotine binding at nicotinic receptor sites (19). Our finding that cotinine does not affect the binding of the nicotinic ligand cytisine argues against inhibition by cotinine of nicotine effects at a nicotinic receptor site. It is of interest that in brain slices cotinine releases dopamine in a mecamylamine-inhibitable manner (35). This would indicate action as a nicotinic agonist at the receptor. There seem to be differences between nicotine and cotinine affinity to other receptor sites, since nicotine, but not cotinine, was shown to displace MK801 binding to rat brain membranes (20). This shows differences in their affinity for the NMDA receptor, and indicates that antagonism at this site can not explain the effect of cotinine on nicotine-induced dopamine release. In our previous study (13), not only nicotinic but also muscarinic and glutamatergic antagonists inhibited nicotine- but not cocaine-induced dopamine release, while dopaminergic antagonists inhibited the effect of nicotine and that of cocaine. We did not test whether cotinine inhibits nicotine action by acting as a dopamine receptor antagonist. While this is not likely, it can not be excluded at the present time.

When several of the effects of nicotine and cotinine were compared, some were found to be similar, but a number of different or opposite effects were also found. In granulosa cell cultures both compounds inhibited progesterone synthesis, but how this would affect pregnancy or endocrine levels in vivo has not been examined (21). In bovine adrenal chromaffin cells, cotinine, like nicotine, increased the activity of protein kinase C, and like nicotine, it increased the release of preloaded norepinephrine from cultured cells (22). The authors suggested that cotinine acts as a low-affinity partial agonist at nicotinic receptor sites on bovine adrenal chromaffin cells, and would have pharmacological activity at high doses at this site (22). It is possible that cotinine could affect nicotine-induced dopamine release at non-nicotinic sites - for example, adrenergic sites. Nicotine and cotinine have been reported to inhibit the conversion of arachidonic acid and prostaglandin H2 into thromboxane, presumably by affecting the cytochrome P450 component of thromboxane synthetase binding to iron (23). The effects of nicotine and cotinine did not seem to be similar in several preparations - in a heart preparation, neither nicotine nor cotinine affected the neuronal or non-neuronal uptake of norepinephrine, but nicotine potentiated stimulation-evoked norepinephrine release, while cotinine inhibited it, thus showing opposite effects at sympathetic nerve endings in this preparation (24). The authors previously noted that nicotine inhibits prostaglandin synthesis in aortic microsomes, whereas cotinine stimulates it (25), and they postulate that prostaglandin changes produce the effects on the adrenergic system (24). Among the opposing endocrine effects reported are findings that nicotine increases blood aldosterone and prolactin levels, whereas cotinine has the opposite effect (26); the different effects of neuroendocrine regulation are probably through mechanisms that are not related to circulatory effects, but occur at the median eminence-pituitary level (26). In an examination of immune response, nicotine was found to inhibit the antibody-forming cell response, but cotinine had no such effect (27).

The heterogeneity of nicotine-induced changes has to be emphasized. Nicotine affects numerous transmitters and numerous receptors in a regionally heterogeneous manner. It induces neurotransmitter release in a regionally heterogeneous manner, with each change regionally specific for each neurotransmitter. The regional pattern of nicotine-induced dopamine release is different from the pattern of induced norepinephrine release, and the pattern of serotonin release is different from that of dopamine or norepinephrine release (12, 28–31). This complex set of changes is probably specific for nicotine, and it is likely that each drug of abuse induces its own specific pattern of changes. This difference in the spectrum of changes between the effects of various drugs explains the ability to discriminate between them. It also explains some of the differences in their pharmacological action, such as the different effects on cognition of nicotine and cocaine. The effects of nicotine on neurotransmitter and other systems occur through several distinct mechanisms, some of which are antagonized by cotinine; others are not affected or

are stimulated by it. The receptor interactions play an important part in the nicotine-induced dopamine release in the nucleus accumbens, as shown by the inhibition of this dopamine release by muscarinic, glutamatergic, and dopaminergic antagonists (13); hence, any disruption of such interaction by cotinine would result in inhibition of the release.

It is clear from the literature that some of the effects of nicotine and cotinine are similar and some are different. Because some systems are affected by cotinine in the opposite direction from nicotine it is possible that several actions of nicotine are inhibited by cotinine. In the present experiments we found that high levels of cotinine antagonize some of the nicotinic effects on dopamine release. It is not possible to examine the effects of low levels of cotinine on nicotinic effects or compare effects of nicotine in the absence of cotinine because cotinine is formed very rapidly after nicotine administration. It is possible that the cotinine formed from nicotine modulates some of its effects, especially at higher nicotine doses, or following chronic nicotine intake. Since cotinine is metabolized slowly while nicotine is rapidly metabolized, cotinine accumulates to levels above nicotine levels in the brain and the blood. The inhibition of dopamine release in nucleus accumbens is of interest in relation to the reward mechanism, since dopamine release seems to play a significant role in these and related behaviors (18). It has to be emphasized that nicotine induces the release of other neurotransmitters (acetylcholine, norepinephrine, serotonin, probably amino acids, peptides, etc.), and affects their levels and dopamine levels in several brain areas. It remains to be explored whether cotinine also affects nicotine-induced release of other neurotransmitters or the release of dopamine in areas other than the nucleus accumbens. The present results indicate the possibility of using nicotine metabolites or their analogs to modulate its actions.

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