(S)-(−)-Cotinine, the Major Brain Metabolite of Nicotine, Stimulates Nicotinic Receptors to Evoke $[^3\text{H}]$Dopamine Release from Rat Striatal Slices in a Calcium-Dependent Manner

LINDA P. DWOSKIN, LIHONG TENG, SUSAN T. BUXTON, and PETER A. CROOKS

College of Pharmacy and Graduate Center for Toxicology, University for Kentucky, Lexington, Kentucky

Accepted for publication August 10, 1998  This paper is available online at http://www.jpet.org

ABSTRACT

Cotinine, a major peripheral metabolite of nicotine, has recently been shown to be the most abundant metabolite in rat brain after peripheral nicotine administration. However, little attention has been focused on the contribution of cotinine to the pharmacological effects of nicotine exposure in either animals or humans. The present study determined the concentration-response relationship for (S)-(−)-cotinine-evoked $[^3\text{H}]$ overflow from superfused rat striatal slices preloaded with $[^3\text{H}]$dopamine ([$[^3\text{H}]$D]A) and whether this response was mediated by nicotinic receptor stimulation. (S)-(−)-Cotinine (1 μM to 3 mM) evoked $[^3\text{H}]$ overflow from [$[^3\text{H}]$D]A-preloaded rat striatal slices in a concentration-dependent manner with an $EC_{50}$ value of 30 μM, indicating a lower potency than either (S)-(−)-nicotine or the active nicotine metabolite, (S)-(−)-nornicotine. As reported for (S)-(−)-nicotine and (S)-(−)-nornicotine, desensitization to the effect of (S)-(−)-cotinine was observed. The classic nicotinic receptor antagonists mecamylamine and dihydro-β-erythroidine inhibited the response to (S)-(−)-cotinine (1–100 μM). Additionally, $[^3\text{H}]$ overflow evoked by (S)-(−)-cotinine (10–1000 μM) was inhibited by superfusion with a low calcium buffer. Interestingly, over the same concentration range, (S)-(−)-cotinine did not inhibit [$[^3\text{H}]$D]A uptake into striatal synaptosomes. These results demonstrate that (S)-(−)-cotinine, a constituent of tobacco products and the major metabolite of nicotine, stimulates nicotinic receptors to evoke the release of DA in a calcium-dependent manner from superfused rat striatal slices. Thus, (S)-(−)-cotinine likely contributes to the neuropharmacological effects of nicotine and tobacco use.

The alcaloidal tobacco constituent (S)-(−)-cotinine is the major peripheral oxidative metabolite of (S)-(−)-nicotine in several animal species, including humans, and is able to pass the blood-brain barrier from the periphery (Gorrod and Wahren, 1993; Benowitz et al., 1994; Crooks et al., 1997). (S)-(−)-Cotinine has been detected in mouse, rat, and cat brain after peripheral nicotine administration (Applegren et al., 1962; Schmiterlow et al., 1967; Stalhandske, 1970; Petersen et al., 1984; Deutsch et al., 1992; Crooks et al., 1995, 1997; Crooks and Dwoskin, 1997) and has been shown to be the most abundant (S)-(−)-nicotine metabolite in the central nervous system after acute s.c. administration of nicotine to rats (Crooks et al., 1997). Interestingly, (S)-(−)-cotinine does not undergo significant biotransformation in brain tissue in vivo and has a much longer half-life in the central nervous system than does (S)-(−)-nicotine (Crooks et al., 1997).

The origin of (S)-(−)-cotinine in brain has not been elucidated and could arise via two different mechanisms: formed oxidatively from nicotine locally in the brain or formed in the periphery and then redistributed to the brain. Although hepatic metabolism of (S)-(−)-nicotine to (S)-(−)-cotinine has been suggested to involve cytochromes P-4502D6, P-4502B6, P-4502E1, P-4502C9, and P-4502A6 (Cashman et al., 1992; McCracken et al., 1992; Flammang et al., 1992; Cholerton et al., 1994), recent evidence demonstrates that P-4502A6 is the major isozyme involved in hepatic C-oxidation of (S)-(−)-nicotine to (S)-(−)-cotinine in humans (Nakajima et al., 1996; Messina et al., 1997). It is important to note that the regional localization of P-4502A6 and its role in local (S)-(−)-nicotine metabolism in brain have not been established to date. Interestingly, it has recently been reported that an individual’s inherent ability to metabolize nicotine to cotinine via CYP2A6 in part determines their tobacco dependence liability (Pianezza et al., 1998).

In contrast to the plethora of studies investigating the neuropharmacological effects of (S)-(−)-nicotine, few studies have investigated the effects of (S)-(−)-cotinine. (S)-(−)-Nicotine has been reported to have intrinsic reinforcing properties suggested to be the result of activation of dopamine (DA) pathways in brain (Fibiger and Phillips, 1987; Corrigall et al., 1992, 1994; Balfour and Benwell, 1993). Nicotine facilitates DA release from striatal nerve terminals in vivo

Received for publication May 26, 1998.

1 This research was supported by grants from the National Institute on Drug Abuse (DA08656) and the Tobacco and Health Research Institute (Lexington, KY).

ABBRIVIATIONS: DHβE, dihydro-β-erythroidine; DA, dopamine; MEC, mecamylamine; ANOVA, analysis of variance.
studies using microdialysis in striatum (Imperato et al., 1986; Toth et al., 1992) and in vitro superfusion studies using striatal slices (Westfall, 1974; Arqueros et al., 1978; Giorgiueff-Chesselet et al., 1979; Westfall et al., 1987; Izenwasser et al., 1991; Harsing et al., 1992; Schulz et al., 1993, Sacaan et al., 1995) and synaptosomes (Takano et al., 1983; Chesselet, 1984; Rowell et al., 1987; Rapier et al., 1988, 1990; Grady et al., 1992; Rowell and Hillebrand, 1994; El-Bizri and Clarke, 1994; Rowell, 1995). Concentrations (0.1–1 µM) of nicotine that correspond to plasma levels in moderate smokers (Russell et al., 1980; Kogen et al., 1981; Benowitz, 1990; Henningfield et al., 1993) evoked DA release in the latter in vitro studies. Moreover, nicotine-evoked striatal DA release was calcium dependent and was inhibited by mecamylamine or dihydro-β-erythroidine (DHβE) (Westfall et al., 1987; Rapier et al., 1988, 1990; Grady et al., 1992; El-Bizri and Clarke, 1994; Sacaan et al., 1995; Teng et al., 1997). Mecamylamine is a centrally active, noncompetitive nicotinic receptor antagonist that blocks the open ion channel of the nicotinic receptor more effectively than the closed channel (Varanda et al., 1985; Loiacono et al., 1993; Peng et al., 1994). DHβE is a selective, competitive nicotinic receptor antagonist that displaces nicotine from its binding site (Reavill et al., 1988; Grady et al., 1992) and inhibits its electrophysiological effects (Vidal and Changeux, 1989; Alkondon and Albuquerque, 1991; Mulle et al., 1991).

Relatively little is known about the effects of (S)-(-)-cotinine on either DA-mediated behaviors or DA neurochemistry. (S)-(-)-Cotinine has a reported Kᵢ of 1 µM for the [³H]nicotine binding site in rat brain, which is approximately 1000-fold weaker affinity than that reported for (S)-(-)-nicotine (Abood et al., 1981). The purposes of the present study were to determine whether (S)-(-)-cotinine evokes ³H overflow from rat striatal slices preload with [³H]DA in a concentration- and calcium-dependent manner and whether (S)-(-)-cotinine-evoked ³H overflow was inhibited by mecamylamine and DHβE, providing evidence for a nicotinic receptor-mediated mechanism.

**Experimental Procedures**

**Materials.** (S)-(-)-Cotinine and pargyline hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Nomifensine maleate, mecamylamine HCl, and DHβE were purchased from Research Biochemicals, Inc. (Natick, MA). [³H]DA (3,4-ethyl-2[N-³H]dihydroxyphenylethylamine; specific activity, 25.6 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Ascorbic acid and α-glucose were purchased from AnalaR (BDH Ltd., Poole, U.K.) and Aldrich Chemical Co. (Milwaukee, WI), respectively. TS-2 tissue solubilizer was purchased from Research Products International (Mount Prospect, IL). All other chemicals were purchased from Fisher Scientific (Pittsburgh, PA).

**Subjects.** Male Sprague-Dawley rats (200–250 g) were obtained from Harlan Laboratories (Indianapolis, IN) and were housed two per cage with free access to food and water in the Division of Lab Animal Resources at the College of Pharmacy, University of Kentucky. Experimental protocols involving the animals were in strict accordance with the National Institutes of Health “Guide for the Care and Use of Laboratory Animals” and were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

**³H][DA Release Assays.** Effects of drug on ³H overflow from rat striatal slices preload with [³H]DA were determined using a previously published method (Dwoskin and Zahniser, 1986). Briefly, rat striatal slices (500 µm, 6–8 mg) were incubated for 30 min in Krebs’ buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl₂, 1.0 mM Na₂HPO₄, 1.3 mM CaCl₂, 11.1 mM glucose, 25 mM NaHCO₃, 0.11 mM L-ascorbic acid, and 0.004 mM ethylenediaminetetraacetic acid, pH 7.4, saturated with 95% O₂/5% CO₂ at 34°C). Slices were then incubated for an additional 30 min in buffer containing 0.1 µM [³H]DA. Each slice was transferred to a superfusion chamber and superfused (1 ml/min) with Krebs’ buffer containing nomifensine (10 µM), a DA uptake inhibitor, and pargyline (10 µM), a monoamine oxidase inhibitor, to ensure that the ³H overflow primarily represented [³H]DA rather than ³H metabolites (Cubeddu et al., 1979; Zumstein et al., 1981; Rapier et al., 1988). When basal outflow was stabilized after 60-min superfusion, two 5-min (5-ml) samples were collected to determine basal ³H overflow followed by superfusion with different concentrations of drugs. For all experiments, slices from a given rat were randomly assigned to all drug concentrations. For concentration-response studies, (S)-(-)-cotinine (1 µM to 3 mM) was added to the superfusion buffer after the collection of the second 5-min sample and remained in the buffer for 60 min. Each superfusion chamber containing one slice was exposed to only one concentration of (S)-(-)-cotinine. Thus, striatal tissue from each rat was exposed to all concentrations of (S)-(-)-cotinine, a repeated-measures design. In each experiment, in addition to slices exposed to (S)-(-)-cotinine, a control slice was superfused in the absence of (S)-(-)-cotinine (i.e., buffer control).

The ability of mecamylamine (100 µM) and DHβE (10 µM) to inhibit (S)-(-)-cotinine (1–100 µM)-evoked ³H overflow was determined in two separate studies. These concentrations of mecamylamine and DHβE were chosen because they were found previously to maximally inhibit (S)-(-)-nicotine-evoked ³H overflow from [³H]DA-preloaded striatal slices (Teng et al., 1997). In one series of experiments, six slices from one rat were superfused in the absence of presence of mecamylamine in each experiment, and in the second series of experiments, six slices from one rat were superfused in the absence or presence of DHβE. Mecamylamine or DHβE was superfused for 60 min before the addition of (S)-(-)-cotinine to the superfusion buffer. Superfusion continued for 60 min in the presence of (S)-(-)-cotinine plus mecamylamine or DHβE. Slices superfused in the absence of mecamylamine or DHβE constituted the (S)-(-)-cotinine control condition. An additional striatal slice from each rat was superfused in the absence of exposure to any drug in each experiment and was referred to as buffer control. Because the purpose of these two studies was to determine the inhibitory effects of the antagonists against (S)-(-)-cotinine [i.e., (S)-(-)-cotinine exposure alone served as control], comparisons were made between the drug-exposure condition and the (S)-(-)-cotinine control rather than between the drug-exposure condition and the buffer control.

To determine whether the effect of (S)-(-)-cotinine was dependent on extracellular calcium, in a separate series of experiments, (S)-(-)-cotinine concentration-response curves were generated in Krebs’ buffer (control buffer) and concurrently in a low-calcium buffer. For the low-calcium buffer, 0.5 mM ethylene glycol bis(β-aminoethyl ether)-N,N',N"-tetraacetic acid was added and CaCl₂ was omitted from the Krebs’ buffer.

At the end of the experiment, each slice was solubilized with TS-2. The radioactivity in the superfuse and tissue samples was determined by liquid scintillation counting (model B1600 TR Scintillation Counter; Packard, Meriden, CT) with an efficiency of 59%. To normalize potential differences in radioactivity between slices of varying weight, fractional release for each sample was calculated by dividing the tritium collected in superfuse by the total tissue tritium at the time of collection and was expressed as percentage of tissue; thus, the unit of fractional release is percentage. Basal outflow was calculated from the average of the fractional release of the two samples just before drug addition. (S)-(-)-Cotinine-evoked total ³H overflow was calculated by summing the increases in fractional release due to drug exposure after subtracting the basal outflow for an equivalent period of drug exposure. Calculation of total ³H overflow also takes...
into account differences among tissue weights, and the unit of total \(^3\)H overflow is percent. Illustrating fractional release as a function of time provides the duration and time course of the effect of drug, and each curve represents the effect of one concentration of the drug. Illustrating the results as total \(^3\)H overflow as a function of drug concentration provides the concentration-response curves allowing determination of pharmacological parameters, which describe the drug-receptor interaction.

**Statistical Analyses.** Repeated-measures two-way analysis of variance (ANOVA) was used to analyze the concentration dependence of (S)-(-)-cotinine-evoked \(^3\)H overflow. The EC\(_{50}\) value for cotinine to evoke \(^3\)H overflow was determined using an iterative nonlinear least-squares curve-fitting program (Prism; GraphPad, San Diego, CA). Repeated-measures two-way ANOVAs also were performed to analyze the time course of the (S)-(-)-cotinine-induced increase in fractional release. The tritium remaining in the striatal slice after (S)-(-)-cotinine exposure was analyzed by repeated-measures one-way ANOVA. Studies determining both the ability of mecamylamine or DHβE to antagonize the effect of (S)-(-)-cotinine and the dependence on external calcium were analyzed by repeated-measures, two-way ANOVA. A protected version of Fisher’s LSD test (i.e., only preplanned comparisons were considered to limit the overall type 1 error rate) was used for post hoc analysis. Results were considered statistically significant when \(P < .05\).

**[\(^3\)H]DA Uptake Assay.** [\(^3\)H]DA uptake was determined using minor modifications of a previously published method (Masserano et al., 1994). Striata were homogenized in 20 ml of ice-cold sucrose solution (0.32 M sucrose and 5 mM sodium bicarbonate, pH 7.4) with 12 passes of a Teflon-pestle homogenizer (clearance, approximately 0.003 in). The homogenate was centrifuged at 2000 \(g\) for 10 min. The supernatant was centrifuged at 12,000 \(g\) for 20 min. The resulting pellet was resuspended in 1.5 ml of ice-cold assay buffer (125 mM NaCl, 5 mM KCl, 1.5 mM KH\(_2\)PO\(_4\), 1.5 mM MgSO\(_4\), 12.5 mM CaCl\(_2\), 10 mM glucose, 0.1 mM l-ascorbate, 25 mM HEPES, 0.1 mM ethylenediaminetetraacetic acid, and 0.1 mM mepazine, pH 7.4). The final protein concentration was 400 \(\mu\)g/ml. Assays were performed in duplicate in a total volume of 500 \(\mu\)l. Aliquots (50 \(\mu\)l of synaptosomal suspension containing 20 \(\mu\)g of protein) were added to assay tubes containing 350 \(\mu\)l of buffer and 50 \(\mu\)l of one of nine concentrations (final concentration, 1 nM to 1 mM) of (S)-(-)-cotinine or vehicle (1 mM HCl). Synapsones were preincubated at 34°C for 10 min before the addition of 50 \(\mu\)l of [\(^3\)H]DA (30.1 Ci/mmol, final concentration 10 nM) and accumulation proceeded for 10 min at 34°C. High-affinity uptake was defined as the difference between accumulation in the absence and the presence of 10 \(\mu\)M GBR 12909. Preliminary studies demonstrate that at 10 min, [\(^3\)H]DA uptake is within the linear range of the time-response curve when experiments are performed at 34°C. Accumulation was terminated by the addition of 3 ml of ice-cold assay buffer containing pyrocatechol (1 mM) and rapid filtration through a Whatman GF/B glass-fiber filter paper (presoaked with buffer containing 1 mM pyrocatechol) using a Brandel Cell Harvester (model MP-43RS; Biochemical Research and Development Laboratories, Inc., Gaithersburg, MD). The filters were washed three times with 3 ml of ice-cold buffer containing 1 mM pyrocatechol and then transferred to scintillation vials and radioactivity determined (model B1600TR scintillation counter, Packard). Protein concentration was determined using bovine serum albumin as the standard (Bradford, 1976).

**Results**

**Effect of (S)-(-)-Cotinine on Superfused Rat Striatal Slices Preloaded with [\(^3\)H]DA.** (S)-(-)-Cotinine evoked an increase in \(^3\)H overflow from rat striatal slices preloaded with [\(^3\)H]DA in a concentration-dependent manner with an EC\(_{50}\) value of 30 \(\mu\)M (Fig. 1). The lowest concentration of (S)-(-)-cotinine to produce a significant increase in \(^3\)H overflow was 10 \(\mu\)M. A plateau in the concentration-response curve was observed beginning at concentrations of 300 \(\mu\)M. A significant main effect of concentration (1 \(\mu\)M to 3 mM) \([F(6,1680) = 133.13, P < .0001]\), a significant main effect of time \([F(13,1680) = 22.89, P < .0001]\), and a significant concentration \(\times\) time interaction \([F(78,1680) = 2.60, P < .0001]\) were found. The time course of these experiments, illustrated in the inset of Fig. 1, shows that basal \(^3\)H overflow under buffer control conditions was stable over the course of the experiment (i.e., no significant differences were found between the first two superfusate samples and later samples collected during the course of superfusion). Because the rate of basal overflow for the buffer control condition was constant over the course of the experiment, (S)-(-)-cotinine-evoked fractional release was compared statistically both with the predrug baseline (within slice basal overflow) and with the control condition (between slices). Fractional release peaked 5 to 10 min after (S)-(-)-cotinine addition to the buffer and the fractional release at the peak was directly dependent on the concentration of (S)-(-)-cotinine. Subsequently, the response to (S)-(-)-cotinine decreased toward basal levels, despite its presence throughout the superfusion period (Fig. 1, inset). Thus, desensitization to (S)-(-)-cotinine was observed using the superfused striatal slice preparation.

After superfusion with (S)-(-)-cotinine (1 \(\mu\)M to 3 mM), the total \(^3\)H remaining in the tissue slices was not different \([F(6,122) = 1.78, P > .05]\) from the control slices. The amount of \(^3\)H remaining in control slices was 176,650 \(\pm\) 8,860 dpm.
The amount of $^{3}\text{H}$ remaining in the slices exposed to the highest concentration (3000 μM) of (S)-(-)-cotinine was 68% of the control slice. Therefore, even though the amount of $^{3}\text{H}$ overflow in superfusate was dependent on the concentration of (S)-(-)-cotinine, the amount of $^{3}\text{H}$ released into superfusate did not significantly decrease the residual tissue $^{3}\text{H}$. Slices typically weighed between 6 and 8 mg wet weight. Therefore, the difference in the weight between slices was relatively small. Additionally, slices were randomly selected for exposure to different concentrations of (S)-(-)-cotinine, such that the influence of weight of the slice was further reduced. Thus, the decreased fractional release after prolonged superfusion with (S)-(-)-cotinine was not due to the depletion of $^{3}\text{H}$DA tissue content.

**Mecamylamine and DHβE Antagonism of (S)-(-)-Cotinine-Evoked $^{3}\text{H}$ Overflow from Rat Striatal Slices Preloaded with $^{3}\text{H}$IDA.** Superfusion with mecamylamine (100 μM) or DHβE (10 μM) alone did not alter $^{3}\text{H}$ overflow, as reported previously (Teng et al., 1997). Mecamylamine (100 μM) significantly inhibited (S)-(-)-cotinine (10–100 μM)-evoked $^{3}\text{H}$ overflow compared with control (absence of mecamylamine; Fig. 2). A significant main effect of (S)-(-)-cotinine concentration $[F(2,21) = 19.30, P < .0001]$, a significant main effect of mecamylamine $[F(1,21) = 23.98, P < .0001]$, and a significant interaction $[F(2,21) = 10.87, P < .001]$ were found. The time course illustrates the complete blockade by mecamylamine of the effect of the highest concentration (100 μM) of (S)-(-)-cotinine tested, such that the response in the presence of mecamylamine was not different from basal efflux before (S)-(-)-cotinine exposure (Fig. 2, inset). Thus, the effect of (S)-(-)-cotinine to evoke $^{3}\text{H}$ overflow was mecamylamine sensitive. Superfusion with DHβE (10 μM) significantly inhibited (S)-(-)-cotinine (10–100 μM)-evoked $^{3}\text{H}$ overflow compared with control (absence of DHβE; Fig. 3). Significant main effects of (S)-(-)-cotinine concentration $[F(2,37) = 10.94, P < .0005]$ and of DHβE $[F(1,37) = 9.22, P < .005]$ were found; however, the (S)-(-)-cotinine × DHβE interaction $[F(2,37) = 2.91, P > .05]$ was not significant. The time course illustrates that similar to mecamylamine, DHβE completely inhibited the effect of the highest concentration (100 μM) of (S)-(-)-cotinine examined, such that the response in the presence of DHβE was not different from basal efflux (Fig. 3, inset). Thus, the effect of (S)-(-)-cotinine to evoke $^{3}\text{H}$ overflow was also DHβE sensitive.

**Calcium Dependence of (S)-(-)-Cotinine-Evoked $^{3}\text{H}$ Overflow from Rat Striatal Slices Preloaded with $^{3}\text{H}$IDA.** Figure 4 illustrates that (S)-(-)-cotinine (10–1000 μM)-evoked $^{3}\text{H}$ overflow was inhibited when slices were superfused in a low-calcium buffer. Low-calcium buffer consisted of Krebs' buffer containing 0.5 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, and CaCl$_2$ was omitted. (S)-(-)-Cotinine was added to superfusion buffer after collection of second 5-min sample. Data are expressed as mean ± S.E.M. total $^{3}\text{H}$ overflow ($n = 8$ rats).
perfused with a low calcium buffer. Significant main effects of (S)(-)cotinine concentration \( F(2,40) = 23.5, P < .0001 \) and of low calcium \( F(1,40) = 25.16, P < .0001 \) were found; however, the \((S)(-)\)-cotinine \times \) low calcium interaction \( F(2,40) = 1.50, P > .05 \) was not significant. Thus, the effect of \((S)(-)\)-cotinine to evoke \(^3\)H overflow was also calcium dependent.

**Effect of \((S)(-)\)-Cotinine on \(^3\)H\)DA Uptake into Rat Striatal Synaptosomes.** (S)(-)Cotinine \((0.1 \) nM to 100 \( \mu\)M) did not inhibit specific uptake of \(^3\)H\)DA into rat striatal synaptosomes (data not shown). Under control conditions (absence of \((S)(-)\)-cotinine), specific \(^3\)H\)DA uptake was 49.0 \pm 7.5 pmol/min/mg protein. The lack of effect of \((S)(-)\)-cotinine over the wide concentration range suggests that \(^3\)H overflow primarily resulted from an increase in DA release rather than an effect on the DA transporter.

**Discussion**

The present study demonstrates that \((S)(-)\)-cotinine evokes \(^3\)H overflow from rat striatal slices preloaded with \(^3\)H\)DA in a concentration- and calcium-dependent manner. Despite the continued presence of \((S)(-)\)-cotinine in the superfusion buffer, a diminished response was observed across the \((S)(-)\)-cotinine exposure period, indicating receptor desensitization. Moreover, the effect of \((S)(-)\)-cotinine was antagonized by the nicotinic receptor antagonists mecamylamine and DHBE. Furthermore, \((S)(-)\)-cotinine had no effect in the \(^3\)H\)DA uptake assay, indicating that the increase in \(^3\)H overflow resulted from an increase in DA release rather than an inhibition of DA uptake. It is important to note that \((S)(-)\)-cotinine had a lower potency than \((S)(-)\)-nicotine, such that the EC\(_{50}\) value for cotinine-evoked \(^3\)H overflow was 30 \( \mu\)M in the present study, whereas that for nicotine has been reported to be 0.1 to 4.0 \( \mu\)M (Izenwasser et al., 1991; Grady et al., 1992, 1994; Sacaan et al., 1995). These findings are consistent with other reports that \((S)(-)\)-cotinine effect may have been due to an insufficient dosing regimen, such that steady-state plasma levels were not attained. Thus, long-term administration of \((S)(-)\)-cotinine may be required to produce pharmacological effects in humans.

Concentrations of cotinine in plasma from smokers have been reported to be in the range of 175 to 500 ng/ml (Benowitz et al., 1983; Kyerematen et al., 1990; Benowitz and Jacob, 1993; Hatsuakumi et al., 1997), although concentrations as high as 900 ng/ml have also been reported (Benowitz et al., 1983). Furthermore, the terminal elimination plasma half-life of \((S)(-)\)-cotinine is approximately 10-fold longer compared with that for \((S)(-)\)-nicotine (Hatsuakumi et al., 1997). Thus, \((S)(-)\)-cotinine plasma concentrations found in smokers correspond to concentrations of 1 to 3 \( \mu\)M, just below the effective concentration in the DA release assay used in the current study. This suggests that the concentration of \((S)(-)\)-cotinine in plasma as a result of tobacco smoking may not reach high enough levels to be pharmacologically active. However, the pharmacokinetics and regional distribution of \((S)(-)\)-cotinine in brain during chronic nicotine administration have not been determined. In a previous study, acute administration (s.c.) of \((S)(-)\)-nicotine to rats afforded brain concentrations of \((S)(-)\)-cotinine approximately 4-fold those of \((S)(-)\)-nicotine at 4 h after injection (Crooks et al., 1997). These relatively higher concentrations of \((S)(-)\)-cotinine are
due to the lack of metabolism of (S)-(−)-cotinine in the brain and to the relatively slower efflux of (S)-(−)-cotinine from brain to periphery compared with (S)-(−)-nicotine (Crooks et al., 1997). The results suggest that (S)-(−)-cotinine may accumulate in brain during chronic smoking and may be present in relatively high concentrations in the brains of chronic smokers. These high concentrations of (S)-(−)-cotinine in the brain could be found to be effective in the DA release assay and may be sufficient to produce neuropharmacological effects. In summary, (S)-(−)-cotinine evokes the release of DA from rat striatal slices in a concentration- and calcium-dependent manner and results in desensitization of nicotinic receptors. The effect of (S)-(−)-cotinine is antagonized by mecamylamine and DHβE, indicating an action at nicotinic receptors. Although the effective concentrations of (S)-(−)-cotinine in the DA release assay are not in the concentration range found in the plasma of tobacco smokers, it is possible that (S)-(−)-cotinine may accumulate in brain after chronic (S)-(−)-nicotine administration and reach effective concentrations to produce neuropharmacological effects. Thus, determination of the pharmacokinetics and regional distribution of (S)-(−)-cotinine in brain is relevant to our understanding of the neuropharmacological effects resulting from tobacco use.

References


Dwoskin et al.


Send reprint requests to: Dr. Linda P. Dwoskin, College of Pharmacy, University of Kentucky, Rose St., Lexington, KY 40536-0082. E-mail: ldwoskin@pop.uky.edu