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COMMENTARY

Contribution of CNS Nicotine Metabolites to the Neuropharmacological Effects of Nicotine and Tobacco Smoking

*Peter A. Crooks and Linda P. Dwoskin**
COLLEGE OF PHARMACY, UNIVERSITY OF KENTUCKY, LEXINGTON, KY 40536-0082, U.S.A.

ABSTRACT. Nicotine, the principal alkaloid in tobacco products, is generally accepted to be the active pharmacological agent responsible for CNS effects resulting from tobacco use. Arguments are presented in this commentary which take issue with this popular dogma, by providing evidence that nicotine metabolites may also be responsible for the CNS effects commonly attributed to nicotine. CNS effects attributed to nicotine include reinforcing effects, mood elevation, arousal, locomotor stimulant effects, and learning and memory enhancement. The reinforcing and locomotor stimulant effects of nicotine have been suggested to be the result of activation of CNS dopaminergic systems, and nicotine-induced modulation of dopaminergic neurotransmission has been studied in detail. Nicotine acts at a family of nicotinic receptor subtypes composed of multiple subunits; however, the exact composition of the subunits in native nicotinic receptors and the functional significance of the receptor subtype diversity are currently unknown. This nicotinic subtype diversity increases the complexity of the potential mechanisms of action of nicotine and its metabolites. Although peripheral metabolism of nicotine has been studied extensively, metabolism in the CNS has not been investigated to any great extent. Recently, studies from our laboratory have demonstrated that several nicotine metabolites are present in the CNS after acute nicotine administration. Moreover, nicotine metabolites are pharmacologically active in neurochemical and behavioral assays. Thus, CNS effects resulting from nicotine exposure may not be due solely to nicotine, but may result, at least in part, from the actions of nicotine metabolites. BIOCHEM PHARMACOL **54**;7: 743–753, 1997. © 1997 Elsevier Science Inc.

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NICOTINE DOGMA

Cigarette smoking is the number one health problem accounting for more illness and death in the U.S. than any other factor [1]. Nicotine is generally accepted to be the active alkaloid in tobacco, and it produces many of its effects on the CNS, some of which may be considered to be beneficial, e.g. mood elevation, arousal, and learning and memory enhancement [2, 3]. The behavioral effects of nicotine are attributed to an action at CNS nicotinic receptors, since most of the effects are blocked by nicotinic receptor antagonists, mecamylamine [2] and DHβE† [4]. For example, mecamylamine blocks nicotine interoceptive cues in behavioral discrimination studies [5], and mecamylamine pretreatment has been shown to influence cigarette smoking behavior and the subjective effects of nicotine [6–8]. Mecamylamine is a CNS-active, noncompetitive nicotinic antagonist, which more effectively blocks the open, rather than the closed, ion channel of the receptor [9–14]; however, it also has been reported to be a noncompetitive channel blocker of NMDA receptors, acting at the MK-801 site within the channel [15–18]. DHβE is a selective, competitive nicotinic receptor antagonist, which displaces nicotine from its binding site [19, 20] and, thereby, inhibits the effects of nicotine in electrophysiological studies [21–23].

Nicotine metabolism has been studied over a period of more than 40 years, and this body of literature represents the most detailed and exhaustive metabolic profile ever compiled for any known xenobiotic [24–26]. Interestingly, almost all of these studies have dealt with the peripheral metabolism of nicotine, and only a few studies have investigated the metabolism of nicotine in the CNS. Surprisingly, little is known about the pharmacological activity of the numerous peripheral nicotine metabolites, which could potentially contribute to the neuropharmacological effects resulting from nicotine exposure and tobacco smoking.

MOLECULAR BIOLOGY OF NICOTINIC RECEPTORS

Molecular biological studies have demonstrated heterogeneity in the composition of nicotinic receptors in both brain and periphery [27–31]. Nicotinic receptors belong to

* Corresponding author. Tel. (606) 257-4743; FAX (606) 257-7564.
† *Abbreviations:* DHβE, dihydro-β-erythroidine; NMDA, *N*-methyl-D-aspartate; DA, dopamine; and MK-801, dizocilpine.

the superfamily of ligand-gated ion channels; stimulation by acetylcholine or nicotine causes the ion channel of the receptor to open, cations to flux, and a resulting rapid (msec) depolarization of the target cell. Expression cloning studies have revealed a surprising diversity among neuronal nicotinic receptors [29, 32–35]. There are two types of neuronal nicotinic receptor subunits, α and β. The α subunits are identified by a pair of adjacent cysteine residues in the amino terminal domain, and are thought to be the agonist-binding subunits. The β subunit also appears to be a co-determinant of the functional properties of the receptor [36]. Eight subtypes of the α subunit (α2–α9) and three subtypes of the β subunit (β2–β4) are found in the vertebrate CNS, and they display different, but overlapping, patterns of CNS expression. The α4 and β2 subtypes are the most common subunits in the brain [37]. The α2, α3, and α4 subunits form functional receptors with either β2 or β4 subunits when co-expressed in *Xenopus* oocytes. These receptors are thought to be pentameric, hetero-oligomers with a stoichiometry of $\alpha_2\beta_3$ [38, 39]; however, α5 coprecipitation indicates that more than two different subunits may assemble together to form a receptor molecule [40–43]. The functional significance of this subtype diversity is still a mystery. However, both native and cloned subtypes are pharmacologically distinct in their response to both nicotinic agonists and antagonists [33, 34, 36, 44–48].

PHARMACOLOGICAL EFFECTS ATTRIBUTED TO NICOTINE

The intrinsic reinforcing properties of nicotine appear to play a major role in the maintenance of smoking behavior in humans [49]. Volunteers who habitually smoke tobacco and are deprived of their cigarettes will self-administer nicotine [7]. Elucidating the mechanism of nicotine's action and determining the effects of chronic nicotine administration are of importance in understanding the initiation and maintenance of tobacco smoking behavior. Evidence for the intrinsic reinforcing property of nicotine includes studies reporting nicotine self-administration by many species on various reinforcement schedules [7, 50–55] and nicotine-induced conditioned place preference in rats [56–58]. Studies clearly indicate that, under the appropriate conditions, nicotine is self-administered avidly by rats [52, 53, 55, 59]. Furthermore, evidence has accumulated that genetic factors play a role in propensity for nicotine self-administration [60]. In contrast to other stimulants, such as amphetamine and cocaine, very precise experimental conditions are required to obtain nicotine self-administration in animal models. Also, nicotine self-administration is decreased in animals and humans by mecamylamine and DHβE [5, 6, 8, 53, 61]. Concomitant with the intrinsic reinforcing effects of nicotine, there is strong evidence indicating that nicotine activates locomotor behavior. In drug-naive rats, acute nicotine administration produces an initial depressant effect on activity, followed by hyperactivity lasting 1 hr or longer [62–64]. With chronic administration, tolerance to the transient hypoactive phase occurs [12, 65–68], whereas sensitization of the hyperactive phase occurs [63, 69–75]. Mecamylamine blocks both the hypoactive and hyperactive phases following acute nicotine injection and also blocks the development of behavioral sensitization following chronic nicotine administration [62, 63]. Thus, nicotine self-administration and nicotine-induced alterations of locomotor behavior are the result of nicotinic receptor stimulation.

Nicotine, amphetamine, and cocaine have been suggested to produce their locomotor stimulant and reinforcing effects by activating the mesolimbic DA system [52, 53, 76, 77]. This neural pathway regulates cognitive and emotional behaviors [78] and is particularly sensitive to the acute effects of psychostimulant drugs [79–81]. Systemic nicotine administration markedly increases the firing rate of mesolimbic DA neurons (ventral tegmental area, VTA) recorded extracellularly [82]. Nicotinic receptors have been localized in the VTA and on DA terminals in the nucleus accumbens [83, 84]. Using intracellular recording techniques, peripheral nicotine administration directly activates rat VTA neurons [85]. Nicotine increases DA turnover [86, 87] and increases DA release in nucleus accumbens *in vitro* [88, 89] and *in vivo* [90–95]. Recently, nicotinic receptors in the VTA have been suggested to be of more importance than those in nucleus accumbens for systemically administered, nicotine-induced stimulation of DA release [96–98]. Furthermore, destruction of nucleus accumbens DA neurons with 6-hydroxydopamine reduces the locomotor stimulant and reinforcing effects of nicotine [52, 71, 98], and causes a reduction in the number of nicotinic binding sites in the nucleus accumbens [84]. These results suggest that a significant portion of the nicotinic receptors are located on DA presynaptic terminals. Thus, the locomotor stimulant and reinforcing effects of nicotine may be due to activation of the mesolimbic DA system. Nicotine metabolites present in the CNS after peripheral nicotine administration may also have locomotor stimulant and reinforcing qualities. This possibility is presently under investigation in our laboratories (see below).

There are many commonalities between the effects of nicotine on the mesolimbic and nigrostriatal DA pathways. Nicotinic receptors are located in the cell body and terminal areas of the nigrostriatal system [10, 11], and 6-hydroxy-dopamine produces a reduction in the number of nicotinic receptors in striatum [83, 84]. In electrophysiologic studies, nicotine activates cell firing of substantia nigra neurons [99, 100]. Nicotine also facilitates DA release from striatal nerve terminals in *in vitro* studies in a stereoselective manner in experiments utilizing slices or synaptosomes [20, 88, 101–118], and in *in vivo* studies [90–93, 119]. Nicotine-evoked DA release is observed at concentrations within the range found in the blood of cigarette smokers (0.2 to 0.8 μM) [120, 121]. Nicotine-evoked DA release in both striatum and nucleus accumbens has been shown to be inhibited by mecamylamine or DHβE and to be Ca^{2+} dependent [20, 96, 109, 112–114, 122]. Pre-exposure of

striatal slices or synaptosomes from mice or rats to nicotine results in functional desensitization, such that subsequent nicotine exposure produces a diminished DA release [112, 115, 117, 118]. The nicotine-induced desensitization is reversible, and is observed even with pre-exposure concentrations that produce no detectable stimulation of DA release. DA release measured using these *in vitro* and *in vivo* assays is more accurately referred to as DA overflow, because it reflects the net events occurring at the dopaminergic presynaptic terminal, i.e. effects on DA release and DA reuptake. Very low concentrations (0.01 to 10 nM) of nicotine have been reported to stereoselectively inhibit DA uptake into striatal minces [106], and tolerance to this effect was observed after chronic nicotine administration [123]. However, the inhibitory effect of nicotine on DA uptake is controversial, and has not been observed by others [124–126]. Interestingly, recent studies report that clearance of exogenously applied DA in nucleus accumbens following systemic administration of nicotine was increased as measured with *in vivo* voltammetry [127], suggested that nicotine facilitates DA transporter activity at least in this brain region. Thus, DA release and uptake assays are useful for determining the ability of nicotine metabolites to interact with nicotinic receptors.

The effect of chronic nicotine administration on DA release is also controversial. When DA release was determined *in vitro* in rodent striatal preparations, either no alteration [128], a decrease [129], or an increase [130] in nicotine-evoked [^{3}H]DA release has been observed after chronic nicotine administration. Similarly, in *in vivo* studies, chronic nicotine administration resulted in either no change [91] or in an increase [73] in DA release in nucleus accumbens in response to nicotine administration. There could be several reasons for these conflicting results such as the species of animal used, the differential regional effects of nicotine in brain, the different dosing regimens, and the different routes of administration utilized. However, no consensus has been reached to explain these variable findings. Consensus has been reached regarding the increase in nicotine binding sites following chronic (intermittent or continuous) nicotine administration [67, 69, 70, 131–137], and is thought to be due to receptor desensitization [115, 119, 131, 138], a decreased rate of nicotinic receptor turnover [14], and/or a recruitment or shift in the equilibrium of a reserve pool of receptors into a mature receptor pool that has the capacity for high affinity ligand binding [139]. Similarly, human brain tissue from cigarette smokers has a higher density of nicotinic receptors compared with that of nonsmokers [140]. Despite the consistent finding of increased receptor number following chronic nicotine exposure, the regulation of nicotinic receptors is complex. Recent studies report up-regulation of nicotinic receptors following chronic mecamylamine administration [141]. Thus, receptor up-regulation can result from chronic administration of either nicotinic agonists or antagonists.

Some of the above discrepancies may be explained eventually by nicotinic receptor subtype diversity and regional distribution. The predominant nicotinic receptor in rodent brain is the $\alpha4\beta2$ subtype, and it has been suggested to correspond to the high affinity [^{3}H]nicotine binding site [14, 142, 143]. Based on the neuronal bungarotoxin sensitivity of nicotine-evoked DA release, the $\alpha3\beta2$ subtype has been implicated as the subtype responsible for nicotine-evoked DA release [20, 44, 144]. However, more recent work based on desensitization kinetics of nicotine-evoked DA release suggests that the $\alpha4\beta2$ subtype or perhaps a hybrid receptor subtype is responsible for modulation of DA release [115], particularly since it has been reported that more than two different subunits may assemble to form a functional receptor [40–43]. More detailed studies of the pharmacology of both cloned and native nicotinic receptors are needed to allow an unambiguous pharmacological identification of nicotinic receptor subtypes involved in functional DA responses to both nicotine and its metabolites.

CENTRAL NICOTINE METABOLISM

Although little is known about the metabolism of nicotine in the human brain, a plethora of information on peripheral nicotine metabolism has been generated over the past 40 years [25, 26]. The complete peripheral metabolic profile of nicotine has been elucidated only recently, i.e. with the recent detection and quantitation of several *major* peripheral glucuronidated nicotine metabolites [145]. In both rats and humans, the primary urinary metabolites are cotinine and secondary cotinine metabolites [145–147], together with variable but small amounts of nicotine *N*-oxide, norcotinine (demethylcotinine), and nornicotine (demethylnicotine).

N-Demethylation is generally recognized as a metabolic pathway for nicotine in the periphery due to the isolation of both nornicotine and norcotinine in urine from a number of animal species following nicotine administration [148–152]. Norcotinine has also been reported as a urinary metabolite in smokers [152–154]. Other studies have shown that norcotinine occurs as a urinary metabolite of cotinine in dogs, mice, and rats, but is not detected after administration of cotinine to humans [148–152, 155]. Norcotinine is also formed *in vitro* from cotinine in hepatic, pulmonary, and renal tissue [156]. It is generally recognized that the metabolism of nicotine to nornicotine in the periphery is a relatively minor pathway. In a study by Cundy and Crooks [157], only 1.6% of the administered (i.p.) dose of [2′-^{14}C]nicotine was found in guinea pig 24-hr urine. Also, after single arterial doses of labeled nicotine to rats, nornicotine accounted for only 8% of the total recovery of administered radioactivity [153]. The plasma half-life of nornicotine (7.2 to 8.5 hr) is longer than that of nicotine (0.9 to 1.4 hr) in both smokers and nonsmokers [147].

Pharmacokinetic studies have quantitated nicotine in brain after various routes of administration in several animal species [134, 158–159]. However, these studies did not determine the relative concentrations of nicotine and

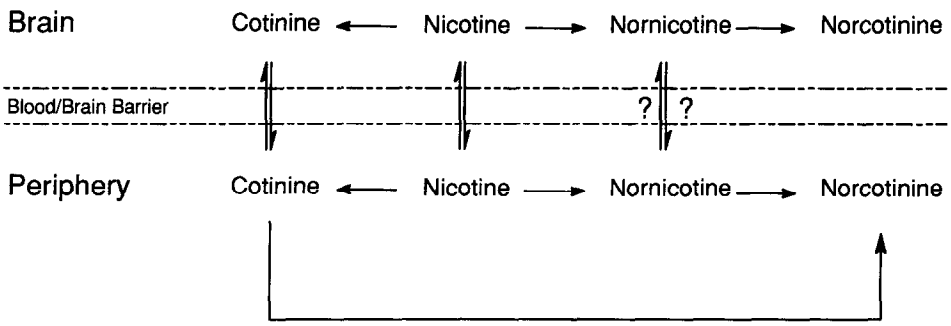

FIG. 1. Current status of knowledge on the origin of CNS metabolites of nicotine after peripheral nicotine administration.

its metabolites in brain. In some studies, fractional analysis of nicotine and its metabolites in brain has been performed. [^{14}C]Cotinine and [^{14}C]nicotine-*N*-oxide were detected by high pressure liquid radiochromatography in mouse brain following i.p. administration of [^{14}C-*N-methyl*]nicotine [160, 161]. N-Demethylated metabolites of nicotine (e.g. nornicotine and norcotinine) cannot be detected in these studies, since the radiolabel is lost during nicotine metabolism. Surprisingly, in one report, cotinine was not found in rat brain after peripheral nicotine injection when analyzed by gas liquid chromatography–mass spectrometry and single ion monitoring [162]. Since we and others have found nicotine metabolites to be pharmacologically active [163–168], experiments to determine the presence and concentrations of all nicotine metabolites in brain are necessary to fully elucidate their contribution to the neuropharmacological effects of nicotine.

Figure 1 summarizes our current understanding of the origin of nicotine metabolites present in brain after peripheral nicotine administration, and is based primarily on our ongoing studies. We recently studied the comparative time course of appearance of nicotine and cotinine in brain after peripheral nicotine administration [169, 170]. Nicotine distribution to brain is rapid, maximum uptake occurring between 30 and 60 min. Also, nicotine efflux out of brain is relatively rapid, since only low levels were detected in the CNS at 4 hr post-injection. Cotinine accumulated in brain much more slowly than nicotine, and was still detectable at 18 hr post-injection. Thus, nicotine is distributed to brain quickly, whereas cotinine is distributed to brain more slowly and has a longer residence time in the CNS than nicotine.

In addition to cotinine, we have identified two other nicotine metabolites in brain after peripheral nicotine administration, i.e. the demethylated metabolites, nornicotine and norcotinine. A fourth, minor, N-demethylated nicotine metabolite, which as yet is unidentified, has also been detected. The metabolite 3-hydroxycotinine and its glucuronide conjugate were not detected in brain even though these are major biotransformation products in the periphery.

Of note is the observation that maximal metabolism of peripherally administered nicotine occurs at 4 hr post-injection. The absolute amounts of nicotine metabolites present in whole brain after acute nicotine administration have also been determined. When a single s.c. dose (0.54 mg/kg) of nicotine was administered to rats, nicotine (12.1 ng/g brain), cotinine (44.6 ng/g brain), nornicotine (11.7 ng/g brain), and norcotinine (3.1 ng/g brain) were found at 4 hr post-injection [170]. Thus, in the case of nornicotine, its concentration in brain approximates 0.1 μM, and this is within the pharmacologically relevant range that evokes DA release from striatal tissue [166, 168]. It is important to note that the latter results were obtained after a single bolus injection; however, the results do not address the important issue of metabolite accumulation after chronic nicotine administration. Compared with nicotine, nornicotine likely has a longer residence time in the CNS. Thus, nornicotine probably accumulates in brain following chronic peripheral nicotine administration, as indicated by the significantly longer plasma half-life for nornicotine [147]. Furthermore, nornicotine levels in brain resulting from exposure to tobacco smoke may also originate from the absorption of nornicotine alkaloid present in the cigarette, since tobacco products contain nornicotine as a major alkaloidal component [171–173]. Thus, even higher concentrations of nornicotine may be present in the CNS of individuals exposed to tobacco smoke as compared with those individuals administered the same amount of nicotine alone. The accumulation of the more polar nicotine metabolites in brain following chronic nicotine administration may lead to their persistence in brain long after smoking ceases. The persistence of these pharmacologically active nicotine metabolites in brain may explain, at least in part, the ensuing neuroadaptation and complex regulation of nicotinic receptors observed after tobacco smoking.

Therefore, the identification of pharmacologically active nicotine metabolites in brain following peripheral nicotine administration provides evidence in support of the contention that metabolites of nicotine contribute to the CNS effect of tobacco product usage, and affords new information pertinent to our understanding of the fundamental processes involved in the neurochemical and behavioral effects of nicotine.

NEUROPHARMACOLOGICAL PROPERTIES OF NICOTINE METABOLITES

In contrast to the wealth of information on the effects of nicotine, relatively little is known about the effect of nicotine metabolites on either DA neurochemistry or

DA-mediated behaviors. From a molecular viewpoint, the structure of nornicotine suggests that it may have significant nicotinic receptor agonist properties. Of the three identified metabolites of nicotine in brain, only two, i.e. cotinine and nornicotine, have been examined in neurochemical and behavioral studies.

Other than our own work, few studies have examined the effects of nicotine metabolites on DA release. We have reported that S(−)nornicotine evokes a concentration-dependent and Ca^{2+}-dependent increase in endogenous DA release from rat striatal slices [166]. (±)Nornicotine was also shown to evoke a concentration-dependent increase in DA release from mouse striatal synaptosomes [20]; however, racemic-nornicotine was used in the latter study. Nicotinic receptor mediation of the effect of S(−)-nornicotine has been assessed by determining the sensitivity to nicotinic antagonists [168]. In a concentration-dependent manner, both mecamylamine and DHβE robustly inhibited nicotine-evoked DA release from rat striatal slices, in good agreement with the results of previous studies [109, 114]. Moreover, mecamylamine and DHβE also effectively inhibited DA release evoked by low concentrations (<100 μM) of S(−)nornicotine, but both antagonists were ineffective in inhibiting the response to high S(−)nornicotine concentrations (100 μM to 3 mM). Mecamylamine-sensitive and DHβE-sensitive nornicotine concentration-response curves revealed the maximal response and EC_{50} values (2.54 ± 0.67 and 0.88 ± 0.31 μM, respectively). Interestingly, S(−)nornicotine has a potency similar to that of S(−)nicotine in the DA release assay. Thus, these results suggest that the effect of S(−)nornicotine at concentrations <100 μM is the result of stimulation of nicotinic receptors, and that high concentrations of S(−)nornicotine may release DA via a nonselective mechanism that is insensitive to the inhibitory effects of mecamylamine and DHβE. Demonstration of the stereoselective effect at low nornicotine concentrations (1–100 μM), such that S(−)nornicotine produced a greater effect than did the R(+)enantiomer over the same concentration range, is consistent with an interpretation of a receptor-mediated effect across this range.

Interestingly, nornicotine has been shown to compete with [^{3}H]nicotine for its binding site in rat brain membranes; however, stereoselective displacement was not observed [19, 174, 175]. Furthermore, in these latter studies nornicotine has been reported to have a 10-fold lower affinity for nicotinic receptors than does nicotine. However, in DA release studies, nornicotine and nicotine were found to have a similar potency. Taken together, these results support the hypothesis that different nicotinic receptor subtypes may be responsible for modulation of DA release and for high affinity nicotine binding.

In behavioral studies, R(+)nornicotine administered to rats decreased locomotor activity, an effect that was blocked by mecamylamine [75]. Moreover, in rats chronically administered nicotine, R(+)nornicotine challenge increased activity, demonstrating cross-sensitization and suggesting a common nicotinic receptor mechanism [75]. More recently, we have found that S(−)nornicotine produces behavioral sensitization following repeated administration, whereas R(+) nornicotine does not [167]. Although, following chronic administration, R(+)nornicotine did not produce an overt behavioral effect, it enabled an enhanced response to nicotine challenge, such that the rats behaved as though they had been chronically administered nicotine [167]. Thus, our results are in agreement with the findings of Stolerman *et al.* [75]. In operant behavioral studies using food reinforcement in monkeys, dogs, and rats [163–165], S(−)nornicotine, R(+) nornicotine, and cotinine were found to be active, and were suggested to contribute to the pharmacologic profile of nicotine, the parent compound. However, mecamylamine did not antagonize the effect of cotinine, suggesting the involvement of a non-nicotinic mechanism. Unfortunately, inhibition of nornicotine by mecamylamine was not examined in the latter studies.

Additionally, we have reported that cotinine evokes DA release from rat striatal slices [176]. Cotinine was ~10-fold less potent than nicotine in producing this effect. Furthermore, chronic administration of cotinine did not produce behavioral sensitization in rats, nor did it enable behavioral sensitization to a challenge dose of nicotine [177]. Cotinine was shown previously to have low affinity for nicotinic receptors [178, 179]. Several studies have clearly shown that cotinine is behaviorally active, and receptor sites other than nicotinic receptors are likely mediators of these behavioral effects [165, 180, 181]. Interestingly, cotinine administration has been reported to reduce abstinence signs of nicotine withdrawal in humans [182, 183]. Cotinine administration (i.v., in amounts that result in blood levels normally seen in moderately heavy smokers) to abstinent smokers was reported to reduce significantly self-reports of the desire to smoke and irritability [182]; however, comparisons with a placebo group were not made, weakening the conclusions that cotinine produces a neuropharmacological effect. More recently, in a study by Keenan *et al.* [183], cotinine administration (i.v.) to abstinent cigarette smokers in amounts sufficient to achieve cotinine serum levels commonly encountered during daily smoking decreased subjective self-reported ratings of abstinence-induced restlessness, anxiety, tension, and insomnia when compared with the placebo group. Thus, cotinine appears to be behaviorally active under the conditions of the latter study, and was suggested to be responsible, in part, for mediating the effects of nicotine. Findings of the latter study are controversial, however, since in more recent studies, cotinine has not been found to be effective in reducing craving for tobacco. Therefore, cotinine, the major CNS nicotine metabolite, may contribute to the neuropharmacological effects of smoking, and determining its pharmacokinetics of accumulation in brain after chronic nicotine administration is relevant to understanding its neuropharmacological action and its role in the CNS effects of tobacco smoking.

IMPORTANCE OF FINDINGS

Taken together, three important conclusions can be drawn: (1) following peripheral administration of nicotine, nicotine metabolites (cotinine, nornicotine, norcotinine, and a fourth unidentified metabolite) are present in brain; (2) nicotine, nornicotine, and cotinine have been shown to evoke DA release from rat striatal slices *in vitro*, and the nornicotine effect was nicotinic-receptor mediated, i.e. mecamylamine-sensitive, DHβE-sensitive, stereoselective, and Ca^{2+}-dependent; and (3) S(−)nornicotine and R(+)nornicotine, similarly to nicotine, were capable of activating the neural mechanism responsible for behavioral sensitization, even though R(+)nornicotine produced no overt behavioral effects after repeated administration. Since behavioral sensitization has been suggested to be dependent on activation of DA systems and since nornicotine evokes DA release, these results suggest a significant role for nornicotine in the behavioral sensitization produced by nicotine.

In conclusion, the neuropharmacological effects of nicotine metabolites and their role in central effects of nicotine exposure as a consequence of tobacco usage, appear to have been under-investigated. More importantly, until recently, very little was known about nicotine biotransformation in brain, and even less information is available on the characterization and quantitation of central nicotine metabolites. However, it is clear from the studies described in this commentary, some of which are very recent, that the CNS effects resulting from nicotine exposure may not be attributed solely to nicotine, but may result, at least in part, from the actions of central nicotine metabolites. Thus, future studies should be directed towards establishing the identity and potential for accumulation of pharmacologically active nicotine metabolites in brain after chronic nicotine administration.

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