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Short communication

Minor alkaloids of tobacco release [^{3}H]dopamine from superfused rat striatal slices

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Abstract

In addition to *S*(−)-nicotine, several minor tobacco alkaloids ((±)-nornicotine, anabaseine, *S*(−)-anabasine, and *S*(−)-*N*-methylanabasine) are present in tobacco smoke. This study demonstrates that these alkaloids increase fractional ^{3}H release in a concentration-dependent manner from rat striatal slices preloaded with [^{3}H]dopamine, with desensitization of this response. The rank order of EC_{50} values was *S*(−)-nicotine (3.0 ± 2.2 μM) > (±)-nornicotine (6.7 ± 2.1 μM) > anabaseine (15.4 ± 6.1 μM) = *S*(−)-*N*-methylanabasine (16.3 ± 4.7 μM) = *S*(−)-anabasine (19.3 ± 3.2 μM). The alkaloids did not modulate fractional ^{3}H release evoked by electrical-field depolarization. Thus, minor tobacco alkaloids may contribute to the apparent neuroprotective effects of smoking in neurodegenerative diseases.

Keywords: Nicotine; Nornicotine; Anabaseine; Anabasine; *N*-Methylanabasine; Dopamine release; Striatum

1. Introduction

Although controversial (Riggs, 1992), an inverse association between tobacco smoking and the incidence of neurodegenerative diseases (Parkinson's and Alzheimer's) has been confirmed in a number of investigations (see reviews: Baron, 1995; Brenner et al., 1993), indicating that men or women who have ever smoked cigarettes have a lower risk compared to never smokers. In animal models of Parkinson's disease in which 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered to mice, continuous cigarette smoke exposure significantly antagonized the MPTP-induced neurotoxicity (Carr and Rowell, 1990). Parkinson's and Alzheimer's patients have also been reported to have fewer brain nicotinic receptors at autopsy (Perry et al., 1987; Whitehouse et al., 1988). Furthermore, nicotine administration has been reported to improve attention and information processing in Alzheimer's patients (Sahakian et al., 1989). Recently, the development of nicotine and structurally related compounds as therapeutic agents for Alzheimer's disease has been reported (Caldwell and Lippiello, 1993).

In addition to nicotine, minor tobacco alkaloids may contribute to the neuropharmacological and neuroprotective effects of smoking tobacco. The present study examines for the first time the effects of the minor tobacco alkaloids, (±)-nornicotine, anabaseine, *S*(−)-anabasine and *S*(−)-*N*-methylanabasine (Fig. 1), on [^{3}H]dopamine release from rat striatal slices.

2. Materials and methods

2.1. Materials

[^{3}H]Dopamine (dihydroxyphenethylamine 3,4-ethyl-2-[*N*-^{3}H]; specific activity, 25.6 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). *S*(−)-*N*-Methylanabasine perchlorate salt was prepared via *N*-1′-methylation of *S*(−)-anabasine (Niranjan and Crooks, unpublished data). *S*(−)-Nicotine ditartrate, *S*(−)-anabasine perchlorate salt and (±)-nornicotine free base were purchased from Research

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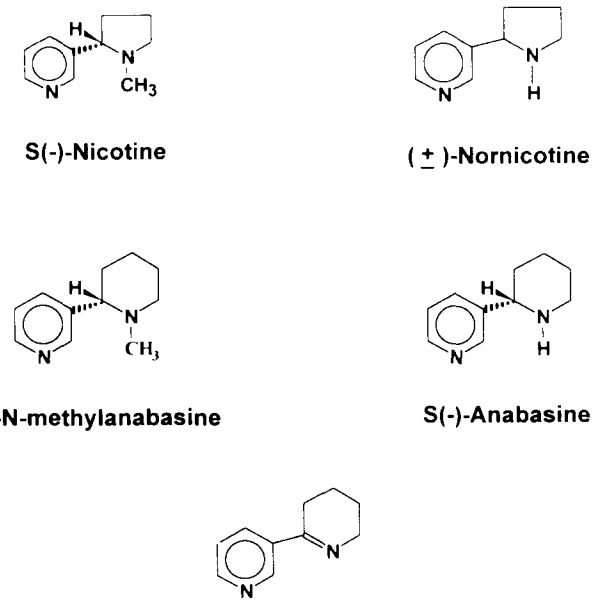

Fig. 1. Structures of $S(-)$-nicotine, (±)-nornicotine, anabaseine, $S(-)$-anabasine and $S(-)$-*N*-methylanabasine.

Biochemicals (Natick, MA, USA), Fluka (Ronkonkoma, NY, USA) and Lancaster Synthesis (Windham, NH, USA), respectively. Pargyline hydrochloride and sodium octylsulfonate were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ascorbic acid and ascorbic acid oxidase were purchased from AnalaR (BDH, Poole, UK) and Boehringer Mannheim Biochemicals (Indianapolis, IN, USA), respectively. TS-1 solubilizer was purchased from Research Products International (Mount Prospect, IL, USA). Anabaseine perchlorate salt and nomifensine maleate were kindly provided by the R.J. Reynolds Tobacco Co. (Winston Salem, NC, USA) and Hoechst-Roussel Pharmaceuticals (Somerville, NJ, USA), respectively. All other chemicals were purchased from Fisher (Pittsburg, PA, USA).

2.2. Animals

Male Sprague-Dawley rats (150–200 g) were obtained from Harlan Laboratories (Indianapolis, IN, USA) and were housed two per cage with free access to food and water in the Division of Lab Animal Resources. Experimental protocols involving the animals were approved by the Institutional Animal Care and Use Committee at the University of Kentucky.

2.3. Dopamine release assay

The effect of the tobacco alkaloids on fractional ^3H release from striatal slices preloaded with [^3H]dopamine was determined using a previously described method (Dwoskin and Zahniser, 1986). Nomifensine (a dopamine uptake inhibitor; 10 μM) and pargyline (an irreversible monoamine oxidase inhibitor; 10 μM) were included in the superfusion buffer from the beginning of superfusion and until the end of the experiment. Superfusate samples were collected at 5 min intervals; collection beginning after 60 min of superfusion, when basal fractional release had stabilized to a low constant level. A single concentration (0.1–100 μM) of $S(-)$-nicotine, (±)-nornicotine, anabaseine, $S(-)$-anabasine or $S(-)$-*N*-methylanabasine was included in the superfusion buffer of individual chambers after collection of the second 5-min sample and remained in the buffer until the end of the experiment. The chambers were assigned randomly to an experimental condition. Each chamber was exposed to only one concentration of alkaloid. One chamber in each experiment was superfused throughout the entire experiment with buffer only, and served as control.

The ability of these minor alkaloids to modulate electrically evoked overflow was also determined. Following the 75 min superfusion with the various concentrations of alkaloid, an electrical-field stimulation was delivered by a Grass stimulator (model SD9, Quincy, MA, USA) and consisted of trains of unipolar, rectangular pulses (1 Hz, 20 mA, 2 ms duration, for 1 min). Subsequently, fifteen 5-min samples were collected. Electrically evoked fractional ^3H release was determined in the absence and presence of alkaloid.

To determine the ^3H content of the tissue at the end of each experiment, each slice was solubilized with 0.5 ml of TS-1 by incubating at 28° C overnight; the pH and volume of the solubilized tissue samples were adjusted to that of the superfusate samples. Radioactivity was determined by liquid scintillation counting (Packard model B1600 TR). Data were analyzed as previously described (Dwoskin and Zahniser, 1986).

3. Results

Basal fractional release of tritium prior to superfusion with the tobacco alkaloids was not significantly different between experiments (1.11 ± 0.03%, range 0.83–1.61; $n = 27$). To determine if the minor tobacco alkaloids increase ^3H overflow from rat striatal slices preloaded with [^3H]dopamine, the effects of $S(-)$-nornicotine, anabaseine, $S(-)$-anabasine and $S(-)$-*N*-methylanabasine were examined. Each of the tobacco alkaloids examined, including $S(-)$-nicotine, produced a concentration-dependent increase in ^3H overflow (Fig. 2, top panel). The lowest concentration of each minor alkaloid which significantly increased ^3H overflow compared to control was as follows: $S(-)$-nornicotine, 1.0 μM; anabaseine, 1.0 μM; $S(-)$-

anabasine, 10 μM; and, S(−)-N-methylanabasine, 10 μM. The effect of the minor alkaloids was also compared to that of the major alkaloidal component of tobacco, S(−)-nicotine. The lowest concentration (0.1 μM) of S(−)-nicotine increased total ^{3}H overflow greater than the same concentration of all the minor tobacco alkaloids examined. In a separate experiment examining the effect of lower concentrations of nicotine, it was found that concentrations below 0.05 μM did not evoke ^{3}H release. Additionally, the ability of the minor tobacco alkaloids to modulate electrically evoked ^{3}H overflow was determined. Concentrations of the minor tobacco alkaloids which did not evoke ^{3}H overflow, also did not modulate electrically evoked ^{3}H overflow (data not shown).

To determine the potency of the alkaloids to evoke ^{3}H overflow from striatal slices, the EC_{50} value was determined for each alkaloid. The rank order of EC_{50} values was S(−)-nicotine (3.0 ± 2.2 μM) > (±)-nornicotine (6.7 ± 2.1 μM) > anabaseine (15.4 ± 6.1 μM) = S(−)-N-methylanabasine (16.3 ± 4.7 μM) = S(−)-anabasine (19.3 ± 3.2 μM). Thus, the minor alkaloids were between 2- and 6-fold less potent compared to S(−)-nicotine-evoked ^{3}H overflow.

The time course for a representative minor tobacco alkaloid, S(−)-anabasine, illustrates the concentration-dependent increase in fractional ^{3}H release from rat striatal slices preloaded with [^{3}H]dopamine and the observed desensitization (Fig. 2, bottom panel). Fractional release was increased immediately following exposure to each S(−)-anabasine concentration, except at the lowest concentration (0.1 μM), which was not different from control over the entire period of superfusion. The peak response occurred after 5–10 min S(−)-anabasine exposure and subsequently returned towards control values despite continued exposure of alkaloid. After 35 min of exposure to 1.0 μM, fractional release was not different from control, whereas higher concentrations increased fractional release over the entire period of superfusion. The other minor alkaloids also exhibited a similar time course of concentration-dependent increase in fractional ^{3}H release (data not shown).

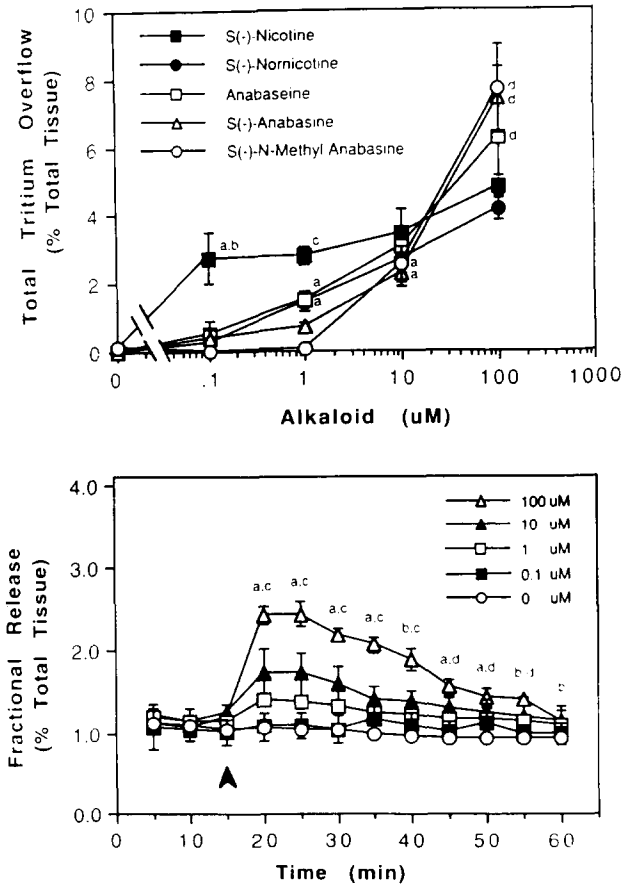

Fig. 2. Minor tobacco alkaloids evoke a concentration-dependent increase in dopamine release from rat striatal slices preloaded with [^{3}H]dopamine. Alkaloid was added to the superfusion buffer after collection of the second 5-min sample and remained in the buffer until the end of the experiment. Top panel illustrates total ^{3}H overflow evoked by alkaloid exposure, which was calculated by summing the increases in fractional ^{3}H release collected during alkaloid exposure and subtracting ^{3}H outflow during an equivalent period of pre-exposure to alkaloid. ^{3}H outflow was calculated from the average of the tritium collected in the two 5-min samples just before the addition of tobacco alkaloid. Data are presented as mean ± S.E.M. total ^{3}H overflow. Two-way ANOVA revealed a significant alkaloid × concentration interaction ($F(16,68) = 4.41$, $P < 0.0001$). Subsequent one-way repeated measures ANOVAs revealed significant concentration-dependent effects of all alkaloids examined: for S(−)-nicotine, $F(4,12) = 32.60$, $P < 0.0001$, $n = 4$; for (±)-nornicotine, $F(4,28) = 55.95$, $P < 0.0001$, $n = 8$; for anabaseine, $F(4,12) = 10.72$, $P < 0.001$, $n = 4$; for S(−)-anabasine, $F(4,8) = 39.57$, $P < 0.0001$, $n = 3$; and, for S(−)N-methylanabasine, $F(4,8) = 23.70$, $P < 0.0005$, $n = 3$. Duncan's new multiple range post hoc test revealed significant ($P < 0.05$) differences: [a] lowest concentration of alkaloid which was different from control; [b] different from (±)-nornicotine, anabaseine, S(−)-anabasine and S(−)-N-methylanabasine at 0.1 μM; [c] different from S(−)-anabasine and S(−)-N-methylanabasine at 1.0 μM; [d] different from S(−)-nicotine at 100 μM. Bottom panel illustrates the time course of the concentration-dependent effect of a representative alkaloid, S(−)-anabasine. Data are presented as mean ± S.E.M. ($n = 3$) fractional release as a function of time after sample collection began. Fractional release was calculated by determining the amount of tritium in each superfusate sample and dividing by the total tritium present in the tissue at the time of the sample collection. Two-way analysis of variance revealed a significant concentration × time interaction ($F(56,112) = 12.64$, $P < 0.0001$). Duncan's new multiple range post hoc test revealed significant ($P < 0.05$) differences at each time point: [a] 1.0–100 μM different from control; [b] 10 and 100 μM different from control; [c] 1.0, 10 and 100 μM different from each other; [d] 100 μM different from 1 and 10 μM.

4. Discussion

Since tobacco contains a number of minor alkaloids structurally related to S(−)-nicotine, these alkaloids may contribute to the neuropharmacological effects of smoking tobacco. (±)-Nornicotine, S(−)-anabasine and S(−)-*N*-methylanabasine are alkaloidal components of *Nicotiana tabacum*, the major plant constituent in the production of cigarette tobacco (Leete and Mueller, 1982). Anabaseine is a degradation product of S(−)-anabasine, and is also present in the leaves of *N. tabacum* (Kisaki and Tamaki, 1966).

The current study demonstrates that several minor tobacco alkaloids ((±)-nornicotine, anabaseine, S(−)-anabasine and S(−)-*N*-methylanabasine), which are structurally related to nicotine, also evoke a robust concentration-dependent increase in fractional release of tritium from striatal slices preloaded with [^{3}H]-dopamine. The results are in agreement with previous reports of a concentration-dependent, S(−)-nornicotine-induced increase in endogenous dopamine release from rat striatal slices (Dwoskin et al., 1993) and (±)-nornicotine-induced release of [^{3}H]dopamine from mouse striatal synaptosomes (Grady et al., 1992). In the present study, the time course of the response to (±)-nornicotine illustrates that after approximately 10 min of exposure, the increase in fractional release returned towards control values, indicative of desensitization.

Compared to the effect of S(−)-nicotine (EC_{50} was 3.0 ± 2.2 μM), (±)-nornicotine afforded a similar (2-fold lower) potency and magnitude of response. In the present study, comparisons were made between the effect of *racemic* nornicotine (i.e., a 1:1 mixture of S(−)- and R(+)-nornicotine) and S(−)-nicotine, because both enantiomers of nornicotine are present in approximately equal amounts in tobacco (Leete and Mueller, 1982) and because nornicotine does not exhibit stereoselectivity in the inhibition of high affinity [^{3}H]nicotine binding to rat brain membranes (Copeland et al., 1991).

In the present study, S(−)-anabasine evoked a concentration-dependent increase in fractional release of tritium which was similar in magnitude to S(−)-nicotine, but S(−)-anabasine was 6-fold less potent than S(−)-nicotine. These results are in agreement with a previous report that (±)-anabasine stimulates [^{3}H]-dopamine release from mouse striatal synaptosomes (Grady et al., 1992). Racemic anabasine has also been reported to inhibit (K_i = 250–300 nM) high affinity binding of [^{3}H]nicotine to rat and mouse striatal membranes (Reavill et al., 1988; Grady et al., 1992). However, direct comparisons between S(−)-anabasine and (±)-anabasine are speculative, since potential stereoisomeric effects of this alkaloid have not been determined.

Apart from nornicotine and anabasine, little is known about the neuropharmacology of the other minor tobacco alkaloids. The current study demonstrates that other minor alkaloids, S(−)-*N*-methylanabasine and anabaseine, also evoke a concentration-dependent increase in fractional release of tritium from rat striatal slices preloaded with [^{3}H]dopamine. The effect of S(−)-anabasine was similar to S(−)-*N*-methylanabasine, when compared to the other three alkaloids studied. S(−)-*N*-Methylanabasine was the most efficacious of the tobacco alkaloids, but it was 5-fold less potent than S(−)-nicotine. The time courses illustrate the immediacy and robustness of the response, as well as the apparent desensitization which develops after 10–15 min of exposure. In contrast, anabaseine was the least efficacious of the tobacco alkaloids. The time course for anabaseine illustrates the increase in fractional release; and that desensitization was still apparent.

In conclusion, this study clearly demonstrates concentration-dependent effects of minor tobacco alkaloids on fractional release of tritium from rat striatal slices preloaded with [^{3}H]dopamine. The results suggest that these minor tobacco alkaloids may contribute to the neuropharmacological effects of tobacco smoke and may contribute to the neuroprotective effect of tobacco smoke in Parkinson's and Alzheimer's diseases. Thus, studies designed to investigate the pharmacological effects of tobacco smoke need to take into account the possible contribution of the minor alkaloidal components of tobacco.

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