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Brief Report

Effect of Chronic Nicotine Administration on Monoamine and Monoamine Metabolite Concentrations in Rat Brain

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Summary: The effects on rat brain tissue monoamine and monoamine metabolite concentrations of chronic nicotine administration at two doses (3 and 12 mg/kg/day) using constant infusion were studied. After 21 days of treatment, tissue concentrations of dopamine (DA), norepinephrine (NE), 5-hyroxytryptamine (5-HT), and several metabolites in striatum, hypothalamus, and frontal cortex were determined by high performance liquid chromatography with electrochemical detection. Compared with a control group, nicotine treatment significantly decreased NE in frontal cortex but not in other regions. The concentration of 5HT also was decreased in frontal cortex but increased in the hypothalamus at the higher dose of nicotine. The 5HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) was not significantly altered in any region. The 5HT index (5-HIAA/5-HT) was significantly decreased in the hypothalamus and increased in frontal cortex at the higher dose. Concentrations of DA and the metabolite homovanillic acid (HVA) were not significantly altered by nicotine. Nevertheless, significant decreases in the DA metabolite dihydroxyphenylacetic acid (DOPAC) were observed in both striatum and hypothalamus. Moreover, the DA index [(DOPAC + HVA)/DA] was significantly decreased in all three brain regions. In contrast to other studies using acute dose and in vitro perfusion paradigms that have reported increased CNS catecholamine release stimulated by nicotine, chronic administration appears to be associated with decreased catecholamine turnover in some brain regions. **Key Words:** Nicotine—Dopamine—Dihydroxyphenylacetic acid—Homovanillic acid—Norepinephrine—5-Hydroxytryptamine.

The clinical neuropsychopharmacology of nicotine is complex, as demonstrated by the paradoxical biphasic acute response of "alerting" followed by

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"calming" that accompanies nicotine administration in the form of inhaled tobacco smoke (1). It appears that the CNS effects of nicotine involve a number of neurotransmitters, including significant effects on noncholinergic systems (2). Most basic science investigations that have examined the effects of nicotine on CNS catecholamines and indoleamines have made use of either in vivo studies using a single acute dose or in vitro perfusion of brain tissue preparations. Such studies of the central and peripheral nervous systems have in general indicated that nicotine stimulates increased release of both dopamine (DA) and norepinephrine (NE) (3–9). With regard to 5-hydroxytryptamine (5-HT), nicotine administration has been reported to decrease CNS turnover, in particular in the hippocampus (2,10,11).

It has been observed that tobacco users vary their smoking habits to maintain a relatively constant blood level of nicotine (12). Because of the short half-life of nicotine, it is obvious that animal studies using a single acute dose or a few repeated injections are less than optimal as models for the chronic effects of nicotine. The present study is an attempt to more closely model the pattern of nicotine administration commonly observed in humans. Rats were implanted with osmotic minipumps that delivered either saline or one of two doses of a nicotine solution as a constant subcutaneous infusion. After chronic treatment, the whole-tissue concentrations of monoamines and several metabolites in various brain regions were determined.

METHODS

Chronic Nicotine Administration

Twenty-seven male Sprague-Dawley rats weighing 300–350 g each were divided into three groups, with 11 rats receiving a control saline solution, 8 receiving nicotine 3 mg/kg/day, and 8 receiving nicotine 12 mg/kg/day via osmotic minipumps. For the duration of the experiment, the rats were housed in wire cages with food and water provided ad libitum. Smoking was not allowed in the area where the animals were housed.

Solutions of nicotine (free base; Sigma Chemical, St. Louis, MO, U.S.A.) were prepared and diluted in sterile saline prior to loading of the solutions into osmotic minipumps (Alzet model 2ML4; Alza Corp., Palo Alto, CA, U.S.A.) designed to deliver the drug at a constant rate for 28 days. The pumps containing either a control solution of isotonic saline or nicotine for delivery at the doses noted above were implanted subcutaneously using ether anesthesia. All animals tolerated the surgery well. The self-contained pumps allowed unrestricted activity with no handling of the animals after implantation.

Quantification of Whole-Tissue Concentrations of Monoamines and Metabolites

After 21 days of infusion, each animal was decapitated and the brain quickly removed. Separate tissue samples from striatum, hypothalamus, and frontal

cortex were dissected on ice, transferred to preweighed conical tubes, and immediately frozen. Samples were stored at −60°C until assay.

The whole tissue concentrations of DA, NE, 5-HT, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) were determined by high performance liquid chromatography (HPLC) coupled with electrochemical detection. Sample preparation was identical to that described by McKay and co-workers (13). All separations were performed on an Altex Ultrasphere ODS (4.6 × 75 mm, 3-μm particles) reverse-phase column (Beckman Instruments, Fullerton, CA, U.S.A.). The mobile phase (flow rate 2 ml/min) consisted of a 0.17 *M* citrate-acetate buffer (pH 3.9) with 10–15% methanol that also contained 0.05 g/L EDTA and 0.10 g/L octanesulfonic acid (sodium salt). Samples were chromatographed using an isocratic HPLC system consisting of a Beckman 114M pump and an Altex 210 injector (50-μl sample loop) (Beckman Instruments), and an ESA 5100A Coulochem dual-detector coulometric electrochemical detector with an ESA 5011 dual-detector analytical cell (ESA, Bedford, MA, U.S.A.). The first detector of the dual detector was set at 0.01 V and the second was set at 0.40 V. The output from the second detector was recorded on a dual-pen Fisher 2000 recorder (Fisher Scientific, Springfield, NJ, U.S.A.). Complete separation and reequilibration of the HPLC system required ~7 min. Dihydroxybenzylamine was employed as an internal standard to calculate recovery. Peak heights were measured for quantification. All monoamine and metabolite values were calculated as total nanograms per gram (ng/g) wet weight of tissue.

Data Analysis

Data obtained regarding the concentration of each monoamine and metabolite in each brain region for the three groups of animals (control, low dose, and high dose) were analyzed using a one-way analysis of variance (ANOVA). As additional indicators of neurotransmitter activity, two indexes were calculated for each animal at each dose for each region. The DA index was calculated as (DOPAC + HVA)/DA and the 5-HT index as 5-HIAA/5-HT, and an ANOVA was also performed on these indexes. In those cases where the ANOVA indicated a significant difference ($p < 0.05$) for a monoamine, a monoamine metabolite, or the DA or 5-HT indexes in a given brain region, between-group comparisons were then done using pairwise *t* tests.

RESULTS

The tissue concentrations of the various monoamines and their metabolites and the values of the two indexes are recorded in Table 1 for each of the three brain regions. The only cases in which significant increases were observed in nicotine-treated animals versus controls involved the 5-HT system. The group receiving the higher dose of nicotine had an increase in 5-HT concentrations in the hypothalamus and an increase in the 5-HT index (Fig. 1) in the frontal cortex. In frontal cortex, however, the concentration of 5-HT decreased significantly at

TABLE 1. *Effect of 21 days of constant nicotine infusion (3 and 12 mg/kg/day) on rat brain tissue amine concentrations*

	DA	DOPAC	HVA	DA index [(DOPAC + HVA)/DA]	NE	5-HT	5-HIAA	5HT index (5-HIAA/5-HT)
Striatum								
Saline control (n = 11)	5,024.8 ± 365.7	3,345.0 ± 282.2	1,282.5 ± 103.2	0.93 ± 0.05	92.1 ± 18.1	207.3 ± 20.9	660.1 ± 49.6	3.31 ± 0.22
Nicotine 3 mg/kg/day (n = 8)	5,166.4 ± 507.4	2,674.1 ± 220.0	1,068.9 ± 64.9	0.75 ± 0.06[a]	50.0 ± 5.2	245.5 ± 36.6	687.9 ± 72.6	2.91 ± 0.12
Nicotine 12 mg/kg/day (n = 8)	5,937.5 ± 341.5	2,092.0 ± 174.5[a]	1,141.7 ± 90.7	0.56 ± 0.05[a]	58.7 ± 7.6	226.6 ± 21.0	619.4 ± 43.3	2.79 ± 0.13
Hypothalamus								
Saline control (n = 11)	325.8 ± 28.9	199.1 ± 25.9	81.5 ± 8.8	0.86 ± 0.05	1,354.4 ± 62.8	454.3 ± 20.7	967.9 ± 38.7	2.19 ± 0.15
Nicotine 3 mg/kg/day (n = 8)	227.5 ± 18.1	92.9 ± 15.0[a]	54.3 ± 10.1	0.68 ± 0.12	1,357.5 ± 138.7	413.3 ± 39.6	877.4 ± 43.6	2.24 ± 0.18
Nicotine 12 mg/kg/day (n = 8)	361.6 ± 56.4	52.3 ± 3.3[a]	62.5 ± 3.4	0.37 ± 0.05[a]	1,639.9 ± 85.5	566.9 ± 26.2[a]	803.7 ± 92.8	1.45 ± 0.18[a]
Frontal cortex								
Saline control (n = 11)	220.3 ± 74.2	73.4 ± 22.7	87.8 ± 12.7	2.20 ± 0.50	170.0 ± 11.4	167.9 ± 13.9	313.4 ± 14.1	1.94 ± 0.11
Nicotine 3 mg/kg/day (n = 8)	249.7 ± 47.9	64.7 ± 9.2	84.7 ± 11.1	0.66 ± 0.06[a]	113.9 ± 7.2[a]	102.0 ± 14.0[a]	261.6 ± 13.6	2.86 ± 0.40
Nicotine 12 mg/kg/day (n = 8)	165.7 ± 48.0	36.1 ± 13.7	91.1 ± 19.6	1.07 ± 0.33[a]	133.5 ± 13.4[a]	113.4 ± 19.5[a]	293.7 ± 26.4	2.87 ± 0.33[a]

Values are group means ± SEM, expressed as ng/g wet weight.

DA, dopamine; DOPAC, dihydroxyphenylacetic acid; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; HVA, homovanillic acid; NE, norepinephrine.

[a] Significant difference versus control group ($p < 0.05$, one-way ANOVA followed by pairwise *t* tests).

both doses. None of the brain regions had a statistically significant alteration of the 5-HT metabolite 5-HIAA. Concentrations of NE also decreased significantly in the frontal cortex, but not in striatum or hypothalamus (Fig. 2).

The concentration of DA was relatively stable in the treated animals compared with the control group. The metabolite DOPAC, however, was decreased in all brain regions at both nicotine doses, with the change reaching statistical significance in both striatum and hypothalamus. Concentrations of HVA also showed a trend toward a decrease in striatum and hypothalamus, but this did not attain statistical significance. The DA index was remarkable for showing significant decreases in all three brain regions (Fig. 3).

DISCUSSION

Nicotine binding sites are widely distributed throughout the CNS (14–16). Moreover, the effects of nicotine are heterogeneous, involving both cholinergic and noncholinergic CNS neurotransmission (2). Thus, the pharmacology of nicotine is complex and may vary in relation to both the CNS anatomic region of interest and the primary neurotransmitter system being investigated.

A number of basic science reports examining the effect of nicotine on CNS catecholamines have indicated that nicotine stimulates increased release of DA and NE (3–9). It is important to note that many of these studies have relied upon in vitro preparations of CNS tissue slices (3–7) or synaptosomes (8). In vivo studies are less common, and typically have relied upon a single acute injection or

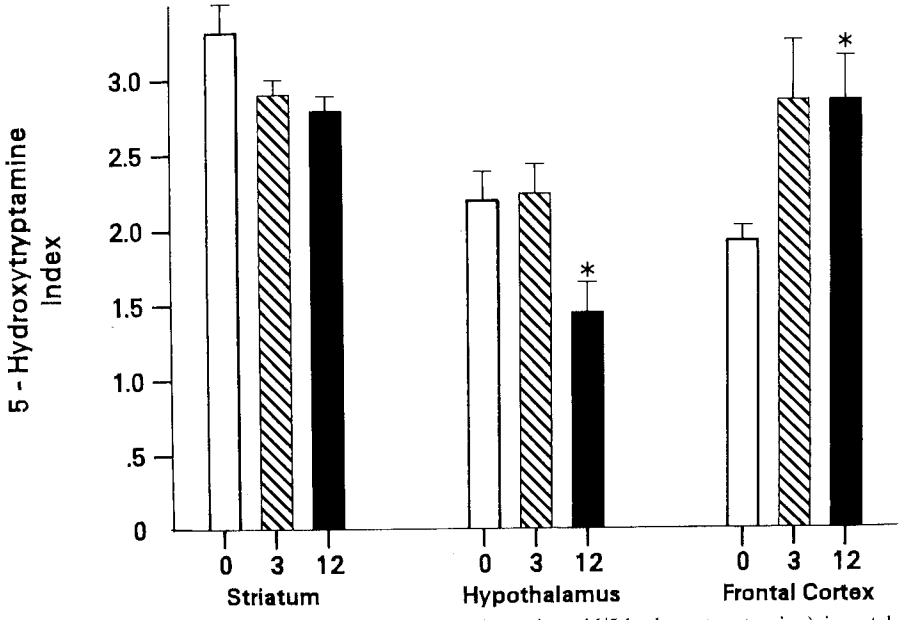

FIG. 1. 5-Hydroxytryptamine index (5-hydroxyindoleacetic acid/5-hydroxytryptamine) in rat brain regions after 21 days of constant nicotine infusion at 0 (n = 11), 3 (n = 8), or 12 (n = 8) mg/kg/day. Asterisks indicate $p < 0.05$, one-way ANOVA followed by pairwise t tests.

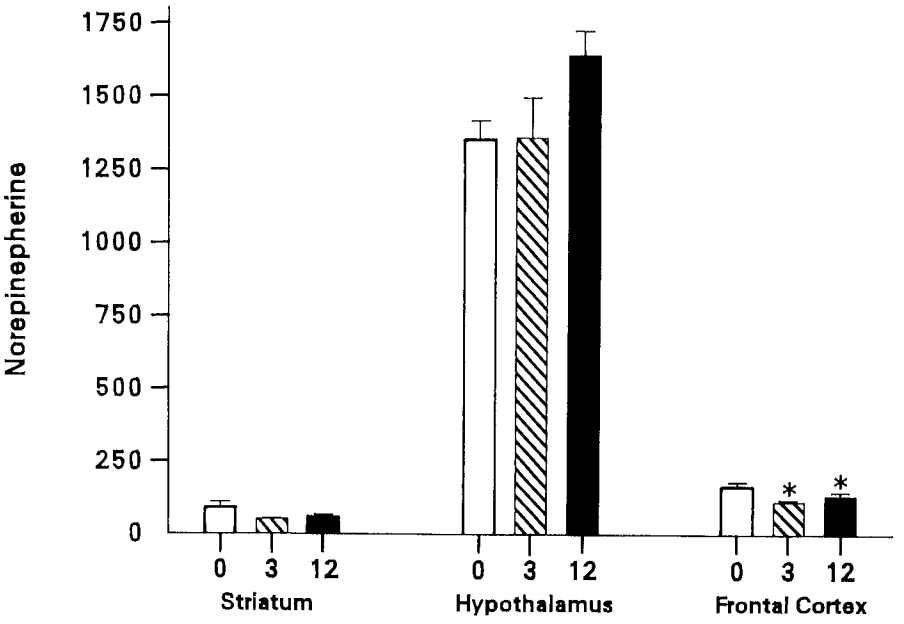

FIG. 2. Rat brain tissue norepinephrine concentrations (ng/g wet weight) after 21 days of constant nicotine infusion at 0 (n = 11), 3 (n = 8), or 12 (n = 8) mg/kg/day.
Asterisks indicate $p < 0.05$, one-way ANOVA followed by pairwise *t* tests.

limited number of injections over a few hours (9). These studies, however, would appear to be inadequate as models for the pattern of chronic nicotine administration typically seen in humans.

Our data after 21 days of constant nicotine administration indicate that rather than an increase in monoamine turnover reflected by increased tissue concentrations of metabolites, the effect of chronic treatment appears to be a decrease in catecholamine turnover, especially with regard to DA (Fig. 3). This decrease in turnover is indicated primarily by a decrease in DOPAC, with a less marked decrease in HVA. The concentration of DA itself, on the other hand, was not significantly decreased, indicating that this downregulation does not appear to be related to a general depletion in tissue DA stores. The only significant effect of nicotine treatment observed regarding NE, the decrease noted in frontal cortex, also runs counter to the observations commonly reported using acute dose and tissue perfusion paradigms. The increase in 5-HT in hypothalamus and decrease in frontal cortex are difficult to reconcile. It should be noted, however, that the significant increase in the 5-HT index in frontal cortex may be deceptive insofar as the absolute tissue concentrations of both 5-HT and 5-HIAA were decreased. In a previous study of the acute effects of nicotine on rat hippocampal 5-HT concentrations, a time-dependent increase followed by a decrease was observed after a single dose (10). It has also been reported that not only nicotine but also cotinine (the major metabolite of nicotine) decreased whole-brain 5-HT turnover in rats (11).

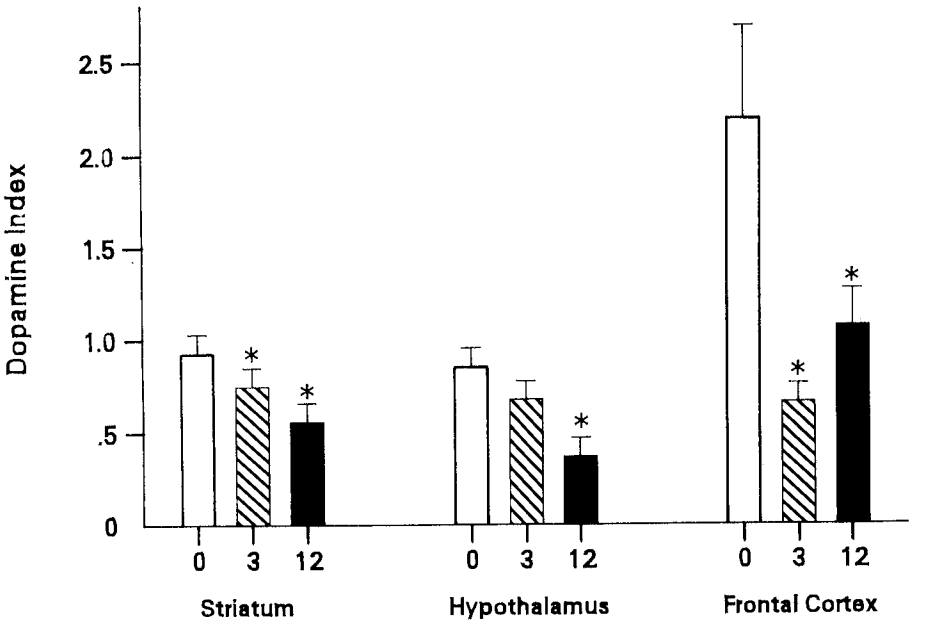

FIG. 3. Dopamine index [(dihydroxyphenylacetic acid + homovanillic acid)/dopamine] in rat brain regions after 21 days of constant nicotine infusion at 0 (n = 11), 3 (n = 8), or 12 (n = 8) mg/kg/day. Asterisks indicate $p < 0.05$, one-way ANOVA followed by pairwise *t* tests.

This preliminary study illustrates the complexity of the pharmacology of nicotine. It appears that the chronic administration of nicotine may have an effect on CNS neurotransmitters that is quite different from that of an acute dose or perfusion of tissue slices, namely, a decrease rather than an increase in catecholamine turnover in specific brain regions. This observation of decreased CNS concentrations of DA metabolites is consistent with the "peripheral" finding that, with chronic nicotine administration, levels of urinary catecholamines return to normal after an initial elevation (17). The reason for the difference between acute and chronic nicotine administration in affecting catecholamine activity is not clear. Various factors may play a role. Acute administration may cause increased release of tissue stores, whereas chronic exposure may downregulate synthesis and/or deplete tissue stores. Chronic administration could increase the activity of metabolic enzymes and/or the elimination of amines and metabolites from brain tissue. The relative roles of these different factors remain to be elucidated.

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