Nicotine-Induced Regional Changes in Brain Noradrenaline and Dopamine Turnover in Rats

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Using high performance liquid chromatography with electrochemical detection, the levels of 3-methoxy-4-hydroxyphenylethylenglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylalanine (DOPA) were determined in various brain regions of the rat 1 h after nicotine administration. Nicotine (1 mg/kg, s.c.) produced an increase in MHPG concentrations in the occipital cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain, pons/medulla and cerebellum. This alkaloid at the same dose also caused an increase in DOPAC concentrations in the hypothalamus, thalamus and pons/medulla. The nicotine-induced increase in MHPG and DOPAC concentrations in the brain regions was inhibited by pretreatment with mecamylamine (5 mg/kg, i.p.) but not by hexamethonium (10 mg/kg, i.p.). Nicotine (1 mg/kg, s.c.) produced an increase in DOPA concentrations under DOPA decarboxylase inhibition with NSD-1015 (200 mg/kg, i.p.) in the hypothalamus, thalamus and pons/medulla. These results indicate that nicotine can increase the turnover of noradrenaline and dopamine in various brain regions of the rat and this effect is mediated via activation of central nicotinic receptors.

Keywords — nicotine; brain; catecholamine turnover; nicotinic receptor; rat

Introduction

Nicotine produces various behavioral and physiological responses via its action on the central nervous system. It is thought that these central effects of nicotine are mediated through changes in the release of a number of different neurotransmitters including the catecholamines noradrenaline and dopamine.

The effect of nicotine on central catecholamine turnover has been determined using in vitro and in vivo techniques. In vitro, nicotine increases the release of both noradrenaline and dopamine from rat brain slices and synaptosomes. In vivo, nicotine increases the turnover of hypothalamic dopamine and noradrenaline. Basal ganglia dopamine is also released by this alkaloid. Although these results indicate that nicotine does increase catecholamine release and turnover in some parts of the brain, little information is available on the effects of nicotine on catecholamine systems at other sites in the brain.

Catecholamine neurons innervate many parts of the brain and nicotinic receptors exist extensively within the brain. In the present study, we determined effects of acute nicotine administration on regional concentrations of noradrenaline and dopamine metabolites in rat brain. In addition, effects of nicotine on regional 3,4-dihydroxyphenylalanine (DOPA) concentration after DOPA decarboxylase inhibition were also examined to determine the effects of nicotine on catecholamine synthesis.

Materials and Methods

Male 5-week-old Sprague-Dawley rats were kept under alternate 12-h periods of dark and light, and given standard rat chow and tap water ad libitum.

First, the effect of nicotine at 0.4 and/or 1 mg/kg on the tissue concentrations of 3-methoxy-4-hydroxyphenylethylenglycol (MHPG) and 3,4-dihydroxyphenylacetic acid (DOPAC) was examined. The concentrations of MHPG and DOPAC give an indication of noradrenaline and dopamine turnover, respectively. Next, to establish if the nicotine effect on catecholamine metabolite concentra-
tions was caused by peripheral or central nicotinic stimulation, effects of pretreatment with hexamethonium and mecaminine were examined for their ability to block the effects of nicotine. In addition, to determine if nicotine alters catecholamine synthesis, the effect of nicotine on DOPA concentration after administration of the decarboxylase inhibitor 3-hydroxybenzylhydrazine (NSD-1015) was examined. Control rats received an injection of saline instead of nicotine.

One hour after nicotine or saline injection, rats were decapitated, and the brain was rapidly removed and placed on ice. Eight brain regions, occipital cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain, pons/medulla and cerebellum were dissected according to the procedure described by Glowinski and Iversen.\textsuperscript{16}

**MHPG Determination** — The brain regions were homogenized in 0.1 M perchloric acid containing 0.1 mM ascorbic acid, and centrifuged. MHPG-SO\textsubscript{4} in the supernatant was deconjugated enzymatically to MHPG using sulfatase (40 U/ml). Following the addition of p-hydroxybenzylalcohol (50 pmol/tube) as an internal standard, MHPG was extracted into ethylacetate. The ethylacetate phase was washed with 2 M KHCO\textsubscript{3} saturated with NaCl and then evaporated under vacuum. The dry residue was reconstituted in 50 μl of 1 mM HCl, which was used for the determination of MHPG. MHPG was assayed using high-performance liquid chromatography (HPLC) with chemical detection (ECD) (Yanaco, Tokyo, Japan); separation was achieved using a Cosmosil/5C18 column and the mobile phase consisted of 0.1 M potassium phosphate buffer (pH 5.8) containing 5% methanol, 0.02% 1-heptanesulfonate sodium and 1 mM disodium ethylenediaminetetraacetic acid (EDTA).

**DOPAC and DOPA Determination** — DOPAC and DOPA were measured according to the procedure described by Westerink and Mulder.\textsuperscript{17} Briefly, tissue homogenate in 500 μl of 0.1 M perchloric acid was centrifuged and the supernatant was put on a Sephadex G-10 column (5 × 20 mm) equilibrated with 0.01 M formic acid. The column was washed with 1.5 ml of 0.01 m formic acid and then DOPA was eluted by successive application of another 1.5 ml of formic acid and 0.5 ml of 0.005 M Na\textsubscript{2}HPO\textsubscript{4}. DOPAC was eluted by next application of 2 ml of 0.005 M Na\textsubscript{2}HPO\textsubscript{4}. DOPAC and DOPA were determined by HPLC-ECD; separation was achieved using a cosmosil 5C18 column and the mobile phase consisting of mixture of 0.1 M citric acid and 0.2 M Na\textsubscript{2}HPO\textsubscript{4} (pH 5.4).

Each value of the data is reported as mean ± S.E. and the statistical significance of the results was calculated using Student's t test. Nicotine free base was obtained from the Research Foundation for Smoking Science, Japan. Mecamylamine hydrochloride and hexamethonium bromide were obtained from Sigma Chem., U.S.A.

**Results**

**Effects on Brain MHPG Concentration**

The noradrenaline metabolite MHPG was measured in the occipital cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain,  

![MHPG concentration (pmol/mg wet weight)]  

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>MHPG Concentration (pmol/mg wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occipital cortex</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Hippocampus</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Striatum</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Hypothalamus</td>
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</tr>
<tr>
<td>Thalamus</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Midbrain</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Pons/medulla</td>
<td><img src="image" alt="Graph" /></td>
</tr>
<tr>
<td>Cerebellum</td>
<td><img src="image" alt="Graph" /></td>
</tr>
</tbody>
</table>

**Fig. 1. Effect of Nicotine on Brain MHPG Concentration**

Nicotine at 1 mg/kg (closed columns) or saline (open columns) was subcutaneously administered and animals were sacrificed 1 h later. Values are mean ± S.E. from 4—6 animals. \textit{a} \textit{p} < 0.05, compared with saline control.
Table I. Effect of Mecamylamine and Hexamethonium on the Nicotine-Induced Increase in MHPG Concentrations of Hippocampus, Hypothalamus and Thalamus

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Hippocampus</th>
<th>Hypothalamus</th>
<th>Thalamus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Nicotine</td>
<td>Saline</td>
</tr>
<tr>
<td>None</td>
<td>0.42±0.01</td>
<td>0.62±0.02a)</td>
<td>0.89±0.06</td>
</tr>
<tr>
<td>Mecamylamine 5 mg/kg</td>
<td>0.44±0.04</td>
<td>0.44±0.04</td>
<td>0.85±0.08</td>
</tr>
<tr>
<td>Hexamethonium 10 mg/kg</td>
<td>0.45±0.01</td>
<td>0.63±0.06a)</td>
<td>1.01±0.06</td>
</tr>
</tbody>
</table>

Nicotine (1 mg/kg) or saline was subcutaneously administered and animals were sacrificed 1 h later. Mecamylamine or hexamethonium was intraperitoneally administered 15 min before nicotine or saline. Values are mean ± S.E. from 4—6 animals. a) p < 0.05, compared with saline value.

Table II. Effect of Mecamylamine and Hexamethonium on the Nicotine-Induced Increase in DOPAC Concentrations of Hypothalamus and Pons/Medulla

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Hypothalamus</th>
<th>Pons/medulla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>Nicotine</td>
</tr>
<tr>
<td>None</td>
<td>0.50±0.02</td>
<td>0.59±0.03a)</td>
</tr>
<tr>
<td>Mecamylamine 5 mg/kg</td>
<td>0.43±0.01b)</td>
<td>0.43±0.03</td>
</tr>
<tr>
<td>Hexamethonium 10 mg/kg</td>
<td>0.41±0.02b)</td>
<td>0.49±0.02a)</td>
</tr>
</tbody>
</table>

Nicotine (1 mg/kg) or saline was subcutaneously administered and animals were sacrificed 1 h later. Mecamylamine or hexamethonium was intraperitoneally administered 15 min before nicotine or saline. Values are mean ± S.E. from 6—7 animals. a) p < 0.05, compared with saline value. b) p < 0.05, compared with none.

Fig. 2. Effect of Nicotine on Brain DOPAC Concentration
Nicotine at 0.4 mg/kg (hatched columns) or 1 mg/kg (closed columns), or saline (open columns) was subcutaneously administered and animals were sacrificed 1 h later. Values are mean ± S.E. from 6—8 animals. a) p < 0.05, compared with saline control.

Nicotine at 1 mg/kg increased MHPG concentrations in all the regions examined (Fig. 1). The nicotine-induced increase in MHPG levels in the hippocampus, hypothalamus, thalamus (Table I) and all other regions (data not shown) was blocked by mecamylamine (5 mg/kg, i.p.), while hexamethonium (10 mg/kg, i.p.) did not affect it. The nicotinic receptor antagonist mecamylamine or hexamethonium alone had no effect on MHPG levels.

Effects on Brain DOPAC Concentration
The dopamine metabolite DOPAC was measured in the striatum, hypothalamus, thalamus, midbrain and pons/medulla (Fig. 2). Nicotine at 1 mg/kg increased DOPAC concentrations in the hypothalamus, thalamus and pons/medulla whereas this alkaloid at 0.4 mg/kg did not affect the concentrations in all the regions examined. The nicotine-induced increase in DOPAC con-
Concentrations in the hypothalamus and pons/medulla was blocked by mcamylamine (5 mg/kg, i.p.), while hexamethonium (10 mg/kg, i.p.) did not block it (Table II). Mecamylamine or hexamethonium alone did not affect pons/medullary DOPAC levels, while DOPAC concentrations in the hypothalamus were decreased by the nicotinic receptor antagonists.

**Effects on DOPA Concentration after DOPA Decarboxylase Inhibition**

DOPA concentrations 1 h after the DOPA decarboxylase inhibitor NSD-1015 (200 mg/kg, i.p.) were measured in the striatum, hypothalamus, thalamus, midbrain and pons/medulla. Nicotine at 1 mg/kg produced an increase in DOPA concentration under the NSD-1015 treatment in the hypothalamus, thalamus and pons/medulla, and a trend for increased DOPA concentration was seen in the striatum and midbrain (Fig. 3).

**Discussion**

The present study indicates that a single dose of nicotine (1 mg/kg) produces an increase in the concentration of brain noradrenaline and dopamine, which has been shown that nicotine can increase noradrenaline turnover in whole brain, the hypothalamus and the hippocampus, and dopamine turnover in the hypothalamus, basal ganglia and brainstem. In this study, we have demonstrated that nicotine enhances the turnover of noradrenaline in almost all the regions in the brain and also enhances the turnover of dopamine in the hypothalamus, thalamus and pons/medulla.

Nicotine produced an increase in the DOPA concentration under DOPA decarboxylase inhibition in the hypothalamus, thalamus and pons/medulla and a tendency for the increase in the DOPA concentration in the striatum and midbrain, indicating that nicotine can also accelerate brain catecholamine synthesis. Previously we could not find any changes in noradrenaline concentrations in these brain regions after nicotine administration (unpublished observation). This failure to find changes in brain noradrenaline concentration after nicotine is probably due to newly synthesized noradrenaline replacing noradrenaline released by nicotine.

Pretreatment with mcamylamine, a centrally acting nicotine antagonist, blocked the nicotine-induced rise in MHPG and DOPAC concentrations in all the regions examined whereas the rise was not affected by pretreatment with hexamethonium, an antagonist poorly penetrating into the brain, suggesting that nicotine affects catecholamine metabolites via activation of central nicotinic receptors.

Receptor binding studies have demonstrated the extensive presence of nicotinic receptors within the brain. Noradrenaline neurons innervate almost all the regions in brain. These findings are compatible with the results of the present study that nicotine can affect noradrenergic metabolites in almost all the brain regions.

It has been shown that nicotine exerts a direct excitatory action on neurons of the zona compacta of rat substantia nigra and also elevates homovanillic acid, another dopamine metabolite, concentrations there. Roth et al. have demonstrated that nicotine produces an elevation in dopamine metabolites in the basal ganglia. In the present study, in contrast, we could not find any changes in DOPAC concent-
trations in the striatum after nicotine administration. More detailed studies will be needed to explain the differences between the results of their studies and ours.

Since mecamylamine alone did not affect the MHPG levels of the brain regions examined, it seems unlikely that there is tonically acting nicotinic input to noradrenergic neurons in the brain regions. In contrast, both the nicotinic receptor antagonists produced a decrease in hypothalamic DOPAC concentrations. Precise mechanisms of this effect by the antagonists remain to be clarified.

Nicotine administration produces various physiological responses in animals,1,20 and some of the effects of nicotine are thought to involve brain catecholamine systems. For example, the release of hormone from the anterior pituitary is, to a large extent, controlled by noradrenaline and dopamine release in the hypothalamus.7,21,22 It is possible that the increase in locomotor activity evoked by nicotine is also related to increased release of dopamine in the nucleus accumbens.23 Nicotine also affects cardiovascular function, in part, via mediation of brain catecholamine systems.24,25 The results of the present study provide further evidence for supporting involvement of brain catecholamine systems in physiological responses to nicotine.

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