Effects of Acute Nicotine on Catecholamine Turnover in Various Rat Brain Regions

Hiroo AMANO,* Yoshio GOSHIMA, Noriko AKEMA,** Hiroshi UEDA,*** Takao KUBO, and Yoshimi MISU

Department of Pharmacology, Yokohama City University School of Medicine, Fuku-ura 3-9, Kanazawa-ku, Yokohama, 236, Japan

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Acute effects of *in vivo* nicotine application on turnover rates of noradrenaline (NA), adrenaline (AD) and dopamine (DA) in rat occipital cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain, pons/medulla and cerebellum were examined by determining the amine concentrations by high performance liquid chromatography. The regional turnover rates of catecholamines were evaluated by comparing the decline in concentrations produced by blockade of synthesis. Intraperitoneally injected fusaric acid (80 mg/kg), an inhibitor of dopamine- β -hydroxylase, significantly decreased NA concentration in all eight regions examined, and AD concentration in the hypothalamus and pons/medulla. α -Methyl-*p*-tyrosine methylester (α -MPT, 400 mg/kg), an inhibitor of the tyrosine hydroxylase, induced significant and uneven decreases in regional DA concentrations 1 h after the i.p. injection. α -MPT also significantly decreased NA concentration in all the regions except the striatum. Subcutaneously injected nicotine 0.4–1 mg/kg significantly enhanced the fusaric acid-induced decline in NA concentrations in the hippocampus and cerebellum. AD decline in the midbrain by fusaric acid was significantly accelerated by nicotine (0.4 mg/kg), but was not affected by a larger dose (1 mg/kg). In the hippocampus, nicotine (1 mg/kg) significantly enhanced the α -MPT-induced decrease in NA concentration, while it reduced the decrease in DA concentration. These findings suggest that nicotine can affect the turnover of brain catecholamine in a regionally specific manner.

Keywords — rat brain; turnover; nicotine; regional catecholamine

Introduction

Central catecholamines have been implicated in the pharmacological effects of nicotine in the central nervous system, such as the elevation of locomotor activity and the effects on the secretion of adenohipophyseal hormones.^{1–3)} It has been demonstrated that nicotine can stimulate some central catecholaminergic systems: nicotine causes the release of noradrenaline (NA) and dopamine (DA) from brain slices and NA release from hypothalamic synaptosomes,^{4,5)} and *in vivo*, nicotine increases the turnover rates of adrenaline (AD), NA and/or DA in some areas under various conditions of administration.^{2,3,6,7)} The purpose of the present study was to investigate more extensively the effects of nicotine on central catecholamine turnover.

Materials and Methods

Materials — Nicotine free base was generously donated by Japan Tobacco Incorporated. All other drugs and reagents were purchased from commercial sources.

Treatments of Animals — All studies were performed using male Sprague-Dawley rats (5 weeks of age, Charles River Japan Inc.). Regional catecholamine turnover was evaluated by determining the decline of amine concentrations after synthesis inhibition either by α -methyl-*p*-tyrosine methylester (α -MPT), 400 mg/kg i.p.) or fusaric acid (80 mg/kg i.p.), inhibitors of tyrosine hydroxylase (TH) and dopamine- β -hydroxylase (DBH), respectively. Nicotine was subcu-

* Present Address: Division of Pharmacology and Microbiology, National Institute of Hygienic Sciences, Osaka Branch, 1-1-43, Hoenzaka, Higashi-ku, Osaka, 540, Japan.

** Present Address: The Second Dept. of Internal Medicine, Yokohama City University School of Medicine, Yokohama, 232, Japan.

***Present Address: Dept. of Pharmacology, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, 606, Japan.

taneously injected immediately after the enzyme inhibitors. All drugs were dissolved in physiological saline. Control animals received the corresponding volume of saline. One hour after the appropriate treatment, rats were killed by decapitation.

Regional Dissection — Immediately after the decapitation, whole brains were removed and dissected into eight discrete regions (occipital cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain, pons/medulla and cerebellum) on an ice-cold glass plate. Striatum, hypothalamus, pons/medulla and cerebellum were dissected as described by Glowinski and Iversen.⁸⁾ Their midbrain region was further divided into two regions, anterior (thalamus) and posterior (midbrain) parts, by a plane cut through the antero-dorsal margin of the colliculus rostralis and the antero-ventral edge of the pons on the midline sagittal section. Dissected brain regions were weighed and stored at -20°C until assayed.

Catecholamine Determination — Each tissue block was homogenized in 500 μl of 1 N HCl containing 100 nM dihydroxybenzylamine, as an internal standard, using a Sonifier cell disruptor, model 185 (Branson). Following centrifugation at 4°C for 20 min at $10000 \times g$, a 400 μl aliquot of the supernatant was cleaned up on alumina.⁹⁾ The resulting sample was subjected to the catecholamine determination with the high performance liquid chromatography-

electrochemical detector (HPLC-ECD) system.

HPLC-ECD Conditions — In all experiments, a Yanaco model L-2000 HPLC-ECD system and Cosmosil 5C₁₈ packed column (4.6×150 mm, Nakarai Chemicals, Ltd.) were used. Other conditions were as follows: mobile phase, 0.1 M potassium phosphate buffer (pH 5.8) containing 1 mM ethylenediaminetetraacetic acid disodium (EDTA·2Na), 0.02% 1-heptanesulfonate sodium and 3% methyl alcohol; flow rate, 1.5 ml/min; applied voltage, 600 mV.

Statistics — Data were analyzed using analysis of variance followed by Student's or Welch's *t* test according to the standard methods. The trend for an effect was defined to be observable when the *t*_{cal} value between the two groups exceed 1.5. Results are presented as the mean and S.E.

Results

Effects of Catecholamine Synthesis Blockade

Intraperitoneally injected fusaric acid (80 mg/kg), an inhibitor of DBH, decreased the concentrations of NA significantly and unevenly in all eight regions examined, 1 h after the injection. The degree of NA decrease was large in the occipital cortex, hippocampus, thalamus and midbrain (over 70% from the control level), small in the striatum and hypothalamus (52 and 50%, respectively) and intermediate in the pons/medulla and cerebellum (63 and 67%, re-

TABLE I. Effect of Fusaric Acid on the Brain Regional NA and AD Concentrations in Rat

	NA concentration (pmol/mg tissue)		AD concentration (pmol/mg tissue)	
	Control	Fusaric acid	Control	Fusaric acid
Occipital cortex	1.80 ± 0.22	$0.54 \pm 0.15^a)$	—	—
Hippocampus	3.09 ± 0.25	$0.88 \pm 0.12^a)$	—	—
Striatum	1.04 ± 0.04	$0.50 \pm 0.08^a)$	—	—
Hypothalamus	17.51 ± 0.93	$8.72 \pm 0.29^a)$	0.166 ± 0.010	$0.123 \pm 0.008^a)$
Thalamus	4.87 ± 0.37	$1.38 \pm 0.17^a)$	0.0118 ± 0.0015	0.0113 ± 0.0011
Midbrain	5.04 ± 0.26	$1.51 \pm 0.14^a)$	0.0158 ± 0.0013	0.0128 ± 0.0015
Pons/medulla	6.33 ± 0.30	$2.33 \pm 0.53^a)$	0.0356 ± 0.0031	$0.0229 \pm 0.0026^a)$
Cerebellum	1.78 ± 0.10	$0.59 \pm 0.05^a)$	—	—

Fusaric acid (80 mg/kg) or saline (control) was intraperitoneally administered and animals were killed 1 h later. Data are expressed as the mean and S.E. from 4–9 animals per group. *a)* Values are significantly different from the control at $p < 0.05$. —: Concentration was below the detection limit.

TABLE II. Effect of α -MPT on the Brain Regional DA and NA Concentrations in Rat

	DA concentration (pmol/mg tissue)		NA concentration (pmol/mg tissue)	
	Control	α -MPT	Control	α -MPT
Occipital cortex	0.062 $\pm$ 0.007	0.016 $\pm$ 0.002 ^{a)}	1.80 $\pm$ 0.22	0.81 $\pm$ 0.21 ^{a)}
Hippocampus	0.063 $\pm$ 0.010	0.013 $\pm$ 0.003 ^{a)}	3.09 $\pm$ 0.25	2.31 $\pm$ 0.08 ^{a)}
Striatum	49.54 $\pm$ 3.19	33.59 $\pm$ 0.59 ^{a)}	1.04 $\pm$ 0.04	1.09 $\pm$ 0.16
Hypothalamus	2.65 $\pm$ 0.11	1.23 $\pm$ 0.05 ^{a)}	17.51 $\pm$ 0.93	12.03 $\pm$ 0.39 ^{a)}
Thalamus	1.45 $\pm$ 0.03	0.84 $\pm$ 0.16 ^{a)}	4.87 $\pm$ 0.37	3.50 $\pm$ 0.14 ^{a)}
Midbrain	0.75 $\pm$ 0.03	0.29 $\pm$ 0.01 ^{a)}	5.04 $\pm$ 0.26	3.58 $\pm$ 0.20 ^{a)}
Pons/medulla	0.276 $\pm$ 0.013	0.090 $\pm$ 0.006 ^{a)}	6.33 $\pm$ 0.30	4.12 $\pm$ 0.11 ^{a)}
Cerebellum	0.030 $\pm$ 0.004	0.011 $\pm$ 0.001 ^{a)}	1.78 $\pm$ 0.10	1.05 $\pm$ 0.09 ^{a)}

α -MPT (400 mg/kg) or saline (control) was intraperitoneally administered and animals were killed 1 h later. Data are expressed as the mean and S.E. from 4–5 animals per group. ^{a)} Values are significantly different from the control at $p < 0.05$.

spectively). The same treatment with fusaric acid also decreased the hypothalamic and pons/medullary AD concentrations significantly but by smaller degrees (26 and 36%, respectively) than with NA. In the thalamus, fusaric acid produced a 22% decrease in AD concentration, which was not statistically significant (Table I). Table II shows the effect of α -MPT administration on the regional DA and NA concentrations. Inhibition of TH by α -MPT 400 mg/kg induced

significant and uneven decreases in regional DA concentrations, the degree of which was largest in the hippocampus (79% from the control level) and smallest in the striatum (32%), 1 h after the i.p. injection. α -MPT also significantly decreased NA concentrations in all the regions except the striatum.

Effect of Nicotine on Regional Catecholamine Turnover

Nicotine (0.4 and 1 mg/kg) significantly en-

Fig. 1. Effect of Nicotine on the Decline in Regional NA Concentrations Produced by Fusaric Acid

Nicotine or saline was subcutaneously administered immediately after fusaric acid (i.p.) and animals were killed 1 h later. Data are expressed as mean and S.E. from 5–9 animals per group. ^{a)} Values are significantly different from the control at $p < 0.05$. □, control; ■, nicotine 0.4 mg/kg; ▨, nicotine 1 mg/kg.

Fig. 2. Effect of Nicotine on the Decline in Regional AD Concentrations Produced by Fusaric Acid

Data are expressed as mean and S.E. from 5–9 animals per group. *a)* Values are significantly different from the control at $p < 0.05$. □, control; ▨, nicotine 0.4 mg/kg; ▩, nicotine 1 mg/kg.

hanced the fusaric acid (80 mg/kg, i.p.)-induced decline in regional NA concentrations in the hippocampus and cerebellum (Fig. 1). The fusaric acid-induced decline in AD concentration in the midbrain was significantly accelerated by nicotine at 0.4 mg/kg, but was not affected by a larger dose (1 mg/kg). Similar biphasic action of nicotine was also observed in the thalamus and pons/medulla. Trend for the nicotine-induced acceleration of AD decline was seen at 0.4 mg/kg ($t_{cal}=1.57$ for the thalamus and $t_{cal}=1.72$ for the pons/medulla), but not at 1 mg/kg (Fig. 2). Figure 3 shows the effects of nicotine (1 mg/kg) on the α -MPT-induced decline in hippocampal DA and NA concentra-

tions. Nicotine significantly enhanced the decrease in NA concentration, while it significantly reduced the decrease in DA concentration. In other regions, nicotine did not show any significant effect on the α -MPT-induced decline either in DA or NA concentration (data not shown).

Discussion

Earlier reports have shown that nicotine can cause release of NA from *in vitro* brain preparations,^{4,5)} and in an *in vivo* experiment, large and repeated doses of nicotine cause accelerated turnover of NA in the hypothalamus.³⁾ More recently, Roth *et al.* reported that a single injection of 1 mg/kg of nicotine enhanced the fusaric acid induced disappearance of NA in the hippocampus,¹⁰⁾ an area with a high concentration of nicotinic receptors.¹¹⁾ We confirmed this finding not only on the fusaric acid-induced NA decline but also on the decline produced by α -MPT, an inhibitor of TH. In addition, we demonstrated that a smaller dose (0.4 mg/kg) nicotine can cause the same effect in size as the 1 mg/kg dose. These results indicate that noradrenergic nerve activity in this area is so sensitive to nicotine that as small a dose as 0.4 mg/kg of nicotine can induce the maximum effect. But this regional specificity cannot be explained by a high density of the hippocampal nicotinic receptor, because nicotine at 0.4 mg/kg was proved to ac-

Fig. 3. Effect of Nicotine on the Decline in Hippocampal DA and NA Concentrations Produced by α -MPT

Nicotine or saline was subcutaneously administered immediately after α -MPT (i.p.) and animals were killed 1 h later. Data are expressed as mean and S.E. from 4–5 animals per group. *a)* Values are significantly different from the control at $p < 0.05$. □, control; ▨, nicotine 1 mg/kg.

celerate NA turnover also in the cerebellum (Fig. 1), an area with a relatively low concentration of nicotinic receptor.¹¹⁾ The proportion of the nicotinic receptors located on the noradrenergic neurons to the total regional nicotinic receptors may be responsible for the regional selectivity of the effect on NA turnover.

Nicotine at 0.4 mg/kg significantly accelerated the AD turnover in the midbrain and showed a similar trend in the thalamus and pons/medulla, although fusaric acid failed to produce a significant AD decrease in the thalamus and midbrain. These effects were unobservable at the larger dose of 1 mg/kg. This biphasic action may have some relation with the biphasic effect of nicotine on behavioral spontaneous activity, on which nicotine acts as stimulant and depressant at small and larger doses respectively.¹²⁾

In the hippocampus, nicotine (1 mg/kg) accelerated NA turnover, but on the contrary, depressed DA turnover. One possible explanation for these phenomena is that nicotine acts in opposite ways on NA and DA neurons innervating the same region. Another is to postulate a blocking effect of nicotine on the vesicular uptake of DA in NA nerve cells, which will inhibit the conversion of precursor (unreleasable) DA to releasable NA and cause increased DA concentration and decreased NA concentration. It does not seem to be due to the possible DBH inhibitory effect of nicotine, which would also increase DA concentration and decrease NA, because the acceleration of NA disappearance was seen not only under the inhibition of TH by α -MPT but also under the full inhibition of DBH by fusaric acid (Figs. 1 and 3). More detailed study is necessary to establish the significance of these effects of nicotine.

Treatment with fusaric acid or α -MPT caused regionally uneven decreases of catecholamine concentrations (Tables I and II). In general, α -MPT-induced DA depletion showed larger regional variations in size (32–79% of control level) than fusaric acid-induced NA depletion (50–72%). An explanation based on the anatomical characteristics of each neuron may be possible. That is, whereas central NA systems project diffusely to a large number of areas, the DA systems innervate specific brain structures.

As the transmitter depletion is expected to occur in the terminal region, restricted distribution of DA terminals may cause various degrees of DA depletion, except in the case of the striatum which receives dense DA innervation from the substantia nigra zona compacta (included in the thalamus region by our dissection method) but showed the smallest disappearance of DA after α -MPT injection. The slow turnover of striatal DA in contrast to the high regional concentration has already been reported and suggested to be evidence for the existence of two distinct DA pools.¹³⁾ In the striatum, α -MPT did not cause any NA decline at all, although TH has been proved to be the common rate-limiting enzyme of catecholamine synthesis. It is generally accepted that there is a small DA pool existing as a precursor in NA nerve terminal regions as shown in the cortical region, hippocampus and cerebellum (Table II), whereas in the striatum, there may exist a fairly large DA population functioning as the NA precursor to compensate for NA loss, in addition to the neurotransmitter DA population.

Our present results provide evidence that systemically administered nicotine expresses its central effects, at least in part, *via* the activation of brain catecholamine turnover. These data provide a useful basis for further studies not only in neurochemical but also behavioral, psychological and other fields.

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