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Effect of Nicotine on Extracellular Levels of Neurotransmitters Assessed by Microdialysis in Various Brain Regions: Role of Glutamic Acid

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We studied the effect of local administration of nicotine on the release of monoamines in striatum, substantia nigra, cerebellum, hippocampus, cortex (frontal, cingulate), and pontine nucleus and on the release of glutamic acid in striatum of rats in vivo, using microdialysis for nicotine administration and for measuring extracellular amine and glutamic acid levels. Following nicotine administration the extracellular concentration of dopamine increased in all regions except cerebellum; serotonin increased in cingulate and frontal cortex; and norepinephrine increased in substantia nigra, cingulate cortex, and pontine nucleus. Cotinine, the major nicotine metabolite, had no effect at similar concentrations. The cholinergic antagonists mecamylamine and atropine, the dopaminergic antagonists haloperidol and sulpiride, and the excitatory amino acid antagonist kynurenic acid all inhibited the nicotine-induced increase of extracellular dopamine in the striatum. The fact that kynurenic acid almost completely prevented the effects of nicotine, and nicotine at this concentration produced a 6-fold increase of glutamic acid release, suggests that the effect of nicotine is mainly mediated via glutamic acid release.

KEY WORDS: Nicotine; microdialysis; glutamic acid; dopamine.

INTRODUCTION

Specific high-affinity binding sites for nicotine have been shown to be present in various brain areas (1-2). The correspondence between the distribution of [^{3}H]nicotine binding sites and the distribution of [^{3}H]acetylcholine is an indication that nicotine binds to nicotinic acetylcholine receptors (3). The nicotinic acetylcholine receptor on striatal dopaminergic neurons was identified as the site of some of the effects of nicotine (4-5). In striatal slices (6-7) and striatal synaptosomal preparations (8) nicotine was shown to stimulate dopamine (DA) release. Acute systemic administration of nicotine resulted in an increase in catecholamine synthesis in some cerebral regions (9) and in stimulation of DA release in nucleus accumbens and dorsal caudate nucleus (10). DA release was also stimulated in nucleus accumbens by the direct application of nicotine with a microdialysis probe (11). In an attempt to identify sites of specific nicotine action in the brain we examined the local effect of nicotine on extracellular monoamine levels at various cerebral sites (striatum, substantia nigra, cerebellum, hippocampus, frontal cortex, cingulate cortex, and pontine nucleus) with the microdialysis technique. To obtain further information about the mechanism(s) of nicotine action, we examined the influence of cholinergic, dopaminergic, and excitatory aminoacidergic antagonists on nicotine-induced neurotransmitter release.

EXPERIMENTAL PROCEDURE

Animals. Male Wistar rats 290-350 g were used in all experiments. The rats were maintained under a 10 h/14 h dark/light cycle

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with food and water constantly available. Experiments were started between 9 and 11 a.m.

Materials. (−)-Nicotine di-(+)tartrate, (−)-cotinine, haloperidol, kynurenic acid, atropine, and sulpiride were purchased from Sigma; mecamylamine, from Merck Sharp and Dohme. Microdialysis probes made by Carnegie Medicine were obtained from Bioanalytical Systems.

Testing of the Microdialysis Probe. Each microdialysis probe was tested for performance before use in animals. In this test the probe was immersed in a standard solution containing catecholamines and indoleamines in concentrations similar to those to which the probe was expected to be exposed in the experiments, and the probe was perfused at 1 μl/min with Ringer solution (147 mM Na^+, 2.3 mM Ca^{2+}, 4 mM K^+, and 155.6 mM Cl^-) for 150 min. Samples of dialysates were collected every 30 min in microtubes containing 5 μl of 1 N perchloric acid and kept on ice. The collected dialysate samples and two aliquots from the standard were kept frozen (−80°C) until analysis. The concentration of the catecholamines in the dialysates was 20-37% of that in the standard solution, and the concentration of HIAA was 33-48% of that of the standard in which the probe was immersed. Variation between probes was 10-20%. Each probe was used several times and was retested after each use with [^{3}H]glutamic acid. When testing the probe with glutamate, the concentration of glutamate in the dialysate was similar to that of catecholamines (20-30% of that in the standard solution).

Permeability Test of the Microdialysis Probe for Nicotine. The microdialysis probe was immersed in 150 μl of water in a 250-μl polypropylene microtube and perfused with 100 mM [^{3}H]nicotine (63 cpm/nmol) in Ringer solution at 1 μl/min. After 30 min the radioactivity was measured in the water and in the perfusate. From the radioactivity recovered in the water the extent of the diffusion of the nicotine through the probe membrane was calculated. In 30 min, 27 ± 3 nmol of the 3000 nmol of nicotine with which the microdialysis probe was perfused was recovered in the water in which the probe was immersed.

Implantation of the Probe in Brain and Microdialysis. The implantation of the microdialysis probe and the microdialysis were performed under anesthesia (for further information see Ref. 14). The rats were injected i.p. with chloral hydrate (0.45 mg/kg) and placed in a Kopf stereotaxic instrument. A hole was drilled in the right side of the skull directly above the site selected for microdialysis. The coordinates, in mm with respect to bregma according to the rat brain atlas of Paxinos and Watson (12), were AP:1.0, ML:2.5, DV:7.0 for striatum; AP: −5.3, ML:2.5, DV:9.5 for substantia nigra; AP: −12.0, ML:0.1, DV:7.0 for cerebellum; AP: −3.8, ML:2.0, DV:4.0 for hippocampus; AP:3.7, ML:2.8, DV:5.0 for frontal cortex; AP:3.4, ML:0.5, DV:4.5 for cingulate cortex; and AP: −7.3, ML:1.0, DV:9.5 for pontine nucleus. Bregma was taken as the zero point and the DV distance was measured from the skull. After the cutting of the dura with a sharp needle, the vertical probe, made of a 0.64-mm OD steel shaft and a 0.5-mm membrane, was implanted. The length of the membrane was 2.0 mm and its molecular cut-off was less than 20 kDa. The probe was perfused with Ringer solution (1 μl/min) for 90 min to establish the basal level of the extracellular monoamines following 60-min equilibration, and the perfusion was continued with Ringer solution containing nicotine or other drugs for 90 min more (13). During the dialysis of different regions of the brain with Ringer solution, the basal level of monoamines and their metabolites did not change significantly. The basal extracellular levels of different monoamines and their metabolites are shown in Table I. A syringe pump CMA/100 (Carnegie Medicine) was used to drive the perfusate. Dialysates were collected every 30 min in polyethylene microtubes containing 5 μl of 1 N perchloric acid, kept on ice (14). After collection the samples were immediately frozen and stored at −80°C until analysis. The rats were killed, and the brains were removed and kept overnight in 10% formaldehyde. The next day they were cut into coronal slices using a rat brain matrix (Harvard Instrument) and the site of the probe was examined under the microscope for verification.

Estimation of Nicotine Level in Striatum Following Microdialysis. The microdialysis probe was implanted in the striatum, which was perfused with Ringer solution containing 100 mM [^{3}H]nicotine. After 90 min of perfusion, the brain was cut into 0.5-mm thick coronal slices. The striatum was dissected out and solubilized in 2% sodium dodecyl sulfate, and aliquots were counted. More than 80% of the radioactivity was within 1 mm of the tip of the microdialysis probe.

Measurement of Monoamines and Their Metabolites. The dialysates were analyzed by reverse-phase liquid chromatography with electrochemical detection. A 100 × 4.6 cm column (Alltech Adsorbosphere, 3 μ) with a 20-μl sample loop was used. The mobile phase was 150 mM monochloroacetic acid, 117 mM NaOH, 2 mM 1-octanesulfonic acid (sodium salt), 100 μM Na_2 EDTA, 1.7% v/v acetonitrile, and 0.5% v/v tetrahydrofuran, pH 3.1. EC detector model 400 (Princeton Applied Research) with a glass carbon electrode vs. Ag/AgCl reference electrode at 0.71 V was used. The flow rate was 1.6 ml/min and the retention times were 2.7, 3.2, 7.0, 8.7, 10.8, and 37 min for norepi-

Table I. Basal Extracellular Levels of Monoamines and Metabolites.

Regions	Dopamine	Dihydroxy-phenylacetic acid	Homovanillic acid	Norepinephrine	Serotonin	Hydroxy-indoleacetic acid
Striatum	31 ± 7	3852 ± 312	4220 ± 160	ND	ND	760 ± 67
Substantia nigra	14 ± 5	97 ± 5	121 ± 39	ND	ND	1123 ± 80
Cerebellum	8 ± 6	16 ± 3	41 ± 22	20 ± 6	ND	216 ± 8
Hippocampus	ND	19 ± 2	ND	ND	ND	ND
Cingulate cortex	5 ± 7	71 ± 3	335 ± 40	ND	8 ± 8	619 ± 77
Pontine nucleus	5 ± 1	66 ± 9	110 ± 15	51 ± 3	ND	1156 ± 73
Frontal cortex	ND	52 ± 33	147 ± 107	ND	ND	470 ± 103

The values are the means of basal levels of monoamines and metabolites (pg per 20-μl dialysate) collected from 3-5 rats.
ND = not detectable.

nephrine, 3,4-dihydroxyphenylacetic acid, 5-hydroxyindoleacetic acid, homovanillic acid, dopamine, and 5-hydroxytryptamine, respectively.

Glutamic Acid Analysis. The glutamic acid content of the dialysates was determined with high-performance liquid chromatography (HPLC) using ultraviolet detection following precolumn 1-naphthylisocyanate derivatization. The mobile phase was 0.0045 M sodium phosphate, 0.0045 sodium acetate pH 5.75, 12.5% methanol, and 2.5% acetonitrile (15).

Statistical Analysis. Results were expressed as means ± SEM (pg/20 μl) of three 30-min dialysates from 3-8 animals. The significance of differences between the basal neurotransmitter amine release and its release in the presence of nicotine or nicotine and antagonist was assessed with the Student's *t*-test.

RESULTS

Effect of Nicotine on Extracellular Monoamine Levels at Different Regions in the Rat Brain. Nicotine at a concentration of 1 mM produced a 262% increase of DA level in the striatum (Table II). The extracellular DA level was increased by nicotine in 6 out of 7 areas tested. The effect of nicotine on the extracellular levels of dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was small, perfusion with 100 mM nicotine causing reduction in HVA in the striatum and elevation in the hippocampus. In the striatum the extracellular concentration of DA increased greatly in a concentration-dependent manner in response to nicotine perfusion. In the substantia nigra the perfusion of 5 mM nicotine had no effect, but 50 mM nicotine increased the concentration of DA greatly. In the hippocampus, cortex, and pontine nucleus 100 mM nicotine increased extracellular DA levels, but in cerebellum it had no effect. The perfusion of 1 mM nicotine had no effect in the cortex or pontine nucleus on DA release, but in the striatum it produced a large increase. Release of norepinephrine (NE) was increased by nicotine in the cingulate cortex, pontine nucleus, and substantia nigra. Nicotine stimulated serotonin (5-HT) release in the cortex and pontine nucleus. No effect on the release of NE or 5-HT was detected in the striatum or hippocampus, a small but not significant effect in the cerebellum (Table III). The effect of nicotine on the release of glutamic acid in the striatum is shown in Table IV. Nicotine produced about a 6-fold increase in the extracellular level of glutamic acid, an effect that was completely antagonized by kynurenic acid.

Effect of Cotinine on Extracellular Monoamine Levels. Cotinine, the primary metabolite of nicotine, at 100 mM concentration had no effect on dopamine or serotonin release in the striatum.

Effect of Various Antagonists on Nicotine-Induced Extracellular DA Increase in the Striatum. Cholinergic antagonists - the nicotinic antagonist mecamylamine and the muscarinic antagonist atropine - inhibited nicotine-induced increase of extracellular DA in the striatum (Tables V and VI). Ninety minutes of perfusion with the

Table II. Effect of Nicotine on Extracellular DA, DOPAC, and HVA Levels in Various Cerebral Areas In Vivo

Area	Nicotine mM	Changes in basal levels: Dopamine		Dihydroxyphenylacetic acid		Homovanillic acid		N
		pg/20 μl	%	pg/20 μl	%	pg/20 μl	%	
Striatum	1	110[a]	262	455	11	540	12	3
	5	367[a]	1265	−405	−12	−743	−18	3
	100	696[a]	6327	−89	−16	−439	−54	8
Substantia	5	2	11	−19	−18	−33	−12	3
nigra	50	47[a]	1000	−52	−21	−47	−36	4
Cerebellum	100	13	68	3	18	−17	−29	3
Hippocampus	100	50	>1000	2	11	24	120	4
Cingulate	1	0	0	−11	−12	9	6	4
cortex	100	50[a]	980	−20	−28	−139	−41	3
Pontine	1	0	0	6	12	7	17	4
nucleus	100	36[a]	720	6	9	−12	−11	3
Frontal	1	0	0	−15	−12	32	12	3
cortex	100	8	>160	−5	−10	51	53	4

The values are the means of changes in amine levels (pg/20 μl) with nicotine infusion and the percent changes over basal levels. The amine levels were measured in 2-4 basal and 2-4 nicotine dialysates collected from 3-8 rats per area. N = number of animals. Statistical differences were assessed between basal and nicotine dialysates with the Student's *t*-test: [a] $P < 0.05$.

Table III. Effect of Nicotine on Extracellular NE, 5-HT, and HIAA Levels in Various Cerebral Areas In Vivo

Area	Nicotine mM	Changes in basal levels: Norepinephrine pg/20 µl	Norepinephrine %	Serotonin pg/20 µl	Serotonin %	Hydroxy-indoleacetic acid pg/20 µl	Hydroxy-indoleacetic acid %	N
Striatum	1	0	0	0	0	−100	−12	3
	5	0	0	0	0	−154	−28	3
	100	0	0	0	0	−196	−48	8
Substantia	5	0	0	0	0	−305	−30	3
nigra	50	30[a]	299	0	0	−518	−37	5
Cerebellum	100	22	115	290	51	117	26	3
Cingulate	1	0	0	0	0	119	25	4
cortex	100	73	>1460	200[a]	2500	−26	5	3
Pontine	1	9[a]	150	0	0	−170	−9	4
nucleus	100	124[a]	243	83	>415	−393	−34	3
Frontal	1	0	0	0	0	85	13	3
cortex	100	0	0	40	200[a]	−16	−3	4

The values are the means of changes in amine levels (pg/20 µl) with nicotine infusion and the percent changes over basal levels. The amine levels were measured in 2-4 basal and 2-4 nicotine dialysates collected from 3-8 rats per area. N = number of animals. Statistical differences were assessed between basal and nicotine dialysates with the Student's *t*-test: [a]$P<0.05$.

Table IV. Inhibition of Nicotine-Induced Release of Glutamic Acid by Kynurenic Acid in the Striatum

Treatment	Glutamic Acid pmol/20 µl	% Increase	N
Ringer	65 ± 14		3
Ringer + Nicotine, 5 mM	354 ± 17	445	3
Ringer + Nicotine, 5 mM Kynurenic acid, 5 mM	90 ± 7	38	3

The values are the means ± SEM of glutamic acid levels in pmol per 20-µl dialysate. Glutamic acid levels were measured in 2-3 basal, nicotine, or nicotine plus kynurenic acid dialysates collected from each rat. Dialysates were collected every 30 min.
N = number of rats used.

Table V. Effect of Mecamylamine and Sulpiride on Nicotine-Induced Increase of Dopamine Release in Striatum

Antagonist	Nicotine mM	Antagonist mM	Increase pg/20 ul	Increase %	Percent Inhibition	N
—	5	—	367	1265		3
Mecamylamine	5	5	157[a]	682	46	3
Sulpiride	5	5	87[a]	241	81	3

The values are the means of increases in dopamine levels produced by nicotine with or without perfusion with antagonist before nicotine. Microdialysis was performed for 90 min with Ringer or Ringer and antagonist followed by Ringer and nicotine for 90 min more.
N = number of animals.
[a]$P < 0.05$.

microdialysis probe of 5 mM mecamylamine before the 90-min perfusion with 5 mM nicotine resulted in a 40% reduction in the nicotine-induced extracellular DA increase. Atropine in 0.6 mM concentration produced 91% inhibition of the 5 mM nicotine-induced extracellular DA increase in the striatum. DA receptor antagonists sulpiride (D2) and haloperidol (D1, D2) and the glutamate receptor antagonist kynurenic acid also inhibited nicotine-induced extracellular DA increase in the striatum by 90%.

DISCUSSION

The microdialysis probe made by Carnegie Medicine has been shown to be suitable for the sampling of extracellular monoamines in rat striatum (16-17). This probe, as our test showed, is also suitable for the local administration of nicotine. Since most of the radioactivity in the striatum during microdialysis with [^{3}H]nicotine was in the area of the site of the probe, the changes produced in the extracellular monoamine levels were due to the local effect of nicotine on the cells surrounding the probe.

There were differences in the responses to nicotine among the various brain areas. The striatal dopaminergic system appeared to be the most sensitive to nicotine of the seven areas tested. At 1 mM, nicotine induced an approx. 2-fold increase in the striatal extracellular DA levels. In the cortex and pontine nucleus 1 mM nicotine

Table VI. Effect of Atropine, Haloperidol, and Kynurenic Acid on Nicotine-Induced Increase of Dopamine Release in Striatum

Antagonist	Nicotine mM	Antagonist mM	Increase pg/20 ul	Increase %	Percent Inhibition	N
—	5	—	367	1265		3
Atropine	5	0.6	117[a]	120	91	4
Haloperidol	5	0.06	76[a]	80	94	4
Kynurenic acid	5	5	23[a]	94	93	3

The values are the means of increases in dopamine levels produced by nicotine in the presence or absence of antagonist in the perfusate. Microdialysis was performed for 90 min with Ringer or Ringer and antagonist followed by Ringer and nicotine for 90 min more.
N = number of animals.
[a]$P < 0.05$.

had no effect on the extracellular DA levels (Table I). Nicotine at 100 mM (0.56% penetrated into the tissue) produced an approx. 60-fold increase in the striatal extracellular DA levels, but in the pontine nucleus, cingulate cortex, frontal cortex, hippocampus, and substantia nigra it produced smaller increases, and none in the cerebellum. In the nucleus accumbens an increase in extracellular DA level was reported following similar local administration of nicotine (11). The observed heterogeneity of the nicotine effect on the central dopaminergic system is probably due to differences in the distribution of binding sites for nicotine and in the level of the compounds that mediate the nicotine effect. Since dopamine regulates motor function (18-19), the nicotine-induced increase of motor activity is probably due to stimulation of striatal dopamine release by nicotine. Nicotine also increased the extracellular NE in the substantia nigra, cingulate cortex, and pontine nucleus. The contribution of changes in the extracellular level of NE and 5-HT to the behavioral effect of nicotine remains to be determined. 5-HT was shown to exert tonic control of acetylcholine turnover in the hippocampus and frontal cortex (20), and the NE agonist isoproterenol increased DA release in the striatum (21).

When cotinine (100 mM), a major metabolite of nicotine, was administered by microdialysis in the striatum, there was no increase in the extracellular DA level. Thus the extracellular monoamine increase produced by local administration of nicotine is due to the direct effect of nicotine and not to the effect of its metabolite. A similar conclusion was reached by Todorov et al. (22) studying the stimulation-evoked release of norepinephrine which was enhanced by nicotine.

The inhibition of nicotine-induced elevation of extracellular DA level in striatum by mecamylamine, and also by atropine, indicates that both nicotinic and muscarinic cholinergic receptors are involved in the action of nicotine. It is also conceivable that the nicotine through enhanced acetylcholine release (23) subsequently releases DA from the axon terminals of nigrostriatal pathway (24) through muscarinic receptor mediation. The classification of such a presynaptic muscarinic receptor (25) remains to be elucidated. Systemic administration of mecamylamine also inhibited the effect of nicotine on the extracellular DA level in the nucleus accumbens (11). The inhibition of nicotine-induced striatal extracellular DA increase by DA and a glutamic acid receptor antagonist suggests that both DA and glutamic acid receptors are also involved in the action of nicotine on extracellular DA levels in the striatum (Tables 3 and 4). The fact that kynurenic acid, a kainate and NMDA receptor antagonist prevented the effect of nicotine on DA release suggests that glutamic acid released by nicotine may be involved in that action. Since glutamic acid (26-28) stimulates dopamine release in the striatum, it is possible that the interaction of nicotine with the glutamatergic system through nicotinic receptors results in an elevated extracellular glutamate level, which then stimulates dopamine release (Figure 1). The finding that nicotine enhanced the release of glutamate in striatum, and this nicotine action was also prevented by kynurenic acid (29, Table 4), substantiates our suggestion that the effect of nicotine on DA release is at least partly indirect, through release of glutamate from the corticostriatal axon terminals. Westfall et al. (30) suggested that the striatal nicotinic effect on the dopamine cells is mediated through the substantia nigra reticulata neurons, producing inhibition of these neurons and also stimulation of the dopamine neurons by disinhibition. Injections of glutamate, which would cause pharmacological stimulation of the striatal output neurons, results in inhibition of substantia nigra reticulata cells, and stimulation of A_9 and A_{10} cells via disinhibition (31).

Our study shows that nicotine produces an increase of extracellular DA levels in most cerebral regions studied, and also of extracellular, glutamate, NE, and 5-HT levels in some areas. These endogenous transmitters, released from synaptic and nonsynaptic axon terminals into the extracellular space, may diffuse long distances to reach target cells equipped with receptors sensitive to the ligand and establish nonsynaptic interactions (31) between neurons. It further shows that the mechanism of action of nicotine is rather complex, at least in the striatum, and involves nicotine receptor-mediated glutamic acid release, which is able to release DA from the dopaminergic axon terminals through stimulation of glutamate receptors.

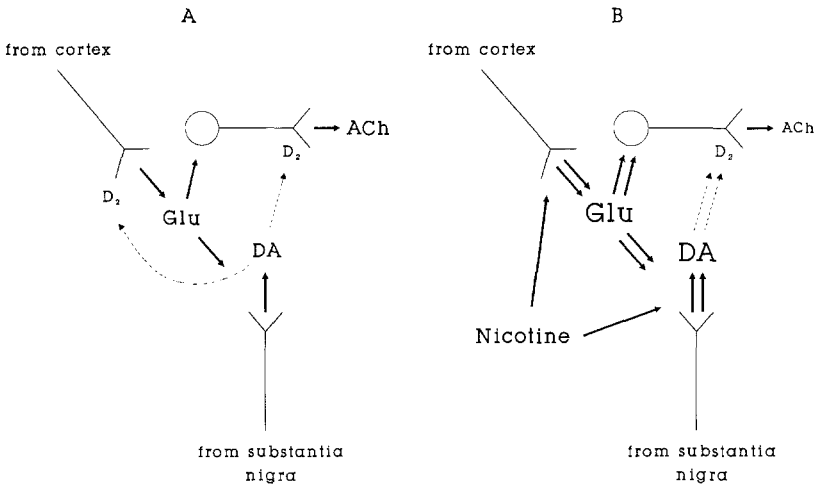

Fig. 1. Scheme of the effect of nicotine on extracellular concentrations of glutamic acid (Glu) and dopamine (DA) in the striatum.
(A) Glutamic acid (Glu) released from the cortico-striatal pathway stimulates the release of dopamine (DA) and acetylcholine (ACh). Dopamine released from the nigro-striatal pathway inhibits both acetylcholine and glutamic acid release, through stimulation by D_2-receptors.
(B) When the striatum was perfused with nicotine (5 mM) the release of glutamic acid was enhanced by 445% (see data of Table 4). The stimulatory effect of nicotine on dopamine release (see data of Table VI) was completely prevented by kynurenic acid, indicating that the effect of nicotine is mainly mediated via glutamic acid release, which is able to release dopamine (26–28).

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