Pharmacology Biochemistry & Behavior, Vol. 41, pp. 57–63. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/92 $5.00 + .00

Chronic Effects of Nicotine on Mesolimbic Dopaminergic System in Rats[1]

YIU K. FUNG

University of Nebraska Medical Center, College of Dentistry, Department of Oral Biology
40th and Holdrege Streets, Lincoln, NE 68583-0740

AND

YUEN-SUM LAU

Creighton University, School of Medicine, Department of Pharmacology, Omaha, NE 68178-0225

Received 31 May 1991

FUNG, Y. K. AND Y.-S. LAU. *Chronic effects of nicotine on mesolimbic dopaminergic system in rats.* PHARMACOL BIOCHEM BEHAV **41**(1) 57–63, 1992.—Rats were pretreated with saline or nicotine (1.5 mg/kg/day) by subcutaneously implanting each animal with an Alzet osmotic minipump which continuously released saline or nicotine (1.5 mg/kg/day) for 14 days. The behavioral and biochemical effects of nicotine on the dopaminergic neuronal system in rat nucleus accumbens were examined. It was found that chronic nicotine treatment increased the affinity of L-[^{3}H]nicotine binding site in the nucleus accumbens. This treatment also potentiated the ability of (+)-amphetamine, but not high potassium, to stimulate formation and release of [^{3}H]dopamine in tissue slices from rat nucleus accumbens. Chronic nicotine treatment did not alter the characteristics of [^{3}H]spiperone binding site, the rate of dopamine turnover and the concentrations of gamma-aminobutyric acid in the nucleus accumbens.

Nicotine Dopamine Nucleus accumbens

TOBACCO products contain high levels of nicotine. With regular use throughout the day, smoking and/or chewing tobacco will continually deliver nicotine to the central nervous system (CNS) (4, 23, 46). Nicotine is the most pharmacologically active component of tobacco products (5, 21, 22). It has been strongly suggested that the pharmacological basis underlying the habitual use of tobacco products is due to the action of nicotine in the brain. Nicotine exerts its psychotropic effects by interacting with the central nicotinic receptors (3, 16, 29, 31, 32, 44). Activation of presynaptic nicotinic receptors on the cell bodies or nerve terminals of the nigrostriatal (striatum) and mesolimbic (nucleus accumbens) dopaminergic (DAergic) neuronal systems stimulates the release of dopamine (DA) (9, 10, 17, 19, 24, 35, 36, 50). Furthermore, this stimulatory effect of nicotine on DA release appears to be greater in the nucleus accumbens than in the caudate-putamen area (45).

It has been suggested that the central actions of nicotine such as alteration of locomotor activity and reinforcement behavior are mediated in part by the central DAergic systems (7, 8, 15, 22, 51). Thus DA may play an important role in eliciting some of the CNS effects produced by the systemic administration of nicotine. The mesolimbic DA system consists of a population of dopamine-secreting neurons with their cell bodies located in the A10 region of the midbrain and projects mainly to two forebrain regions, the nucleus accumbens and the olfactory tubercle. The DAergic stimulation in the nucleus accumbens is thought to be associated with an initiation of locomotor activity and involved in reinforcement and reward behaviors (20, 25, 37). Thus DA release caused by nicotine in the mesolimbic area may be responsible for nicotine-dependent behaviors such as positive reinforcement and psychotropic stimulation.

Acute administration of nicotine activates mesolimbic DAergic neurons, increasing the tyrosine hydroxylase activity and enhancing DA outflow in the nucleus accumbens (6, 7, 24). It has been suggested that chronic and continuous administration of nicotine can exert behavioral and biochemical changes on DA neurons quite different from those seen after single or multiple intermittent nicotine administration (7). This study was designed to evaluate the DAergic-mediated behavioral and biochemical changes in the nucleus accumbens following chronic (14 days) administration of nicotine through osmotic minipump infusion. This method of delivering nicotine avoids instantaneous bursts of nicotine to the brain as with intermittent administration (41). Consequently, biochemical and behavioral alteration in the brain due to chronic continuous administration of nicotine can be correlated to provide important information regarding the effects of nicotine on DAergic system in the nucleus accumbens.

In our previous studies, we found that continuous administra-

[1]A preliminary report of this study appeared in Pharmacologist 33(3):193; 1991.

tion of nicotine (1.5 mg/kg/day) to rats via osmotic minipumps increased the total number (B_{max}) of postsynaptic dopaminergic receptor binding sites and decreased the rate of DA turnover in the striatum (16,17). In this study, we further evaluated the chronic effects of nicotine on neurochemical changes in the mesolimbic DAergic system. Nicotine was continuously administered to rats for 14 days; we then examined the locomotor activity of these animals in response to intraaccumbens injections of DA and measured the ability of gamma-aminobutyric acid (GABA) to inhibit the DA-induced hyperactivity. We also studied the characteristics of nicotinic and DAergic receptor binding sites, the stimulation of [^{3}H]DA synthesis and release by (+)-amphetamine or potassium, the rate of DA turnover and concentrations of gamma-aminobutyric acid (GABA) in the nucleus accumbens.

METHOD

Animals and Nicotine Pretreatment

Male Sprague-Dawley rats (Dominion Inc., Omaha, NE) weighing between 180–200 g were used. They were housed in groups of 3 per cage in a light- and temperature (23 ± 1°C)-controlled environment with a 12-h light-dark cycle and free access to food (Purina Lab Chow, St. Louis, MO) and water.

Animals were anesthetized with a mixture of isoflurane/oxygen and were implanted subcutaneously between the shoulders with Alzet osmotic minipumps model 2002 (Alza Corp., Palo Alto, CA). These pumps were filled with saline or nicotine (1.5 mg/kg/day). Before implantation, each pump was primed overnight at 37°C in saline solution. The dose of nicotine administered was calculated as the free base using nicotine tartrate (Sigma Chemical Co., St. Louis, MO) dissolved in saline. Alzet osmotic minipump model 2002 delivers a 0.5-μl/h solution of nicotine tartrate in saline. The dose of nicotine in mg/kg/day was calculated from the pumping rate and the average body weight of the animals. All studies were conducted 14 days after pump implantation.

Intraaccumbens Drug Injections

Preliminary studies had been conducted to establish the stereotaxic coordinates for intraaccumbens drug injections. This was achieved by examining the tissue stain resulted from an injection of an aqueous 10% (w/v) methylene blue solution into the nucleus accumbens. On the day of the experiment, each rat was injected with a monoamine oxidase inhibitor, nialamide (100 mg/kg, IP) and was allowed to adapt to the Digiscan animal activity monitor (model RXYZCM-16, Omnitech Electronic Inc., Columbus, OH) for an hour. The rat was removed from the activity monitor and was anesthetized with an isoflurane/oxygen mixture. Each rat was then secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA). A midline incision was made in the skull and holes were drilled on each side of the skull at the coordinates of the nucleus accumbens: A 9.4 mm; L ± 2.4 mm (28). The needle of a 10-μl Hamilton syringe (Hamilton Co., Reno, NV) was inserted at a 10° angle (to avoid puncturing the ventricles) into the holes to a depth of V = − 1.0 mm. Under isoflurane/oxygen anesthesia, saline or DA (20 μg) was first injected bilaterally into each side of the nucleus accumbens in a volume of 0.5 μl. Animals usually recovered from isoflurane anesthesia within 2–3 min after removal of the anesthetic and showed no evidence of discomfort from the surgery. The rat was returned to the activity monitor and recorded for locomotor activity. After thirty minutes, the animal was again removed from the activity monitor and secured in the stereotaxic frame. Under isoflurane/oxygen anesthesia, each rat was again injected via the accumbens route with either saline or GABA (200 μg) in a volume of 0.5 μl. Injection of the drug into each side of the nucleus accumbens was performed over a 1-min period. The microsyringe was left in place for an additional minute to allow for drug diffusion away from the injection needle. After this treatment, the skin incision was closed with wound clips and covered with lidocaine ointment to relieve any pain. Upon recovery from the anesthetic, each rat was placed back in the activity monitor for the assessment of locomotor activity. Cumulative locomotor activity was measured at every 15-min interval for a duration of 2 h.

Assessment of Locomotor Activity

Horizontal activity was recorded every 15 min for a duration of 2 h. Horizontal movement sensors directed 16 beams from front to back (x-axis) and 16 beams from side to side (y-axis). Interruption of these beams generated data that were collected by an analyzer and the results printed automatically at the end of each time period. All testing was conducted between 8:00 a.m. and 4:00 p.m.

L-[^{3}H]Nicotine Binding Assay

The nucleus accumbens preparation and L-[^{3}H]nicotine binding assay were carried out according to the methods described by Marks et al. (35). Nucleus accumbens was dissected and homogenized in 0.5 ml of a buffer containing HEPES (20 mM), NaCl (118 mM), KCl (4.8 mM), $CaCl_2$ (2.5 mM), $MgSO_4$ (1.2 mM) and NaOH for adjusting the pH to 7.5. The homogenate was incubated for 5 min at 37°C and was then centrifuged at 17,500 × g for 30 min. The pellet was suspended and lysed in 20 volumes of ice-cold glass-distilled water at 4°C for 1 h, followed by incubating at 37°C for 5 min. The sample was centrifuged as above. The pellet was resuspended and washed twice in fresh homogenizing buffer. Membranes were used for the L-[^{3}H]nicotine binding assay.

The L-[^{3}H]nicotine binding assay (in a final volume of 0.25 ml) contained Tris HCl (0.2 M), pH 7.5, a single concentration of L-[^{3}H]nicotine (9 nM) and 0.1 ml of tissue membranes (0.4–0.7 mg protein). Increasing concentrations of unlabeled L-nicotine (2–200 nM) were added in assays for constructing a competition curve. The assay tubes were incubated at 4°C with agitation for 90 min. The incubation was terminated by pipetting 3 ml of the homogenizing buffer into the tube, and the mixture was poured immediately (within 3 s) onto a GF/C glass microfiber filter disc (Whatman, Inc., Clifton, NJ) that had been presoaked in 0.5% polyethyleneimine in the homogenizing buffer. The filtration was carried out at 4°C under constant vacuum (5 inches of Hg). Each filter disc was washed with 3 ml fresh buffer for three times then dried and counted for radioactivity in a liquid scintillation counter. From each competition curve, the binding parameters (K_D and B_{max}) of the L-[^{3}H]nicotine binding sites were determined using the nonlinear least square regression LIGAND analysis (40).

[^{3}H]Spiperone Binding Assay

Nucleus accumbens from the saline-treated or nicotine-treated animals was homogenized in 4 ml of Tris-HCl (50 mM) buffer (pH 7.4, 4°C) containing ascorbic acid (0.1%), pargyline (0.01 mM), NaCl (120 mM), KCl (5 mM), $CaCl_2$ (2 mM) and $MgCl_2$ (1 mM). The homogenate was centrifuged at 27,000 × g for 20 min at 4°C. The pellet was additionally washed twice with fresh

buffer in the same manner. [^{3}H]Spiperone binding assay was performed according to our previous method (30) using (+)-butaclamol (1.0 μM) for determining the nonspecific binding sites and mianserin (1.0 μM) for masking the [^{3}H]spiperone binding to 5-hydroxytryptamine receptors. A dose-dependent [^{3}H]spiperone (0.04–2.0 nM) binding curve was determined in each of the control and nicotine-treated animals. The binding parameters (K_D and B_{max}) of the high-affinity [^{3}H]spiperone binding sites were determined by using the nonlinear least-squares regression LIGAND analysis (40).

Determination of [^{3}H]DA Synthesis and Release

This study was conducted according to the method as described by Fung and Uretsky (18). Rats were killed and tissue from the nucleus accumbens was dissected, weighed and sliced into 0.25 × 0.25-mm square sections using a McIlwain tissue chopper. The tissue slices were dispersed in ice-cold normal medium containing NaCl (118.4 mM), KCl (4.73 mM), KH_2PO_4 (1.2 mM), $MgSO_4 \cdot 7H_2O$ (1.18 mM), $CaCl_2 \cdot 2H_2O$ (1.25 mM) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (22 mM) and dextrose (2 mg/ml). The solution was aerated with 95% O_2 and 5% CO_2 for 30 min and adjusted to pH 7.2 with 1 N NaOH. The slices were then centrifuged at 500 × g for 5 min. The supernatant fluid containing amino acids released from the slices was discarded (2). The slices were then resuspended in a volume of cold normal medium such that each 0.25-ml aliquot of this suspension would contain 25 mg of the tissue slices. In some experiments, (+)-amphetamine (10^{-6} M) was added to the suspension in a final volume of 3 ml. In other experiments, slices were incubated in potassium-enriched medium containing 30 or 50 mM K^+ substituting for an equimolar concentration of Na^+. The slices were aerated with 95% O_2 and 5% CO_2 and incubated in a shaking water bath at 37°C for 8 min. L-[^{3}H]Tyrosine (55 Ci/mmol) was then added to a final concentration of 10 μM. The incubation was continued for an additional 20 min and the reaction was stopped by cooling the flasks on ice. Replicate slices that were kept on ice throughout the experiment served as blanks. Tissues were separated from the medium by centrifugation, and both fractions were assayed for newly synthesized [^{3}H]DA. [^{3}H]DA was separated from [^{3}H]tyrosine by alumina absorption and ion exchange (Amberlite CG 50) chromatography. The radioactivity present in the predetermined eluate fractions was measured by liquid scintillation counting. The total [^{3}H]DA formation was obtained by adding the activity from both tissue and medium fractions. The release of newly synthesized [^{3}H]DA from the tissue was calculated by dividing the amount of [^{3}H]DA in the medium by the total amount of [^{3}H]DA formed (18).

Determination of the Levels of GABA in Rat Nucleus Accumbens

The GABA content in the nucleus accumbens of rats was estimated according to the method of Earley and Leonard (14), using disposable Bio-Rad columns packed with Sephadex G-10. Animals were killed by decapitation. Tissues from the nucleus accumbens were dissected on an ice-cold surface, weighed and then homogenized in 1 ml of 0.2 N perchloric acid (4°C) containing EDTA (1 mM). The sample was centrifuged at 4800 × g for 20 min at 4°C, and the supernatant was applied onto the column. Tissue extracts containing known amounts of GABA were included throughout the whole procedure. For the separation of GABA, the elution pattern of Earley and Leonard (14) was followed. The concentration of GABA was determined by a fluorimetric method (48). Aliquots of the eluate (0.1 ml) were transferred to separate tubes. Glutamate reagent (0.15 ml) and ninhydrin reagent (0.2 ml) were added consecutively. The samples were well mixed and heated at 60°C for 30 min. After the samples were cooled to room temperature, 5 ml copper tartrate reagent was added. The samples were left standing at room temperature for 15 min and read at 380 nm (excitation)–460 nm (emission). The recovery of GABA was determined to be within 85–90%.

Rate of DA Turnover

Dopamine turnover was measured as the rate at which the striatal DA level declined after intraperitoneal administration of the tyrosine hydroxylase inhibitor, alpha methyl-p-tyrosine (300 mg/kg, IP) (11). Rats were killed at 0, 1 and 2 hours after the injection of the inhibitor and the nucleus accumbens was collected for determining its DA content by using an HPLC. Nucleus accumbens from saline- or nicotine-pretreated rats was suspended in 1 ml of 0.2 N perchloric acid. The sample was sonicated and centrifuged at 11,000 × g for 5 min at 4°C. The supernatant was filtered through a nylon syringe filter unit (0.45 μm). An aliquot of the filtrate was injected into a high-performance liquid chromatography (HPLC) (Waters, Milford, MA) in a mobile phase consisting of sodium acetate (100 mM), citric acid (20 mM), sodium octyl sulfate (Eastman Organic Chemicals, Rochester, NY) (100 mg/l), EDTA (50 mg/l) and methanol (4% v/v), pH 4.1. The sample was chromatographed by a μBondapak C_{18} reversed-phase column (3.9 × 150 mm, Waters, Milford, MA) at a constant flow rate of 2 ml/min. Dopamine concentration in each sample was determined by electrochemical detection at a potential of 0.6 V.

Drugs

The following drugs were purchased from Sigma Chemical Co. (St. Louis, MO): nicotine tartrate, nialamide, DA hydrochloride, pargyline, GABA and alpha methyl-p-tyrosine methyl ester. DA was dissolved in nitrogen-bubbled distilled water containing 0.1% sodium metabisulfite and was adjusted to pH 6 with 0.5 N sodium hydroxide. Both L-[^{3}H]nicotine (60.4 Ci/mmol) and [^{3}H]spiperone (23.2 Ci/mmol) were purchased from New England Nuclear (Boston, MA). L-[^{3}H]Tyrosine (55 Ci/mmol) was purchased from Amersham (Chicago, IL).

Statistical Analysis

For the biochemical data, statistical comparisons were made using analysis of variance followed by Newman-Keuls for comparison between and within groups and the two-tailed Student's *t*-test for independent means. Analysis of variance (ANOVA) was used to analyze the data on locomotor studies followed by least significance difference test. A p value of less than 0.05 was considered to be significant.

RESULTS

Effect of Chronic Nicotine Treatment on DA-Induced Locomotor Activity

Dopamine (20 μg), when injected into each side of the nucleus accumbens, caused a time-dependent increase of locomotor activity (Fig. 1). This DA-induced locomotor hyperactivity in rats, however, was not affected by chronic nicotine treatment (Fig. 1).

Effect of Chronic Nicotine Treatment on GABA Inhibition of DA-Induced Hyperactivity

Bilateral injections of GABA (200 μg) into each side of the nucleus accumbens 30 min after injection of DA (20 μg) inhib-

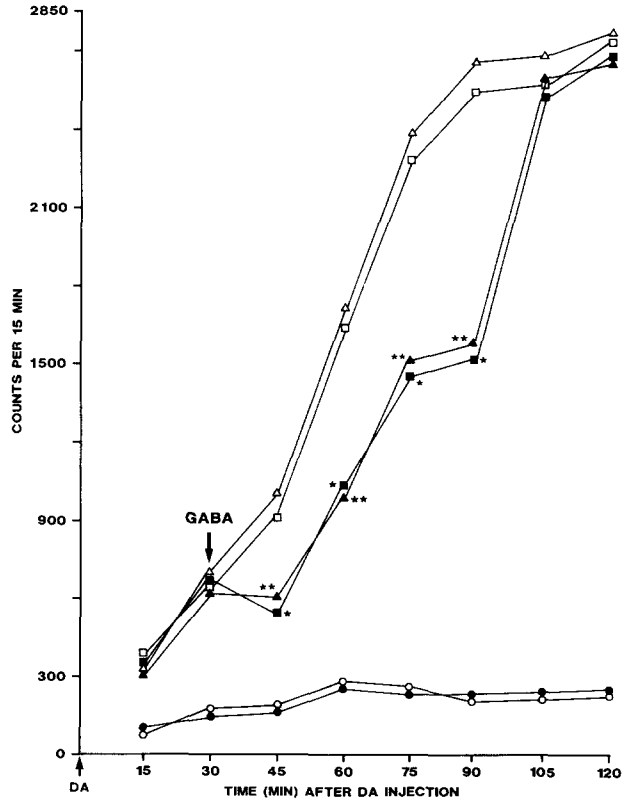

FIG. 1. Inhibitory effect of GABA on DA-induced hyperactivity in chronic saline- or nicotine-treated rats. Rats were implanted with osmotic minipumps containing either saline or nicotine (1.5 mg/kg/day). Fourteen days later, they were injected with nialamide (100 mg/kg, IP) and were allowed to adapt in Digiscan animal activity monitors for an hour. Under isoflurane anesthesia, saline or DA (20 μg) in a volume of 0.5 μl was injected bilaterally into the nucleus accumbens. Either saline or GABA (200 μg) in a volume of 0.5 μl was injected 30 min later. Locomotor activity was measured at 15-min intervals for a duration of 2 h. Each data point represents the mean activity of 7 animals at the end of each 15-min period. Since the standard errors were less than 10% of the mean, they were left out of the graph for the sake of clarity. ○—○ Chronic saline-treated rats which received intraaccumbens injections of saline followed by saline, ●—● chronic nicotine-treated rats which received intraaccumbens injections of saline followed by saline, □—□ chronic saline-treated rats which received intraaccumbens injections of DA followed by saline, △—△ chronic nicotine-treated rats which received intraaccumbens injections of DA followed by saline, ■—■ chronic saline-treated rats which received intraaccumbens injections of DA followed by GABA, ▲—▲ chronic nicotine-treated rats which received intraaccumbens injections of DA followed by GABA. *$p<0.05$ when compared to saline-treated rats which received intraaccumbens injections of DA and saline. **$p<0.05$ when compared to chronic nicotine-treated rats which received intraaccumbens injections of DA and saline.

ited the DA-induced locomotor hyperactivity. The effect of GABA was observed immediately after its injection and continued for 60 min. This ability of GABA to inhibit DA-induced hypermotility was not altered by chronic nicotine treatment (Fig. 1).

Effect of Nicotine Treatment on L-[^{3}H]Nicotine Receptor Binding

L-[^{3}H]Nicotine was used in this study to quantitate the nicotinic receptor binding in rat nucleus accumbens. The binding characteristics of L-[^{3}H]nicotine to the nucleus accumbens membranes from saline- and nicotine-treated rats were compared. No significant difference in the apparent maximum number of binding (B_{max}) was detected in these two animal groups. However, an increase in nicotinic receptor binding affinity as demonstrated by a decrease of the apparent K_D was found in chronic nicotine-treated animals (Table 1).

TABLE 1

EFFECT OF CHRONIC NICOTINE ADMINISTRATION ON L-[^{3}H]NICOTINE BINDING SITES IN RAT NUCLEUS ACCUMBENS

	L-[^{3}H]Nicotine Binding	
Chronic Treatment	B_{max} (fmol/mg protein)	K_D (nM)
Saline	29.1 ± 2.5	5.6 ± 0.9
Nicotine	32.9 ± 3.1	2.2 ± 0.7*

Animals were implanted with osmotic minipumps containing either saline or nicotine (1.5 mg/kg/day) for 14 days. The nucleus accumbens were isolated and used for L-[^{3}H]nicotinic binding studies. Values are presented as mean ± S.E.M. of 5–6 independent experiments. *Significantly lower than chronic saline-treated controls ($p<0.05$, Student's t-test).

Effect of Chronic Nicotine Treatment on D_2 Dopamine Receptor Binding

[^{3}H]Spiperone was used in this study to quantitate the D_2 receptor binding sites in the rat nucleus accumbens. The apparent K_D and B_{max} values for [^{3}H]spiperone binding from chronic nicotine-treated rats were not significantly different from chronic saline-treated controls (Table 2).

Chronic Nicotine Treatment on GABA Levels in Rat Nucleus Accumbens

The effect of chronic nicotine treatment on GABA concentrations was measured. The GABA content (405 ± 37 μg/g) in chronic nicotine-treated rats was not significantly different from the GABA concentration (394 ± 32 μg/g) in chronic saline-treated controls.

Effect of Chronic Nicotine Treatment on (+)-Amphetamine or High Potassium-Stimulated Formation and Release of [^{3}H]DA in Nucleus Accumbens Slices

(+)-Amphetamine (10^{-6} M) or a high concentration of potassium (30 or 50 mM) significantly stimulated the formation

TABLE 2

EFFECT OF CHRONIC NICOTINE ADMINISTRATION ON [^{3}H]SPIPERONE BINDING SITES IN RAT NUCLEUS ACCUMBENS

	[^{3}H]Spiperone Binding	
Chronic Treatment	B_{max} (fmol/mg protein)	K_D (nM)
Saline	70.2 ± 1.5	0.073 ± 0.016
Nicotine	60.5 ± 4.7	0.075 ± 0.011

Animals were implanted with osmotic minipumps containing either saline or nicotine (1.5 mg/kg/day) for 14 days. The nucleus accumbens were isolated and used for DA receptor binding studies. Values are presented as mean ± S.E.M. of 5 independent experiments. All values between the chronic saline- and chronic nicotine-treated groups are not statistically significant in difference (Student's t-test, $p>0.05$).

TABLE 3

EFFECTS OF CHRONIC NICOTINE TREATMENT ON (+)-AMPHETAMINE OR HIGH POTASSIUM-STIMULATED FORMATION AND RELEASE OF [^{3}H]DA IN SLICES FROM RAT NUCLEUS ACCUMBENS

Animal Groups	Treatment	Agent Added to Medium	[^{3}H]DA Formed (nmol/g/20 min)	[^{3}H]DA Released (%)
1	Saline	Saline	1.6 ± 0.3	22.1 ± 3.4
2	Nicotine	Saline	1.5 ± 0.2	25.5 ± 1.5
2A	Saline	Amph. (10^{-6} M)	3.5 ± 0.2*	55.0 ± 2.5*
2B	Nicotine	Amph. (10^{-6} M)	4.3 ± 0.1*†	68.1 ± 1.3*†
3A	Saline	Potassium (30 mM)	3.4 ± 0.2*	59.3 ± 1.8*
3B	Nicotine	Potassium (30 mM)	1.9 ± 0.3‡	33.2 ± 3.4*‡
4A	Saline	Potassium (50 mM)	5.7 ± 0.4*	62.5 ± 0.3*
4B	Nicotine	Potassium (50 mM)	2.0 ± 0.3‡	44.0 ± 1.0*‡

Tissue slices (25 mg) from nucleus accumbens of chronic saline or chronic nicotine-treated (1.5 mg/kg/day) rats were incubated in normal medium containing saline (groups 1 and 2), (+)-amphetamine (10^{-6} M) (groups 2A and 2B) or in high-potassium media (30 mM in groups 3A and 3B; 50 mM in groups 4A and 4B). The slices were preincubated in respective medium for 8 min. L-[^{3}H]Tyrosine was added and the reaction was continued for 20 min. [^{3}H]DA formation and release were then determined. Data are mean ± S.E.M. of 6–10 determinations. *Significantly higher than chronic saline-treated rat slices incubated with saline in the medium (group 1) ($p<0.05$, Newman-Keuls test). †Significantly higher than chronic saline-treated rat slices incubated with (+)-amphetamine in the medium (group 2A) ($p<0.05$). ‡Significantly lower than chronic saline-treated rat slices incubated with corresponding concentrations of potassium in the medium (30 mM in group 3A and 50 mM in group 4A, respectively) ($p<0.05$).

and release of [^{3}H]DA in nucleus accumbens slices of chronic saline-treated rats (Table 4). It is noteworthy that the (+)-amphetamine-stimulated [^{3}H]DA formation and release were potentiated in rats which were treated chronically with nicotine. Paradoxically, the stimulation of [^{3}H]DA formation and release by high concentrations of potassium was blunted in chronic nicotine-treated rats (Table 3).

Effect of Chronic Nicotine Treatment on DA Turnover

The rate of DA turnover in the nucleus accumbens of chronic nicotine-treated rats was not significantly different from chronic saline-treated animals ($p>0.05$) (Fig. 2).

DISCUSSION

In this study, administration of nicotine (1.5 mg/kg/day) via osmotic minipump implantation in rats did not cause any overt sign of tissue necrosis or infection. In our previous study, we reported that nicotine administration to rats using this method had caused no effect on daily gain in body weight or daily food and water consumptions (17). When nicotine is administered to rats at a dose of 1.5 mg/kg/day, the rat plasma nicotine levels are found to be comparable to that of a human individual who chews 12 g of moist ground snuff or smokes one pack of cigarettes a day (5, 17, 23, 41, 46). Thus the present study is designed to investigate nicotine effects using a rat model that closely resembles the conditions of tobacco use in humans.

The activation of mesolimbic DAergic neurons in the nucleus accumbens has been suggested to subserve some of the behavioral effects of nicotine (7, 8, 20). In this study, we examined the chronic effect of nicotine on DA-induced hyperactivity via the nucleus accumbens. In agreement with other studies (12, 13, 25, 26), bilateral injections of DA into the nucleus accumbens of rats pretreated with a monoamine oxidase inhibitor resulted in a long-lasting stimulation of locomotor activity due to the direct stimulation of DA receptors in the nucleus accumbens. The ability of DA to stimulate locomotor hyperactivity was not modified by chronic pretreatment of nicotine, suggesting that nicotine did not alter the sensitivity of DAergic receptors in the nucleus accumbens. This behavioral observation was further supported by our DA receptor binding data which demonstrated that chronic nicotine produced no discernible change in [^{3}H]spiperone binding characteristics (the apparent K_D and B_{max}) in the nucleus accumbens. In contrast, Reilly et al. (43) reported that chronic but intermittent administration (5 days per week for 6 weeks) of nicotine (0.8 mg/kg, once daily) in rats led to an increase in the values for K_D and B_{max} of [^{3}H]domperidone binding. However, our results are more in consent with a recent study showing that chronic (14 days) infusion of nicotine (3 mg/kg/day) to rats via subcutaneously implanted osmotic minipumps produced no significant effect on [^{3}H]spiperone binding in the nucleus accum-

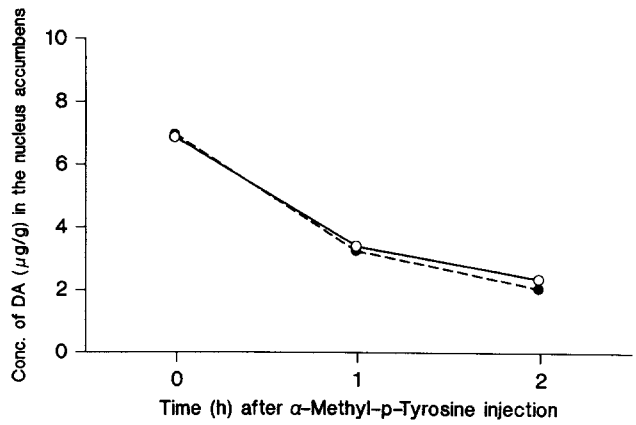

FIG. 2. Dopamine turnover in chronic saline- or nicotine-treated rats. The rate of DA depletion (μg/g) in the nucleus accumbens of chronic saline (○) and chronic nicotine-treated rats (●) at 1 and 2 h after injecting the animals with alpha methyl-p-tyrosine methyl ester (300 mg/kg, IP). The nucleus accumbens were dissected, and their DA concentrations were determined by high-performance liquid chromatography, N = 5–6 for each data point. Since the standard errors were less than 10% of the mean, they were left out of the graph for the sake of clarity. All data points between the chronic saline and chronic nicotine-treated groups are not statistically significant in difference ($p>0.05$, Student's t-test).

bens (6). The differences in duration and method of nicotine administration may account for the discrepancy in these results.

Several studies have shown that GABA interacts functionally with DA in the nucleus accumbens and that GABA plays an inhibitory role in the behavioral expression of DA receptor stimulation (38, 42, 49). Our results showed that the ability of GABA to inhibit DA-induced hyperactivity in the nucleus accumbens was not altered by chronic nicotine treatment. Furthermore, chronic nicotine treatment did not affect GABA concentrations in the nucleus accumbens. These observations suggest that chronic nicotine treatment did not change the GABAergic system significantly in the nucleus accumbens.

Chronic administration of nicotine produced an upregulation (an increase in number) of L-[^{3}H]nicotine receptor binding sites in several brain regions including striatum, midbrain, hindbrain, hippocampus and hypothalamus (16, 29, 34). In nucleus accumbens, however, we detected an increase in the affinity of L-[^{3}H]nicotine binding sites, whereas the receptor was not upregulated after chronic nicotine treatment.

It has been shown in striatal slices that (+)-amphetamine and high potassium stimulated DA synthesis as well as release (47). Similar results were obtained with these agents in this study using nucleus accumbens slices. It is particularly interesting to find that the ability of (+)-amphetamine to stimulate formation and release of [^{3}H]dopamine was potentiated in nucleus accumbens slices from chronic nicotine-treated rats. Surprisingly, chronic nicotine treatment inhibited the effects of high potassium on DA synthesis and release in the nucleus accumbens. Our results suggest that long-term administration of nicotine produced a supersensitive response to the stimulatory effects of (+)-amphetamine. On the other hand, a subsensitive response was developed to the action of high potassium. The mechanisms underlying this paradoxical observation in response to (+)-amphetamine and high K^+ on [^{3}H]DA formation and release in chronic nicotine-treated rat nucleus accumbens remain unclear.

Andersson et al. (1) has reported an increase of DA turnover in the nucleus accumbens following acute administration of nicotine (1 mg/kg, IP). Although chronic nicotine treatment has resulted in a reduction in the striatal DA turnover (17,27), no significant change in DA turnover was found in the nucleus accumbens from this study. Furthermore, nicotine appears to exert differential effects on nigrostriatal and mesolimbic DAergic neuronal systems (16,17). By comparing this study with others, we conclude that nicotine can produce differential effects on behavior and DA function, depending upon whether nicotine is administered continuously by osmotic minipump infusion or by multiple intermittent injections. The results of this study suggest that, although activation of the mesolimbic DAergic system is implicated in some of the behavioral effects of nicotine, the DAergic neurochemical parameters that we so far examined are less affected by the chronic exposure of nicotine.

ACKNOWLEDGEMENTS

This study was supported by a grant (#0225-01) from the Smokeless Tobacco Research Council, Inc., NY. The technical assistance of L. Fiske and the advice of Dr. Norman Uretsky in preparing this manuscript are gratefully acknowledged.

REFERENCES

1. Andersson, K.; Fuxe, K.; Agnati, L. F. Effects of single injection of nicotine on the ascending dopaminergic pathways in the rats. Evidence for increases dopamine turnover in the mesostriatal and mesolimbic dopamine neurons. Acta Physiol. Scand. 112:345–347; 1981.
2. Balcar, V. J.; Johnston, G. A. Liberation of amino acids during the preparation of brain slices. Brain Res. 83:173–175; 1975.
3. Balfour, D. J. K. The effects of nicotine on brain neurotransmitter systems. Pharmacol. Ther. 16:269–282; 1982.
4. Benowitz, N. L. Clinical pharmacology of nicotine. Annu. Rev. Med. 37:21–32; 1986.
5. Benowitz, N. L. Pharmacologic aspects of cigarette smoking and nicotine addiction. N. Engl. J. Med. 319:1318–1330; 1988.
6. Carr, L. A.; Powell, P. P.; Pierce, W. M., Jr. Effect of subchronic nicotine administration on central dopaminergic mechanisms in the rats. Neurochem. Res. 14:511–515; 1989.
7. Clarke, P. B. S. Mesolimbic dopamine activation—the key to nicotine dependence. In: The biology of nicotine dependence. CIBA Foundation symposium. 152:153–168; 1990.
8. Clarke, P. B. S.; Fu, D. S.; Jakubovic, A.; Fibiger, H. C. Evidence that mesolimbic activation underlies the locomotor stimulant action of nicotine in rats. J. Pharmacol. Exp. Ther. 246:701–708; 1988.
9. Clarke, P. B. S.; Pert, A. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res. 348:355–358; 1985.
10. Connelly, M. S.; Littleton, J. M. Lack of stereoselectivity in ability of nicotine to release dopamine from rat synaptosomal preparations. J. Neurochem. 41:1297–1302; 1983.
11. Costa, E.; Neff, N. H. Estimation of turnover rate to study the metabolic regulation of the steady-state level of neuronal monoamines. In: Lajtha, A., ed. Handbook of neurochemistry. vol. 4. New York: Plenum Press; 1969:45–90.
12. Costall, B.; Naylor, R. J. The behavioral effects of dopamine applied intracerebrally to areas of the mesolimbic system. Eur. J. Pharmacol. 32:87–92; 1975.
13. Dozanti, B. A.; Uretsky, N. J. Effects of excitatory amino acids on locomotor activity bilateral microinjection into the rat nucleus accumbens: Possible dependence on dopaminergic mechanisms. Neuropharmacology 22:3–6; 1983.
14. Earley, C. J.; Leonard, B. E. Isolation and assay of noradrenaline, dopamine, 5-hydroxytryptamine and several metabolites from brain tissue using disposable Biorad columns packed with Sephadex G-10. J. Pharmacol. Methods 1:67–79; 1978.
15. Fung, Y. K. The importance of nucleus accumbens in nicotine-induced locomotor activity. J. Pharm. Pharmacol. 42:595–596; 1990.
16. Fung, Y. K.; Lau, Y.-S. Receptor mechanisms of nicotine-induced locomotor hyperactivity in chronic nicotine-treated rats. Eur. J. Pharmacol. 152:263–271; 1988.
17. Fung, Y. K.; Lau, Y.-S. Effect of nicotine pretreatment on striatal dopaminergic system in rats. Pharmacol. Biochem. Behav. 32:221–226; 1989.
18. Fung, Y. K.; Uretsky, N. J. The effect of dopamine uptake blocking agents on the amphetamine-induced circling behavior in mice with unilateral nigrostriatal lesions. J. Pharmacol. Exp. Ther. 214: 651–656; 1980.
19. Giorguieff-Chesselet, M. F.; Kemel, M. F.; Wandscheer, D.; Glowinski, J. Regulation of dopamine release by presynaptic nicotinic receptors in rat striatal slices: Effect of nicotine in low concentration. Life Sci. 25:1257–1262; 1979.
20. Grenhoff, J.; Svensson, T. H. Selective stimulation of limbic dopamine activity. Acta Physiol. Scand. 133:595–596; 1988.
21. Grenhoff, J.; Svensson, T. H. Pharmacology of nicotine. Br. J. Addict. 84:477–492; 1989.
22. Henningfield, J. E.; Goldberg, S. R. Pharmacologic determinants of tobacco self-administration by humans. Pharmacol. Biochem. Behav. 30:221–226; 1988.
23. Hill, P.; Haley, N. J.; Wynder, E. L. Cigarette smoking: Carboxyhemoglobin. Plasma nicotine, cotinine and thiocyanate vs self-reported smoking data and cardiovascular disease. J. Chronic Dis. 26: 439–449; 1985.
24. Imperato, A.; Mulas, A.; Chiara, G. D. Nicotine preferentially stimulates dopamine release in the limbic system of freely moving rats. Eur. J. Pharmacol. 132:337–338; 1986.

25. Jackson, D. M.; Anden, N. E.; Dahlstrom, A. A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. Psychopharmacologia 45:139–149; 1975.
26. Kalivas, P. W.; Miller, J. S. Dopamine microinjection into the nucleus accumbens: Correlation between metabolism and behavior. Biochem. Pharmacol. 34:284–286; 1985.
27. Kirch, D. G.; Gerhardt, G. A.; Shelton, R. C.; Freedman, R.; Wyatt, R. J. Effect of chronic nicotine administration on monoamine and monoamine metabolite concentrations in rat brain. Clin. Neuropharmacol. 10:376–383; 1987.
28. Konig, J. F. R.; Kippel, R. A. The rat brain: A stereotaxic atlas of the forebrain and lower parts of the brain stem. Huntington, NY: Krieger; 1963.
29. Ksir, C.; Hakan, R.; Hall, D. P., Jr.; Kellar, K. J. Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases binding of [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 24:527–531; 1985.
30. Lau, Y. S.; Runice, C.; Dowd, F. Role of Calmodulin-dependent phosphorylation in chronic sulpiride-induced striatal dopamine receptor supersensitivity. J. Pharmacol. Exp. Ther. 229:32–37; 1984.
31. Lee, E. H. Y.; Tsai, M. J.; Tang, Y. P.; Chai, C. Y. Differential biochemical mechanisms mediate locomotor stimulation effects by caffeine and nicotine in rats. Pharmacol. Biochem. Behav. 26:427–430; 1987.
32. Lichtensteiger, W.; Hefti, F.; Felix, D.; Huwyler, T.; Melamed, E.; Schlumpf, M. Stimulation of nigrostriatal dopamine neurons by nicotine. Neuropharmacology 31:963–968; 1982.
33. Makanjoula, R. O. A.; Dow, R. C.; Ashcroft, G. W. Behavioral responses to stereotactically controlled injections of monoamine neurotransmitters into the accumbens and caudate-putamen nuclei. Psychopharmacology (Berlin) 71:227–235; 1980.
34. Marks, M. J.; Romm, E.; Gaffney, D. K.; Collins, A. C. Nicotine induced tolerance and receptor changes in four mouse strains. J. Pharmacol. Exp. Ther. 237:809–819; 1986.
35. Marks, M. J.; Stitzel, E.; Romm, E.; Wehner, J. M.; Collins, A. C. Nicotinic binding sites in rat and mouse brain: Comparison of acetylcholine, nicotine and alpha bungarotoxin. Mol. Pharmacol. 30:427–436; 1986.
36. Martino-Barrows, A. M.; Kellar, J. K. [^{3}H]Acetylcholine and [^{3}H](−)nicotine label the same recognition site in rat brain. Mol. Pharmacol. 31:169–174; 1987.
37. Mogenson, G. J.; Jones, D. L.; Yim, C. Y. From motivation to action: Functional interface between the limbic system and the motor system. Prog. Neurobiol. 14:69–97; 1980.
38. Mogenson, G. J.; Nielsen, M. A. Evidence that an accumbens to subpallidal GABAergic projection contributes to locomotor activity. Brain Res. Bull. 11:309–314; 1983.
39. Moore, K. E.; Wuerthele, S. M. Regulation of nigrostriatal and tuberoinfundibular hypophyseal dopaminergic neurons. Prog. Neurobiol. 13:325–329; 1979.
40. Munson, P. J.; Rodbard, D. LIGAND: A versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220–239; 1980.
41. Murrin, L. C.; Ferrer, J. R.; Zeng, W.; Haley, N. J. Nicotine administration to rats: Methodological considerations. Life Sci. 40: 1699–1708; 1987.
42. Pycock, C. J.; Horton, R. W. Dopamine-dependent hyperactivity in the rat following manipulation of GABA mechanisms in the region of the nucleus accumbens. J. Neural Transm. 45:17–33; 1979.
43. Reilly, M. A.; Lapin, E. P.; Maker, H. S.; Lajtha, A. Chronic nicotine administration increases binding of [^{3}H]domperidone in rat nucleus accumbens. J. Neurosci. Res. 18:621–625; 1987.
44. Rosecrans, J. A.; Meltzer, L. T. Central sites and mechanisms of action of nicotine. Neurosci. Biobehav. Rev. 5:497–501; 1981.
45. Rowell, P. P.; Carr, L. A.; Garner, A. C. Stimulation of [^{3}H] dopamine release by nicotine in rat nucleus accumbens. J. Neurochem. 49:1449–1454; 1987.
46. Russell, M. A. H.; Wilson, C.; Patel, U. A.; Feyerabend, C.; Cole, P. V. Plasma nicotine levels after smoking cigarettes with high, medium and low nicotine yield. Br. Med. J. 2:414–416; 1975.
47. Schwarz, R. A.; Uretsky, N. J.; Bianchine, J. R. The relationship between the stimulation of dopamine synthesis and release produced by amphetamine and high potassium in striatal slices. J. Neurochem. 35:1120–1127; 1980.
48. Uchida, T.; O'Brien, R. I. The effects of hydrazines on rat brain 5-hydroxytryptamine, norepinephrine and gamma-aminobutyric acid. Biochem. Pharmacol. 13:725–730; 1964.
49. Van Rossum, J. M.; Broekkamp, C. L. E.; Pejnenburg, A. J. J. Behavioral correlates of dopaminergic function in the nucleus accumbens. Adv. Biochem. Psychopharmacol. 16:201–207; 1977.
50. Westfall, T. C.; Grant, H.; Perry, J. Release of dopamine and 5-hydroxytryptamine from rat striatal slices following activation of nicotinic-cholinergic receptors. Gen. Pharmacol. 14:321–325; 1983.
51. Wise, R. A.; Rompre, P. P. Brain dopamine and reward. Annu. Rev. Psychol. 40:191–225; 1989.