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Behavioral and Biochemical Effects of Nicotine in an MPTP-Induced Mouse Model of Parkinson's Disease

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SERSHEN, H., A. HASHIM AND A. LAJTHA. *Behavioral and biochemical effects of nicotine in an MPTP-induced mouse model of Parkinson's disease.* PHARMACOL BIOCHEM BEHAV **28**(2) 299–303, 1987.—This study examined the effects of nicotine on locomotor activity and on the level of dopamine (DA) and its metabolies 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum and olfactory tubercle of mice that had been treated with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP significantly lowered the spontaneous locomotor activity 1–2 weeks and 2 months after 2 injections of MPTP (30 mg/kg SC, 24 hr apart) in young adult (3 months) and old mice (22–24 months old). The effect of nicotine on locomotion was biphasic; an initial stimulation of locomotor (0–5 min after nicotine) followed by a depressant period lasting from 5 to 20 min after injection. Tolerance to the depressant effect of nicotine developed after the 5th day of daily injections of nicotine (0.4 mg/kg SC, twice daily). Tolerance did not occur by day 8 to the initial stimulatory effect of nicotine. A similar effect of nicotine on locomotor activity was seen in mice treated with MPTP. The levels of DOPAC and HVA in the striatum were reduced by about 20% in the chronic nicotine-treated animals. The levels of DOPAC, DA, and HVA were reduced in the MPTP-treated mice; however, acute and chronic nicotine did not cause an additional change in the amine levels. The results suggest that nicotine has an influence on locomotor activity in MPTP-treated mice and that this effect is not due to changes in DA receptor activity in the striatum caused by chronic nicotine.

Nicotine MPTP Parkinson's disease Locomotor activity

THE causation of Parkinson's disease remains obscure; according to epidemiological studies cigarette smoking may have pathophysiological and/or therapeutic implications [1, 2, 6]. An early uncontrolled observation reported benefits from injections of nicotine in patients with parkinsonism [17]. Enhanced locomotor response after injections of nicotine has been observed in rodents [4,11]. Recently, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been shown to produce nigrostriatal dopaminergic deficits in humans, primates [13], and mice [9,22] that may provide a useful model to study the mechanisms involved in Parkinson's disease. We attempted to test whether there is any experimental support for a therapeutic effect of nicotine in an MPTP-induced animal model of Parkinson's disease.

The present study examined the degree of motor deficit produced in mice after MPTP and the subsequent effects of nicotine on motor behavior and catecholamine response in this animal model of Parkinson's disease.

METHOD

Animals

Adult female BALB/cBy mice bred in our colony were used. The animals were kept on a 12-hour light/dark cycle, with food and water available ad lib.

Drugs

Two injections of MPTP·HCl (30 mg/kg SC; Research Biochemicals Incorporated, MA) were given in a period of 24 hours. Nicotine (0.4 mg/kg as free base, SC) was injected twice daily at approximately the same time of day (once in the morning and once in the afternoon) for up to 9 days.

Locomotor Activity

Locomotor activity was measured over several 24-hour periods in MPTP-treated young and old mice. Two cages each containing 3 animals were placed in an activity monitor, and activity measured over 2-hour segments. Control animals were tested concurrently in another monitor.

Locomotor activity was also measured in individual animals given nicotine. Mice were housed in separate cages (27×17×12 cm) during the period of locomotor activity measurements as described by Reith *et al.* [19]. Behavioral testing was started by placing the animal in its own home cage in an Opto-Varimex-Minor activity monitor (Columbus Instruments) and replacing the lid with a flat top without food and water. After basal activity was measured for 10 min in 5-min segments, the animal was injected with either saline or nicotine and locomotor activity measured for an additional 20 min (in 5-min segments). The number of activity

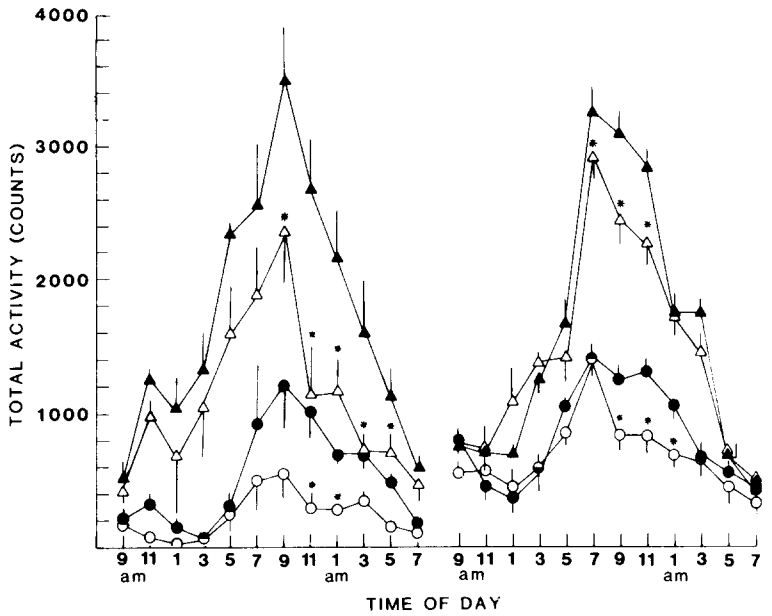

FIG. 1. Spontaneous locomotor activity in young (▲) and old (●) BALB/cBy mice after MPTP (2 injections; 0.4 mg/kg, SC, 24 hours apart) (open symbols); 2 weeks (left side) and 2 months (right side) after treatment. The average total activity ±S.E.M. over a 2-hour period covering a 24-hour cycle taken 7 times is shown. *Significantly different ($p<0.05$) from untreated animals (n=7 per time period, Student's t-test).

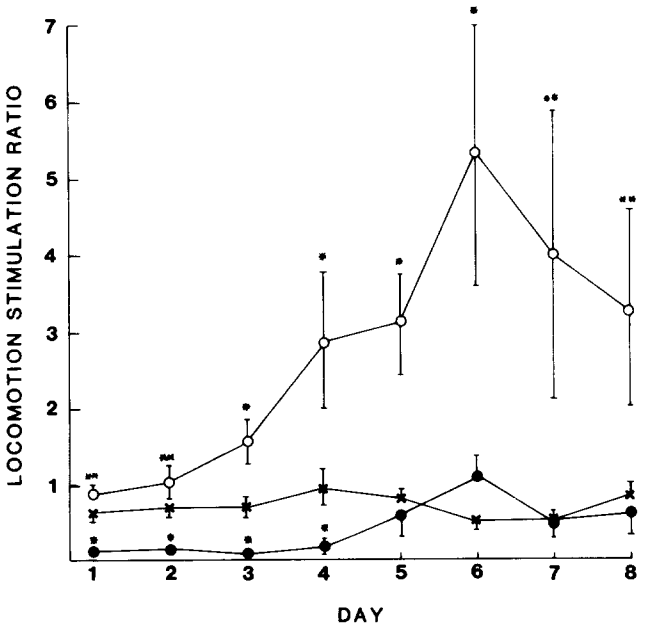

FIG. 2. Effect of nicotine on the locomotor stimulation ratio (number of activity counts produced in a 5-min postinjection period divided by the average counts produced during a 5-min segment in the preinjection period). Locomotor stimulation ratio for saline treated (x), and nicotine treated (○=0.5 min and ●=5–10 min after nicotine injection). Significantly different (*$p<0.01$, **$p<0.05$, n=20 animals) from saline injected by Student's t-test. The absolute activity levels preinjection was 141±68; mean preinjection activity counts during the 8 test days ±S.D., n=32.

counts produced by interrupting consecutive infrared beams in the postinjection period was divided by the average counts produced during a 5-min segment in the preinjection period. This ratio was defined as the locomotor stimulation ratio. Animals were injected at approximately the same time of day when tested daily. Saline- and nicotine-treated animals were tested concurrently. MPTP-treated mice were tested with nicotine 1–2 weeks after MPTP.

Determination of Amine Levels

Animals were killed by decapitation at the end of the behavioral studies, and the striatum was quickly removed and frozen on dry ice. The tissue was disrupted by sonication in 0.2 N $HClO_4$ containing 0.1% cysteine. The clear supernatant after centrifugation was injected into an ODS 5 μm Biophase column and eluted with 3.5% acetonitrile/96.5% 0.15 M monochloracetic acid buffer pH 3.0 containing 0.86 M sodium octylsulfate and 18 ml tetrahydrofuran per liter. The detector was an LC-4B (Bioanalytical Systems) with a glassy carbon working electrode at 800 mV detector potential against a Ag/AgCl reference electrode.

RESULTS

Total spontaneous locomotor activity over a 24-hour period was measured in young and old mice, and at 1–2 weeks and 2 months after MPTP (Fig. 1). The average total activity of seven 24-hour periods is shown. Locomotor activity was greatly reduced in old (20–24 months old) mice as compared to young animals (3–6 months old). Peak locomotor activity was observed during the nighttime. The peak activity (7:00 p.m. to 5:00 a.m.) was significantly reduced ($p<0.05$ versus untreated animals, n=7 per time period, Student's t-test) in both young and old mice given two injections

TABLE 1

EFFECT OF CHRONIC AND ACUTE NICOTINE ON CATECHOLAMINE LEVELS IN STRIATUM AND OLFACTORY TUBERCLE

Treatment		DOPAC	DA	HVA	DOPAC + HVA/DA
		(ng/mg protein)			
Striatum					
Saline		7.9 ± 0.4	134 ± 3	23.5 ± 0.7	0.233 ± 0.006
Saline/Nicotine	5 min	7.8 ± 0.7	126 ± 4	20.5 ± 0.5*	0.225 ± 0.011
Saline/Nicotine	10 min	6.4 ± 0.3	132 ± 5	20.0 ± 0.7*	0.202 ± 0.010†
Nicotine/Nicotine	5 min	5.8 ± 0.2*	135 ± 5	19.9 ± 1.0*	0.189 ± 0.006*
Nicotine/Nicotine	10 min	5.9 ± 0.2*	136 ± 7	19.2 ± 0.7*	0.186 ± 0.004*
Olfactory Tubercle					
Saline		7.9 ± 0.4	74 ± 4	10.9 ± 0.4	0.256 ± 0.010
Saline/Nicotine	5 min	7.8 ± 0.3	74 ± 3	10.1 ± 0.3	0.242 ± 0.007
Saline/Nicotine	10 min	8.5 ± 0.4	74 ± 4	10.0 ± 0.4	0.253 ± 0.005
Nicotine/Nicotine	5 min	6.5 ± 0.2†	76 ± 4	8.8 ± 0.3*	0.202 ± 0.005*
Nicotine/Nicotine	10 min	7.3 ± 0.5	78 ± 6	9.4 ± 0.4†	0.216 ± 0.009*

Mice were injected with either saline or nicotine (0.4 mg/kg as free base, SC) twice daily for eight days. On day 9, a group of saline-treated animals were given an acute injection of nicotine, and the nicotine-treated animals an injection of nicotine. Animals were killed 5 and 10 min after injection and amine levels determined in striatum and olfactory tubercle.

Significantly different (*$p<0.001$; †$p<0.05$; n=10) as compared to saline group by ANOVA.

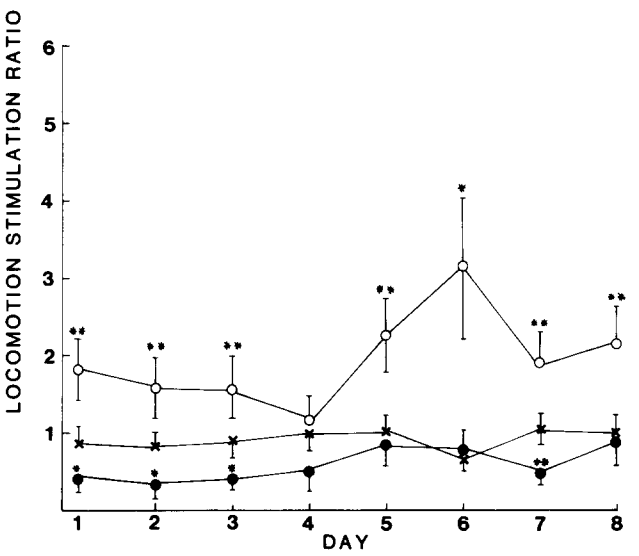

FIG. 3. Effect of nicotine on locomotor stimulation ratio in MPTP-treated mice. Symbols as in Fig. 2. Significantly different (*$p<0.01$, **$p<0.05$, n=20) from saline injected mice. The absolute activity levels preinjection was 85±24; mean preinjection activity counts during the eight test days ±S.D., n=32.

of MPTP. This reduction in activity persisted even 2 months after MPTP.

The effect of nicotine on locomotor activity was measured in the daytime between 9:00 a.m. and 5:00 p.m. (Fig. 2). Each animal was tested at approximately the same time of day throughout the chronic nicotine study. Activity was defined as the ratio of postactivity locomotor counts after nicotine injection to preactivity counts (locomotor stimulation ratio). Nicotine has been shown to have a biphasic effect on behavior in rats, an initial depression followed by stimulation [4]. We observed a similar biphasic response in mice; however, an initial stimulation was followed by a depressant effect seen 5–10 min after injection of nicotine (Figs. 2 and 3). This depression lasted up to 20 min, but the period of depression decreased with repeated daily nicotine injections (data not shown), and by day 5, tolerance to this depressant effect of nicotine occurred (Figs. 2 and 3). The locomotor stimulation ratio also increased during the 0–5 min-period after nicotine, starting on day 1, with a peak stimulation at day 6, and persisted even after the 8th day of nicotine injections (Figs. 2 and 3).

The levels of dopamine and its metabolites were measured on day 9, in chronic saline-treated animals after saline or acute nicotine, and in chronic nicotine-treated animals 5 and 10 min after nicotine, in striatum and olfactory tubercle (Table 1). DA levels were not significantly changed in either brain region in control mice given nicotine acutely, or in chronic nicotine-treated mice. The level of DOPAC was reduced by approximately 25% in the chronic nicotine-treated animals, and was not changed in controls given an acute injection of nicotine. The level of striatal HVA was also reduced by 15–18% in the chronic nicotine-treated mice, and was reduced by 13–15% in animals given acute nicotine. HVA was reduced by 14–19% in the olfactory tubercle under chronic nicotine treatment. The ratio of the dopamine metabolites (DOPAC AND HVA) to DA was reduced by about 20% in both the striatum and the olfactory tubercle after chronic nicotine treatment.

In MPTP-treated mice, the effect of nicotine was similar on locomotor activity (Fig. 3). The preinjection activity of the MPTP-treated mice (Fig. 3) was lower by 40% than the untreated animals shown in Fig. 2 (85±24 versus 141±68;

TABLE 2

EFFECT OF CHRONIC AND ACUTE NICOTINE ON CATECHOLAMINE LEVELS IN STRIATUM AND OLFACTORY TUBERCLE OF MICE TREATED WITH MPTP

Treatment		DOPAC	DA	HVA	DOPAC + HVA/DA
		(ng/mg protein)			
Striatum					
Saline (control)		6.0 ± 0.2	118 ± 5	15.7 ± 0.7	0.186 ± 0.004
Saline (MPTP)		2.9 ± 0.2	45 ± 3	13.9 ± 0.7	0.374 ± 0.024
Saline/Nicotine	5 min	3.5 ± 0.4	54 ± 6	13.1 ± 0.9	0.316 ± 0.010
Saline/Nicotine	10 min	2.9 ± 0.2	46 ± 2	12.4 ± 0.5	0.330 ± 0.010
Nicotine/Nicotine	5 min	3.5 ± 0.4	51 ± 4	11.8 ± 1.4	0.309 ± 0.014
Nicotine/Nicotine	10 min	3.4 ± 0.4	45 ± 4	11.6 ± 0.5	0.347 ± 0.023
Olfactory Tubercle					
Saline (control)		6.6 ± 0.2	78 ± 3	9.3 ± 0.5	0.204 ± 0.004
Saline (MPTP)		4.7 ± 0.4	59 ± 5	7.9 ± 0.8	0.213 ± 0.006
Saline/Nicotine	5 min	4.7 ± 0.3	53 ± 4	7.2 ± 0.4	0.228 ± 0.006*
Saline/Nicotine	10 min	4.9 ± 0.2	51 ± 2	7.8 ± 0.4	0.252 ± 0.006*
Nicotine/Nicotine	5 min	4.3 ± 0.2	56 ± 3	6.8 ± 0.3	0.202 ± 0.005
Nicotine/Nicotine	10 min	4.3 ± 0.4	53 ± 3	6.9 ± 0.3	0.214 ± 0.006

Mice were given two injections of MPTP·HCl (30 mg/kg in a period of 24 hr). One to two weeks later nicotine was given twice daily as in Table 1.

Significantly different (*$p<0.05$; n=10) as compared to saline (MPTP group) by ANOVA.

mean preactivity counts during the eight test days ±S.D.; $p<0.001$, tind=4.117, n=32, Student's t-test). Tolerance to the depressant effect of nicotine measured at 5–10 min after nicotine injection developed after the 5th day of injections. Locomotor stimulation did occur at 0–5 min after injection of nicotine, with a 2–3-fold increase in the locomotor stimulation ratio seen between days 5 and 8, although the increase seen was not as large as with the non-MPTP-treated mice. In absolute terms, the activity after nicotine in MPTP-treated mice increased to slightly greater than the preinjection activity of non-MPTP-treated mice.

The level of DA was reduced by about 55–60% in the striatum of MPTP-treated animals (Table 2). The ratio of the DA metabolites to DA increased by about 80% in the striatum. The level of DA in the olfactory tubercle was affected less by MPTP (reduced by about 25%), with a less than 25% increase in the ratio of DA metabolites to dopamine. Acute or chronic nicotine had no effect on the level of DA, DOPAC, or HVA in MPTP-treated mice.

DISCUSSION

Epidemiologic evidence has suggested a negative association between cigarette smoking and the risk of Parkinson's disease [1, 2, 6], and thus that nicotine may have some therapeutic value [11,17]. Since a deficiency of striatal dopamine is fundamental in parkinsonism, by providing chronic nicotine exposure, cigarette smoking could exert a central dopaminergic influence to alter the dopamine deficiency characteristic of parkinsonism. We attempted to test this possibility in a mouse model of parkinsonism induced by exposure to the neurotoxin MPTP. This highly selective, irreversible neurotoxicity that is responsible for a parkinsonian syndrome in man and monkey following the administration of MPTP results from the destruction of dopamine neurons in the substantia nigra.

MPTP does induce a deficit in locomotor activity, clearly seen when the animals are most active during the night. Although the effect of nicotine was measured during the day cycle, the preinjection activity of the MPTP-treated mice was also lower than control mice during this time period. Nicotine has been shown to have a biphasic effect on behavior in rats, an initial depression followed by stimulation [4]. We observed biphasic response in mice; however the depression followed an initial stimulation of locomotor activity. By day 5, tolerance to the depressant effect occurred (Figs. 2 and 3). Tolerance to the stimulant effect of nicotine did not occur up to the eighth day of injection. Although the locomotor activity was lower in MPTP-treated animals a similar profile of activity was seen after nicotine treatment. Although the time frame of stimulation was only 5 minutes, the lack of tolerance to this effect after daily injections could suggest that repeated exposure to nicotine would prolong the stimulation; for example, in humans who maintain a plasma nicotine concentration by repeated puffs from cigarettes over several hours. Additionally, the effect of nicotine could be different if measured when the animals are more active during the night. Be that as it may, it is possible that MPTP-treated mice have low levels of activity, and that nicotine stimulates activity back towards normal levels, and this could explain the possible therapeutic action of nicotine as seen by Moll [17].

A major biochemical change after MPTP is the marked reduction of dopamine in the striatum. Since nicotine does release striatal dopamine [7,15], it was of interest to examine the effect of nicotine in MPTP-treated mice on catecholamine metabolism.

Amine levels were slightly reduced after chronic nicotine, as indicated by a 20% reduction in the ratio of DA metabolites to DA in striatum and olfactory tubercle. In MPTP-treated animals, this reduction in ratio was seen only in the olfactory tubercle. The olfactory tubercle has been shown to

be less affected by MPTP neurotoxicity [20]. Changes do, however, occur in the surviving terminals after MPTP treatment. The increase in the ratio of DA metabolites to DA indicate compensatory mechanisms to increase DA release after 6-hydroxydopamine [8], in parkinsonian brain [10], or after MPTP (here and [21]). Although nicotine increases striatal dopamine release [7,15] the present results on catecholamine levels did not indicate an increase in DA release as far as the interpretation of the increase in the ratio of metabolites of dopamine to dopamine has been suggested to indicate. Since monoamine levels remain fairly constant during periods of enhanced release, an increase in the metabolite to transmitter ratio has been assumed to be indicative of an increased turnover of the transmitter, and this, in turn, equated with increased neuronal activity. In the present experiments, we did not find any indication of increased DA release by nicotine as measured by the ratio of DA metabolites to DA. Although, caution has been urged in relating changes in DA metabolite levels to neuronal release processes [5,23], we do note in Table 1 indications that chronic nicotine decreases dopamine release in striatum from the decrease in the ratio of dopamine metabolites to dopamine. This finding agrees with our previous study in rats after 6 weeks of chronic nicotine [14], suggesting that nicotine acts at other sites to enhance locomotion. The sustained decrease in dopamine turnover induced by repeated nicotine may be a factor in the reduced tendency of individuals who later develop Parkinson's disease to acquire the smoking habit. D_2 receptor activity in striatum, as measured by labeled spiperone binding was not changed after acute or chronic nicotine treatment (data not shown). This suggests that the effect of nicotine on postsynaptic dopamine activity was not involved.

Our results show that nicotine has an influence on locomotor behavior in MPTP-treated mice. This effect may not be due to changes in DA receptor activity caused by chronic nicotine, but may be related to the observed increase in the number of nicotinic cholinergic receptors seen after chronic nicotine [12,16]. The present results afford some support for a therapeutic action of nicotine in a Parkinsonian animal model.

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