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BRIEF COMMUNICATION

Effect of Long-Term Cigarette Smoke Exposure on Locomotor Activity and Brain Monoamine Levels in Rats

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SUEMARU, K., R. OISHI, Y. GOMITA, K. SAEKI AND Y. ARAKI. *Effect of long-term cigarette smoke exposure on locomotor activity and brain monoamine levels in rats.* PHARMACOL BIOCHEM BEHAV 41(3) 655–658, 1992.– Rats were chronically exposed to cigarette smoke for 20 min twice daily using a smoking machine. On days 1, 4, and 14, locomotor activity and rearing were measured for 15 min in an open-field apparatus. On day 1, exposure to cigarette smoke increased locomotor activity and rearing in the latter half of the observation period. This effect became more pronounced on days 4 and 14. Chronic cigarette smoke exposures for 21 days significantly decreased the norepinephrine levels in the hypothalamus, thalamus, and pons-medulla, but not the levels of dopamine, 5-hydroxytryptamine, or their metabolites. These results suggest that repeated cigarette smoke exposure increasingly stimulates locomotor activity and rearing and affects norepinephrine metabolism, especially in the brainstem.

Cigarette smoke Locomotor activity Rearing Monoamine levels Rat

TOBACCO smoking affects CNS arousal, eliminates stress, suppresses appetite, and improves performance on certain tasks in humans (15). In behavioral studies of animals, acute systemic administration of nicotine has been reported to stimulate or depress locomotor activity depending on the dose, sex of the animal, time after administration, and route of administration (4,7,8). In addition, both suppression (tolerance) and enhancement of the nicotine-induced locomotor-stimulating action have been reported after repeated systemic administration(2,4,5). However, there are few reports of the effect of tobacco smoking on locomotor activity in rats. Furthermore, there is no report of the relationship between locomotor activity and brain monamine levels when tobacco smoke was chronically inhaled. In the present study, we measured locomotor activity and rearing in an open-field apparatus, and also examined brain monoamine levels after long-term exposure to cigarette smoke.

METHOD

Animals

Male Wistar strain rats (supplied by Charles River Lab., Japan) weighing 180–190 g were kept in groups of five animals each in plastic walled cages (26 × 36 × 25 cm) in a room with a 12 L:12 D cycle (lights on from 0700–1900) at 22 ± 1°C and approximately 60% relative humidity. Animals were allowed free access to food and water during the experiment.

Cigarette Smoke Exposure

The cigarette used was "Long-Pease,®" supplied by Japan Tobacco Inc., Tokyo. The nicotine and tar contents were 2.0 and 22 mg per cigarette, respectively. Rats were exposed to cigarette smoke using the Hamburg II Smoking Machine as previously described (10). Briefly, the cigarette was smoked at a rate of 15 puffs/min with an inhalation duration of 2 s. Smoke was mixed with 7 volumes of air and sent to the exposure chamber. When animals were exposed to the same cigarette smoke for 10 min, the nicotine plasma levels reach 212 ng/ml (11). In the present study, animals were exposed to the smoke for 20 min in the chamber twice daily (0700 and 1900) for 21 days. Control rats were handled in the same manner as the smoke-exposed rats except for cigarette smoking.

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Open-Field Test

Locomotor activity and rearing were measured after the cigarette smoke exposure (1700) on days 1, 4, and 14 using Hall's open-field apparatus (12), which was 60 cm in diameter with a gray painted stainless steel wall 50 cm high. The field floor was divided into 19 blocks almost similar in area. The number of blocks traversed (locomotor activity) and rearing were counted every 3 min for 15 min.

Assay of Brain Monoamines and Their Metabolites

Rats were killed 24 h after the last exposure to cigarette smoke. The brain was immediately removed and seven brain regions (cerebral cortex, hippocampus, striatum, hypothalamus, thalamus, midbrain, and pons-medulla oblongata) were dissected as previously described (24). Tissue was homogenized with 0.4 M perchloric acid containing 0.1% L-cysteine and an appropriate amount of epinine as an internal standard. After centrifugation, a 50-μl aliquot of the supernatant was injected into a high-performance liquid chromatograph (HPLC) with an electrochemical detector to determine concentration of norepinephrine (NE), 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), and 3,4-dihydroxyphenylacetic acid (DOPAC). The HPLC system was composed of a pump (LC-6A, Shimadzu, Kyoto, Japan), a reverse-phase column (Wakopak ODS $5C_{18}$, 4.6 $\times$ 250 mm), and a coulometric detector (Coulochem 5100A, ESA, Inc., Bedford, MA). The mobile phase used was 0.08 M phosphate buffer containing 0.8 mM sodium octanesulfonate, 6% acetonitrile, and 10 μM EDTA, pH 4.0.

Statistical Analysis

Behavioral data were analyzed by the Mann-Whitney *U*-test and biochemical data were analyzed by the two-tailed Student's *t*-test.

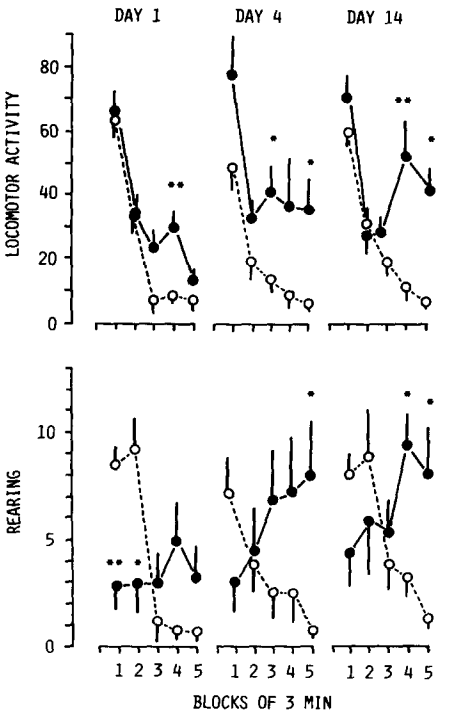

FIG. 1. Effect of long-term cigarette smoke exposure on locomotor activity and rearing in rats. Activity was measured after the second exposure of the day on days 4 and 14 as indicated. Each point represents the mean number of blocks traversed (locomotor activity) or the rearing every 3 min in an open-field. Vertical bars are the SEM. ○, nonsmoking control group; ●, cigarette-smoke-exposed group (n = 6 for each). *$p < 0.05$; **$p < 0.01$.

RESULTS

Figure 1 shows the effects of chronic cigarette smoke exposure on locomotor activity and rearing. On day 1, locomotor activity in the latter half of the observation period was slightly higher in the smoke-exposed rats than in the control rats. This stimulating effect was marked on days 4 and 14. During the first 6 min of observation on day 1, the smoke-exposed rats showed less rearing and instead showed marked grooming and preening. However, in the latter half of the observation period the smoke-exposed rats showed more rearing, but not significantly more than control. The increase in rearing by the smoke exposure became more pronounced on days 4 and 14.

As shown in Table 1, NE levels were significantly lower in hypothalamus, thalamus, and pons-medulla oblongata in the smoke-exposed rats. However, levels of 5-HT, 5-HIAA, DA, and DOPAC were not different from the control in any brain region examined.

DISCUSSION

There have been many reports of acute and chronic effects of nicotine on locomotor activity (4,5,8,21). In general, small doses of nicotine have a stimulating effect that is enhanced by chronic administration. On the other hand, large doses of nicotine have depressant effects including ataxia, particularly when measured soon after administration, but there is tolerance to these depressant effects with repeated administration. In the present study, cigarette smoke exposure produced hyperactivity and repeated exposure enhanced the stimulation of locomotor activity and rearing. The enhancing effect may be attributed to the changes of sensitivity to smoke. Namely, these may be due to a chronic action of a small amount of nicotine contained in the cigarette smoke.

It has been reported that nicotine receptors are present in noradrenergic and dopaminergic nerve terminals (6,25) and that nicotine accelerates catecholamine release (13,14) and turnover (19,21,22). Acute cigarette smoke exposure to rats also decreases NE and DA levels in the hypothalamus and enhances NE turnover (1). However, chronic nicotine administration decreases the catecholamine turnover in the rat brain (9,16). The present study clearly showed that long-term cigarette smoke exposure decreases NE levels in the hypothalamus, thalamus, and pons-medulla in the rat brain. These changes might be to a decrease in NE turnover in these areas, although further study is required to show clear evidence.

It has been suggested that the locomotor-stimulating action of acute administration of nicotine coincides with dopaminergic activation in the mesolimbic system (3,23), but enhancement of the stimulating effect by chronic administration coincides with an increase in the number of nicotinic and dopaminergic receptors (9,17,18) and decreased dopamine turnover (9,16,20), in the rat brain. However, decreases in the DA

TABLE 1

EFFECT OF LONG-TERM CIGARETTE SMOKE EXPOSURE ON LEVELS OF MONOAMINES AND THEIR METABOLITIES IN THE RAT BRAIN

Brain Region	Treatment	Brain Levels (ng/g)				
		NE	5-HT	5-HIAA	DA	DOPAC
Cerebral cortex	Control	197 ± 11	185 ± 11	254 ± 18	152 ± 18	57 ± 4
	Smoke	192 ± 11	176 ± 9	256 ± 9	167 ± 13	68 ± 6
Hippocampus	Control	378 ± 25	140 ± 7	333 ± 15		
	Smoke	294 ± 13	145 ± 8	328 ± 11		
Striatum	Control		257 ± 8	488 ± 24	9022 ± 297	2942 ± 136
	Smoke		230 ± 10	477 ± 16	8197 ± 295	2831 ± 72
Hypothalamus	Control	1699 ± 40	669 ± 26	527 ± 20	601 ± 46	111 ± 6
	Smoke	1297 ± 61*	604 ± 42	532 ± 15	499 ± 29	108 ± 4
Thalamus	Control	744 ± 43	452 ± 41	562 ± 32		
	Smoke	596 ± 41*	406 ± 34	576 ± 20		
Midbrain	Control	954 ± 41	1095 ± 57	1602 ± 89	451 ± 9	149 ± 11
	Smoke	864 ± 53	1154 ± 104	1667 ± 91	462 ± 37	163 ± 8
Pons-medulla oblongata	Control	512 ± 18	391 ± 26	46 ± 28		
	Smoke	445 ± 20*	404 ± 24	50 ± 21		

Rats were exposed to cigarette smoke twice daily for 21 days and killed 24 h after the last exposure. Each value represents the mean ± SEM (ng/g) for 10 rats. *Significantly different from control ($p < 0.05$).

and DOPAC levels in the striatum with long-term cigarette smoke exposure were slight and were not statistically significant.

In conclusion, the present study shows that long-term cigarette smoke exposure produces behavioral sensitization to the locomotor-stimulating effects of the smoke and a significant decrease in NE levels in the rat brain, especially in the upper and lower brainstem. Aside from changes in the dopaminergic system, changes in the noradrenergic system may also contribute to the behavioral effects of long-term cigarette smoke exposure.

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REFERENCES

1. Andersson, K.; Fuxe, K.; Eneroth, P.; Agnati, L. F.; Harfstrand, A. Effects of acute continuous exposure of the rat to cigarette smoke on amine levels and utilization in discrete hypothalamic cathecholamine nerve terminal systems and on neuroendocrine function. Naunyn-Schmiedeberg's Arch. Pharmacol. 335:521-528; 1987.
2. Clarke, P. B. S. Nicotine and smoking: A perspective from animal studies. Psychopharmacology (Berl.) 92:135-143; 1987.
3. Clarke, P. B. S.; Davina, S. F.; Jakubovic, A.; Fibiger, H. Evidence that mesolimbic dopaminergic activation underlies the locomotor stimulant action of nicotine in rats. J. Pharmacol. Exp. Ther. 246:701-708; 1988.
4. Clarke, P. B. S.; Kumar, K. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78: 329-337; 1983.
5. Clarke, P. B. S.; Kumar, K. Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br. J. Pharmacol. 80:587-594; 1983.
6. Clark, P. B. S.; Pert, A. G. U. Autoradiographic evidence for nicotine receptors on nigrostriatal and mesolimbic dopaminergic neurons. Brain Res. 348:355-358; 1985.
7. Cronan, T.; Conrad, J.; Bryson, R. Effects of chronically administered nicotine and saline on motor activity in rats. Pharmacol. Biochem. Behav. 22:897-899; 1985.
8. Freeman, G. B.; Sherman, K. A.; Gibson, G. Y. Locomotor activity as a predictor of time and dosages for studies of nicotine's neurochemical actions. Pharmacol. Biochem. Behav. 26:305-312; 1987.
9. Fung, Y. K.; Lau, Y. S. Effect of nicotine pretreatment on striatal dopaminergic system in rats. Pharmacol. Biochem. Behav. 32: 221-226; 1989.
10. Gomita, Y.; Eto, K.; Furuno, K.; Araki, Y. Effects of standard cigarette and nicotine-less cigarette smoke inhalings on nicorandil plasma levels in rats. Pharmacology 40:312-317; 1990.
11. Gomita, Y.; Furuno, K.; Eto, K.; Okazaki, M.; Suemaru, K.; Araki, Y. Effect of cigarette smoking on theophylline pharmacokinetics in rats. J. Pharm. Pharmacol. 43:621-624; 1991.
12. Hall, C. S. Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Physiol. 18:385-403; 1934.
13. Hall, G. H.; Turner, D. M. Effects of nicotine on the release of ^{3}H-noradrenaline from the hypothalamus. Biochem. Pharmacol. 21:1829-1839; 1972.
14. 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.
15. Jarvik, M. E.; Henningfield, J. E. Pharmacological treatment of tobacco dependence. Pharmacol. Biochem. Behav. 30:279-294; 1988.
16. 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.
17. Ksir, C.; Hakan, R. L.; Hall, D. P.; Kellar, K. J. Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases of [^{3}H]acetylcholine to nicotine receptor. Neuropharmacology 24:527-531; 1985.
18. Ksir, C.; Hakan, R. L.; Kellar, K. J. Chronic nicotine and loco-

motor activity: Influences of exposure dose and test dose. Psychopharmacology (Berl.) 92:25–29; 1987.
19. Kubo, T.; Amano, H.; Kurahasi, K.; Misu, Y. Nicotine-induced regional changes in brain noradrenaline and dopamine turnover in rats. J. Pharmacobiodyn. 12:107–112; 1989.
20. Lapin, E. P.; Marker, H. S.; Sershen, H.; Lajtha, A. Action of nicotine on accumbens dopamine and attenuation with repeated administration. Eur. J. Pharmacol. 160:53–59; 1989.
21. Lee, E. H. Y. Effects of nicotine on exploratory behavior in rats: Correlation with regional brain monoamine levels. Behav. Brain Res. 17:59–66; 1985.
22. Margen, W. W.; Pfeil, K. A. Mecamylamine blockade of nicotine enhanced noradrenaline turnover in rat brain. Life Sci. 24:417–420; 1979.
23. Museo, E.; Wise, R. A. Microinjections of nicotinic agonist into dopamine terminal fields: Effect on locomotion. Pharmacol. Biochem. Behav. 37:113–116; 1990.
24. Oishi, R.; Nishibori, M.; Saeki, K. Regional distribution of histamine and tele-methylhistamine in the rat, mouse and guinea-pig brain. Brain Res. 280:172–175; 1983.
25. Svenssen, T. H.; Engberg, G. Effect of nicotine on single cell activity in the noradrenergic locus coeruleus. Acta Physiol. Scand. 479:31–34; 1980.