Br. J. Pharmac. (1970), **38**, 86–92.

Effects of chronic administration of nicotine on storage and synthesis of noradrenaline in rat brain

B. BHAGAT

Department of Physiology, St. Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, Missouri 63104, U.S.A.

Summary

1. Chronic administration of nicotine (0·5 mg/kg, subcutaneously four times a day, 5 days a week, for 6 weeks) did not affect the growth rate and water intake in rats. In these animals food intake was normal for the first 5 weeks, but was significantly increased during the sixth week of treatment.

2. Nicotine administration increased the blood pressure of rats from 120 mm Hg to 151 mm Hg.

3. The concentrations of endogenous noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine in the brain remained unaltered. However, chronic treatment with nicotine increased the turnover rate of noradrenaline. Initial accumulation of ^{3}H-noradrenaline was also significantly increased.

4. It is concluded from these studies that changes in the turnover of cerebral noradrenaline caused by chronic administration rather than changes in the concentration of noradrenaline may be an important factor in nicotine-induced behavioural changes.

Introduction

Nicotine plays an important part in the tobacco smoking habit. Johnston (1942) showed that the craving of habitual smokers could be appeased by nicotine injection instead of smoking. Many of the actions of nicotine on the central nervous system have been attributed to the release of noradrenaline or other amines from central neurones. However, some investigators have failed to find any definite changes in the noradrenaline content of the brain after a single or repeated injection of nicotine (Hansson, Masuoka & Clark, 1964; Westfall, Fleming, Fudger & Clark, 1967). We have presented evidence which indicated that even very small doses of nicotine cause an increased release of ^{3}H-noradrenaline from the brain (Bhagat, Kramer & Seifter, 1967). Assuming that the release of ^{3}H-noradrenaline reflects an accompanying release of endogenous noradrenaline, it is possible that the failure to find a decrease in the concentration of cerebral noradrenaline reflects a rate of replenishment equal to the rate of release.

The present study was undertaken to determine the effect of chronic administration of nicotine on catecholamine concentrations in the rat brain. In order to gain additional information, the concentrations of the other biogenic amines, dopamine, 5-hydroxytryptamine (5-HT), and acetylcholine were determined.

Methods

Animals

Groups of four male (Holtzman strain) rats weighing about 60 g were used throughout. Each group occupied a single cage and was allotted a treatment at random. All cages were kept under similar conditions of lighting and humidity in a room maintained at a temperature within the range of 21° ±0·5°. Food and water were supplied *ad libitum*. Body weight was recorded weekly. Food and water intake was measured daily. Nicotine hydrochloride (0·5 mg/kg) was injected subcutaneously, four times a day, 5 days a week, for 6 weeks. Control animals were injected with saline only. All animals were used 12 hr after the last injection.

Measurement of systolic blood pressure

The systolic blood pressure was measured weekly in unanaesthetized animals using a pulse transducer applied to the tail.

Radioactive studies

Rats were anaesthetized with pentobarbitone sodium (50 mg/kg) and 5 μCi of (±)-^{3}H-noradrenaline (5 Ci/mmol), obtained from the New England Nuclear Corporation, Boston, Massachusetts, was injected into the right lateral ventricle using a procedure previously described (Kramer, Seifter & Bhagat, 1967). Thirty minutes later the rats were decapitated, the brains were removed rapidly and rinsed in ice-cold 0·9% NaCl solution. In all these experiments, controls were run in parallel with nicotine pretreated animals and the brains were handled in an identical manner.

Estimation of catecholamines

The brains were homogenized in ice-cold 0·4 N perchloric acid. The catecholamines in the protein-free supernatant solution obtained after centrifugation were absorbed on to alumina at pH 8·6 and then eluted with 0·05 N perchloric acid (Anton & Sayre, 1962). The endogenous noradrenaline was converted to its fluorescent trihydroxyindole derivative by oxidation with potassium ferricyanide at pH 6·5 (von Euler & Lishajko, 1961). The radioactivity in a 1·0 ml. aliquot of the alumina eluate was estimated by liquid scintillation counting.

In some experiments 5-HT, noradrenaline and dopamine were determined simultaneously according to the fluorimetric method of Fleming, Clark, Fenster & Towne (1965).

Acetylcholine was extracted from the brain according to the procedure of Toru & Aprison (1966) and assayed against acetylcholine chloride on eserinized frog rectus muscle (Bhagat & Lockett, 1962). Throughout 2 × 2 assays of Latin square design were used.

The following drugs were used: acetylcholine chloride ; nicotine hydrochloride ; α-methyl-*p*-tyrosine.

The doses of all drugs (except nicotine) are expressed in terms of salt. The doses of nicotine are given in terms of free base. Details of dosages, time schedule and route of administration are given in the appropriate places under **Results.**

Results

The growth of the animals receiving nicotine treatment was normal. The food and water intake of these animals was initially lower than that of control groups but was almost normal in the third, fourth and fifth week. In the sixth week water intake remained normal but food intake was significantly increased. The results are presented in Fig. 1. The blood pressure of the control group showed little change throughout the 6 weeks. The mean blood pressure was 120 mm Hg. Animals treated with nicotine showed a significant increase in blood pressure by the fourth week of treatment. The blood pressure continued to rise progressively to reach a mean value of 151 mm Hg at the end of the sixth week of treatment (Fig. 2).

The rats treated with nicotine were hyperactive and aggressive.

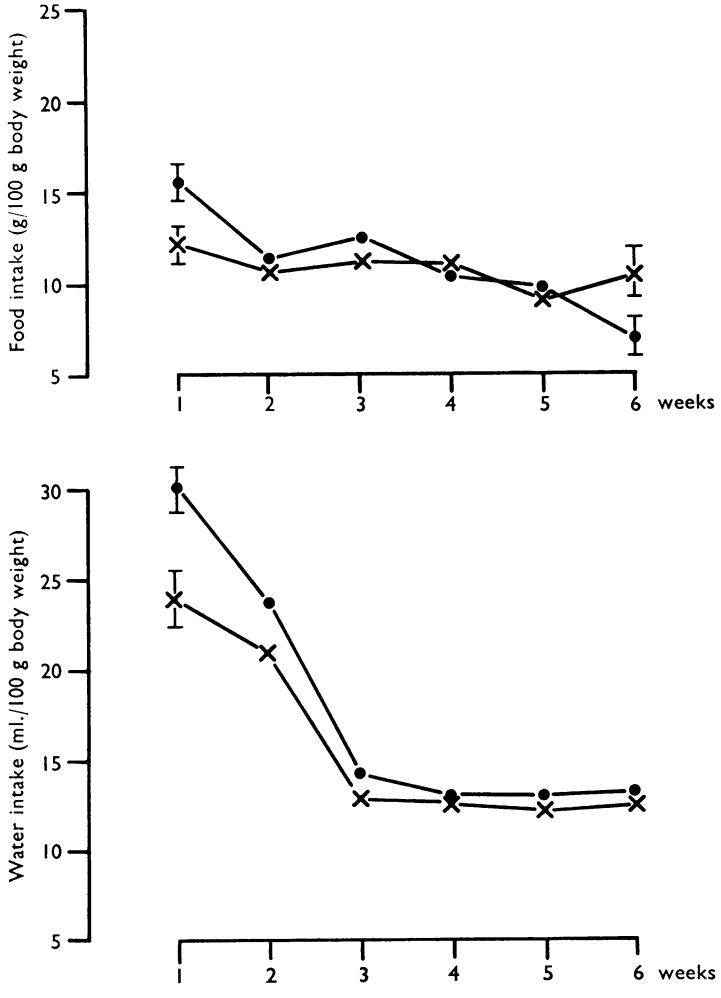

FIG. 1. Effect of repeated administration of nicotine on the food and water intake of rats. Rats were given nicotine 0·5 mg/kg subcutaneously four times a day, 5 days a week, for 6 weeks. Food and water intake was determined daily. Each point represents the mean food intake or water intake between fourteen and sixteen animals. Vertical bars indicate the standard errors of the means. Nicotine treated (×——×); controls (●——●).

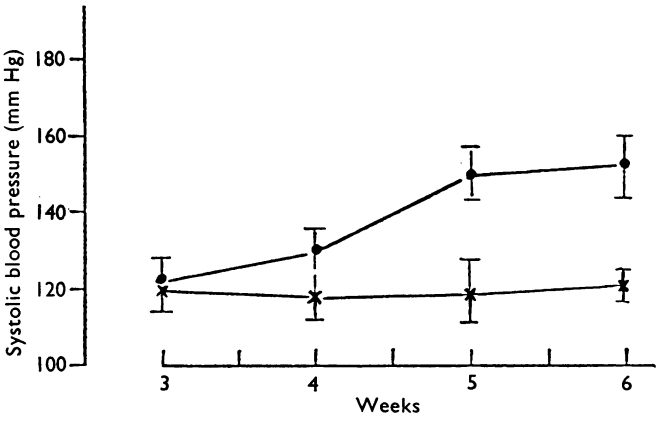

FIG. 2. Influence of nicotine administration on systolic blood pressure. Rats were given nicotine 0·5 mg/kg subcutaneously four times a day, 5 days a week, for 6 weeks. Systolic blood pressure was measured once a week with a pulse transducer applied to the tail. Each point represents the mean blood pressure of between fourteen and sixteen animals. Vertical bars indicate the standard errors of the means. Nicotine treated (●——●); controls (×——×).

TABLE 1. *Lack of effects of chronic administration of nicotine on various biogenic amines and acetylcholine levels in the rat brain*

	Tissue concentration μg/g wet tissue			
Treatment	Noradrenaline	Dopamine	5-Hydroxytryptamine	Acetylcholine
None	0·40±0·03 (8)	0·84±0·11 (7)	0·68±0·24 (8)	3·29±0·03 (10)
Nicotine	0·38±0·02 (10)	0·72±0·04 (8)	0·66±0·05 (8)	3·08±0·04 (10)

Rats were injected with nicotine (0·5 mg/kg) subcutaneously four times a day, 5 days a week, for 6 weeks. Twelve hours after the last injection of nicotine the animals were killed and their brains were analysed for noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine.

The number of animals on which each mean is based is given in parentheses. Results given as mean ± S.E.M.

TABLE 2. *Effect of chronic administration of nicotine on the initial accumulation of ^{3}H-noradrenaline in the rat brain*

Treatment	Concentration of ^{3}H-noradrenaline in brain. Mean c.p.m./mg±S.E.M.	% of control	Significance of difference
Control	963±24 (6)		
Nicotine	1,240±30 (8)	127	$P<0·001$

Rats were injected with nicotine (0·5 mg/kg) subcutaneously four times a day, 5 days a week, for 6 weeks. Twelve hours after the last injection these animals as well as controls received 5 μCi of ^{3}H-noradrenaline by direct injection into the lateral ventricle. The animals were killed 30 min later and their brains assayed for ^{3}H-noradrenaline.

Number in parentheses indicates the number of animals.

TABLE 3. *Turnover rate of brain noradrenaline in rats pretreated chronically with nicotine*

Treatment	Steady state noradrenaline levels (in fresh tissue) (μg/g±S.E.)	Rate constant (hr^{-1})	Turnover rate (μg/g per hr)	Change in turnover rate (%)
None	0·40±0·02 (8)	0·225±0·021	0·09	
Nicotine	0·42±0·02 (10)	0·380±0·030	0·16	+77

Rats, normal as well as nicotine pretreated, were given 100 mg/kg of α-methyl tyrosine intravenously and the rats were killed at 1, 2, 4 and 6 hr. A second dose of 100 mg/kg was administered 2 hr after the first, to the animals to be killed at 4 and 6 hr. Catecholamines in the brain were determined and expressed in terms of noradrenaline. The rate constant was calculated by the method of least squares.

Effect of chronic administration of nicotine on the concentrations of biogenic amines in the rat brain

The results presented in Table 1 show that the concentrations of endogenous noradrenaline, dopamine, 5-hydroxytryptamine and acetylcholine in the brain were not changed appreciably by 6 weeks of nicotine treatment.

Effect of chronic administration of nicotine on the initial accumulation of ^{3}H-noradrenaline in the brain of rats

Rats, nicotine pretreated as well as controls, were given 5 μCi of ^{3}H-noradrenaline into the lateral ventricle of the brain. All the animals were killed 30 min after the injection of the labelled amine. It is usually assumed that the radioactive noradrenaline in the brain after short periods of time is representative of the initial accumulation (Thierry, Javoy, Glowinski & Kety, 1968) because factors modifying the disappearance of the labelled amine would have operated for only a brief time. The results are presented in Table 2. There was a 27% increase in the accumulation of ^{3}H-noradrenaline in the nicotine treated rats when compared with respective controls.

Effect of chronic administration of nicotine on noradrenaline turnover rate

Turnover rates of noradrenaline in rat brains were measured from the decline in its concentration after blockade of synthesis (Brodie, Costa, Dlabac, Neff & Smookler, 1966) at a rate limiting step by administration of α-methyl-*p*-tyrosine, a potent inhibitor of tyrosine hydroxylase (Nagatsu, Levitt & Udenfriend, 1964).

Rats, nicotine pretreated as well as controls, were given an intravenous dose of 100 mg/kg of α-methyl-*p*-tyrosine at time zero ; 2 hr later a second dose of 100 mg/kg was given to the rats to be killed at 4 and 6 hr after the first injection of this drug. The rats (both experimental and controls) were killed in batches of five or six at 1, 2, 4, and 6 hr. Their brains were analysed for their noradrenaline content. The logarithms of the mean endogenous noradrenaline concentrations were plotted against the time after the first administration of α-methyl-*p*-tyrosine and regression lines were fitted according to the method of least squares. The turnover rates were estimated from the product of the steady state concentration and of the rate constant of the decline in endogenous amine (Brodie *et al.*, 1966).

The results are summarized in Table 3. The reduction in noradrenaline concentration was greater in animals pretreated with nicotine than in the controls. The calculated turnover rate indicates that there was a 77% increase in turnover in the brains of nicotine treated animals when compared with that in controls.

Discussion

The results demonstrate that chronic administration of nicotine increased the initial accumulation of ^{3}H-noradrenaline and the rate of turnover of noradrenaline in the brain with no decrease in the concentration of endogenous noradrenaline. The results are in contrast to those observed in the hearts of such rats. In the heart, nicotine pretreatment reduced the ability to accumulate ^{3}H-noradrenaline, did not affect the turnover of noradrenaline, but caused a significant decrease in the concentration of the endogenous amine (Bhagat, 1968).

The concentrations of dopamine, 5-hydroxytryptamine and acetylcholine in the brain also remained unaltered by such treatment.

In contrast to these findings, Westfall *et al.* (1967) found a significant increase in the concentration of 5-hydroxytryptamine after administration of nicotine in doses of 0·5 and 1·0 mg/kg, four times a day for 7 days. However, no increase was observed when the administration of nicotine was continued up to 14 days. It seems that with time, adaptive changes can take place in order to maintain the biogenic amines at their normal concentrations.

In the present study, chronic treatment with nicotine caused an increase in the initial accumulation of ^{3}H-noradrenaline. Enhanced accumulation of noradrenaline may be an additional measure to meet the requirement for noradrenaline in the face of continued enhanced utilization.

Autoradiographic studies (Schmiterlöw & Hansson, 1965) and experiments with ^{3}H-nicotine (Bhagat, unpublished) have indicated that nicotine reached the brain almost immediately after intravascular injection. The majority (95%) disappeared over a period of 15 to 60 min. Since the measurements of turnover rate and concentration of noradrenaline were made 12 hr after the last injection of nicotine, at a time when no or very little nicotine was left in the brain, it is concluded that the administration of nicotine induced a sustained increase in the synthesis and utilization of noradrenaline.

The results may help to explain the increase in the lever pressing activity of rats given nicotine frequently (Armitage, Hall & Morrison, 1968) and the improvement in the learning ability of rats and mice in several different tests after small subcutaneous doses of nicotine (Bovet, Bovet-Nitti & Oliverio, 1967).

It is concluded from these studies that changes in the turnover of cerebral noradrenaline caused by chronic administration rather than changes in the concentration of noradrenaline may be an important factor in nicotine-induced behavioural changes.

This work was supported by a grant from the Council for Tobacco Research, U.S.A.

REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). A study of the factors affecting the aluminium oxide trihydroxyindole procedure for the analysis of catecholamines. *J. Pharmac. exp. Ther.*, **138**, 360–375.

ARMITAGE, A. K., HALL, G. H. & MORRISON, C. F. (1968). Pharmacological basis for the tobacco smoking habit. *Nature, Lond.*, **217**, 331–334.

BHAGAT, B. (1968). Reduced accumulation of ^{3}H-norepinephrine (NE) and nicotine-induced hypertension. *Pharmacologist*, **10**, No. 2, 194.

BHAGAT, B. & LOCKETT, M. F. (1962). The synthesis of acetylcholine by acetone dried powders from the brains of normal rats and of thiamine-deficient rats. *J. Pharm. Pharmac.*, **14**, 37–40.

BHAGAT, B., KRAMER, S. Z. & SEIFTER, J. (1967). The effects of nicotine and other drugs on the release of injected ^{3}H-norepinephrine and on endogenous norepinephrine levels in the rat brain. *Eur. J. Pharmac.*, **2**, 234–235.

BOVET, D., BOVET-NITTI, F. & OLIVERIO, A. (1967). Action of nicotine on spontaneous and acquired behavior in rats and mice. *Ann. N.Y. Acad. Sci.*, **142**, 261–267.

BRODIE, B. B., COSTA, E., DLABAC, A., NEFF, N. H. & SMOOKLER, H. H. (1966). Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamine. *J. Pharmac. exp. Ther.*, **154**, 493–498.

EULER, U. S. VON & LISHAJKO, F. (1961). Improved technique for the fluorimetric estimation of catecholamine. *Acta physiol. scand.*, **51**, 348–355.

FLEMING, R. M., CLARK, W. G., FENSTER, E. D. & TOWNE, J. C. (1965). Single extraction method for the simultaneous fluorometric determination of serotonin, dopamine and norepinephrine in brain. *Analyt. Chem.*, **37**, 692–695.

HANSSON, E., MASUOKA, D. T. & CLARK, W. G. (1964). Effect of nicotine on catecholamine and serotonin levels *in vivo*. *Archs int. Pharmacodyn. Thér.*, **148**, 153–159.

JOHNSTON, L. M. (1942). Tobacco smoking and nicotine. *Lancet*, **2**, 742.

KRAMER, S. Z., SEIFTER, J. & BHAGAT, B. (1967). Regional distribution of tritiated acetylcholine in rat brain. *Nature, Lond.*, **217**, 184–185.

NAGATSU, T., LEVITT, M. & UDENFRIEND, S. (1964). Tyrosine hydroxylase, the initial step in norepinephrine biosynthesis. *J. biol. Chem.*, **239**, 2910–2917.

SCHMITERLÖW, C. G. & HANSSON, E. (1965). In *Tissue Distribution of C^{14}-nicotine. Tobacco Alkaloids and Related Substances*, Proc. 4th Int. Symp., ed. Euler, U. S. von, p. 75. Stockholm: Wenner Gren Center. New York: Pergamon Press.

THIERRY, A., JAVOY, F., GLOWINSKI, J. & KETY, S. S. (1968). Effects of stress on the metabolism of norepinephrine, dopamine and serotonin in the central nervous system of the rat. I. Modification of norepinephrine turnover. *J. Pharmac. exp. Ther.*, **163**, 163–171.

TORU, M. & APRISON, M. H. (1966). Brain acetylcholine studies: a new extraction procedure. *J. Neurochem.*, **13**, 1533–1544.

WESTFALL, T. C., FLEMING, R. M., FUDGER, M. F. & CLARK, W. G. (1967). Effect of nicotine and related substances upon amine levels in the brain. *Ann. N.Y. Acad. Sci.*, **142**, 83–100.

(*Received June* 20, 1969)