Short communications

Effect of chronic administration of nicotine on the concentrations of adrenal enzymes involved in the synthesis and metabolism of adrenaline

B. BHAGAT AND M. W. RANA

Department of Physiology, Saint Louis University School of Medicine, Saint Louis, Missouri, USA

Chronic administration of nicotine caused an increase in tyrosine hydroxylase and catecholamine concentrations in rat adrenals, but failed to affect adrenal monoamine oxidase, catechol-*O*-methyl transferase or phenylethanol-amine N-methyl transferase activities.

Nicotine is an important alkaloid of tobacco. Administration of nicotine causes a widespread activation of the sympathetic nervous system and a release of catecholamines from sympathetic nerve endings and adrenal medulla. When adrenal sympathetic activity is prolonged, increased neural release of adrenal catecholamine is associated with an increase of tyrosine hydroxylase activity (Mueller, Theonen & Axelrod, 1969), the probable rate limiting enzyme in the biosynthesis of catecholamines (Levitt, Spector, Sjoerdsma & Udenfriend, 1965). This increase in enzyme activity appears to be dependent upon the production of new quantities of tyrosine hydroxylase (Mueller *et al.*, 1969). This study was undertaken to determine whether chronic administration of nicotine can lead to induction of adrenal tyrosine hydroxylase and also the induction of adrenal enzyme phenyl-ethanol-amine N-methyl transferase (PNMT) which catalyses the conversion of noradrenaline to adrenaline.

Methods.—*Animals.* Male rats (Holtzman strain) weighing about 70 g were used in all experiments. They were grouped six to a cage and were alloted 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°±05° C. Food and water were supplied *ad libitum*.

Nicotine (1 mg/kg) was injected subcutaneously 5 times each day for 6 days a week over a period of 12 weeks. Control animals were injected with saline only. All animals were used 12 h after the last injection.

Biochemical studies. Animals were killed by a blow on the head and the adrenal glands were rapidly removed and homogenized in 2 ml of ice-cold 0·25 M sucrose. One millilitre of each homogenate was acidified with 9·0 ml of 0·4 N-perchloric acid for assay of catecholamines (Anton & Sayre, 1962). The catecholamines were isolated from the clear supernatant fluid of the tissue homogenates by absorption on alumina and elution with 0·05 N perchloric acid (Anton & Sayre, 1962). The adrenaline was converted to its fluorescent trihydroxyindole derivative by oxidation with potassium ferricyanide at pH_2. The catecholamines are expressed as adrenaline per pair of adrenal glands.

Tyrosine hydroxylase was assayed by the method of Levitt *et al.* (1965) with modifications described by Mueller *et al.* (1969).

PNMT activity was measured by Axelrod (1962) using normetadrenaline as the substrate and ^{14}C-s-adenosylmethionine (52·3 Ci/mM Amersham/Searle, Illinois) served as a methyl donor.

Monoamine oxidase (MAO) was assayed by measuring the conversion of ^{14}C-tryptamine to indolacetic acid according to the method of Wurtman & Axelrod (1963), catechol-*O*-methyl transferase (COMT) was assayed by measuring the formation of ^{3}H-metadrenaline on incubation with (−) adrenaline and ^{3}H-methyl-s-adenosylmethionine (specific activity 8·9 Ci/mM, Amersham/Searle, Illinois) as described by Axelrod (1959).

Results.—The results presented in Table 1 show that chronic administration of nicotine increased tyrosine hydroxylase concentration, but did not affect PNMT, COMT, or MAO. The catecholamine concentration of the glands was increased.

Discussion.—The increase in tyrosine hydroxylase is consistent with the reports from several laboratories that nerve stimulation results in an increase in the activity of this enzyme. This increase is not a response to depletion of catecholamine by nicotine, since the adrenal catecholamine levels were slightly raised.

TABLE 1. *Effect of chronic administration of nicotine on the activity of adrenal enzymes*

Treatment	Body wt. (9)	Adrenals wt. (mg/pair ad. wet tissue)	Catechol-amine (μg/pair ad.)	Enzymes activities: Tyrosine hydroxylase	PNMT ((nmol/h)/pair ad.)	COMT ((nmol/h)/pair ad.)	MAO ((nmol/h)/pair ad.)
Saline	429·0±7·5	49·2±1·6	32·6±2·0	38·4±1·39	8·6±0·6	·012±·001	·15±·03
Nicotine	410·2±5·2	53·6±2·1	42·2±4·2	55·0±1·38	9·1±0·3	·014±·001	·15±·02
	>·05	>·05	<·05	<·001	>·05	>·05	>·05

Rats were injected with nicotine (1 mg/kg) subcutaneously 5 times a day, 6 days a week for 12 weeks. Twelve hours after the last injection of nicotine the animals were killed and their adrenals were analysed for catecholamines (expressed as adrenaline) and enzymes. Each value represents the mean ± S.E.M. of five–ten experiments.

The activity of PNMT increases in response to various conditions or treatments which increased the tyrosine hydroxylase activity. These include insulin induced hypoglycemia (Patrick & Kirshner, 1971, Wurtman & Axelrod, 1966) administration of reserpine (Mueller *et al*, (1969), Bhagat, unpublished results) or 6 hydroxydopamine (Thoenen, Mueller & Axelrod, 1969, 1970) immobilization stress (Kvetnansky, Weise & Kopin, 1970) and stress by prolonged isolation or by repeated exposure to cold (Bhagat, unpublished results). In all these conditions, the activation of the sympathetic nervous system and enhanced secretion of catecholamines are the common denominator. In the present study nicotine treatment appears to be specific for the tyrosine hydroxylase since it failed to affect adrenal monoamine oxidase, catechol-*O*-methyl transferase or PNMT activities.

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