THE INHIBITION OF CHOLINE ACETYLASE BY NICOTINE

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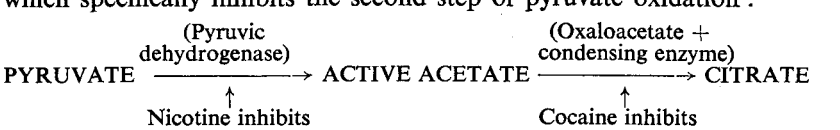
Recent investigations[1] into the action of nicotine on cellular metabolism have shown that the depressant effect of the drug on the respiration of brain-tissue may be attributed to its inhibitory action on the oxidation of pyruvate. A detailed study of the effects of nicotine on isolated steps of the citric acid cycle has revealed that pyruvic dehydrogenase, which catalyses the first step in pyruvate oxidation, is inhibited more strongly than are any of the other enzymes of the pyruvic oxidase system.

It is of interest to compare this action of nicotine with that of cocaine which specifically inhibits the second step of pyruvate oxidation[2].



Evidence has been obtained that some of the pharmacological effects of nicotine are increased by administration of cocaine[3].

Since the formation of active acetate is an essential reaction in the pyruvic dehydrogenase system it becomes of interest to discover whether or not nicotine interferes with other mechanisms in which active acetate is involved. We have therefore investigated the action of nicotine on purified choline acetylase of brain and on the sulphanilamide acetylating system of liver.

Experimental

Effect of nicotine on purified choline acetylase

Choline acetylase was prepared from acetone-dried rabbit brain and purified by dialysis after fractionation with ammonium sulphate according to the method of Nachmansohn, Hestrin and Voripaieff[4]. A boiled extract of washed brewer's yeast was used as the source of coenzyme A.

Mixtures (5·0 ml.), with and without nicotine, containing buffered enzyme solution (1·0 ml. ≡ 250 mg. of dried brain), yeast extract (3·0 ml.) and, in final concentrations, potassium chloride (0·04M), choline (0·02M), acetate (0·02M), cysteine (0·02M), calcium chloride (0·002M), magnesium chloride (0·0002M), adenosine triphosphate (0·003M) and physostigmine (0·001M) were incubated at *p*H 7·0 and 37° C. in nitrogen-filled, stoppered tubes. Samples (2·0 ml.), withdrawn at zero time and after 1 hour, were deproteinised with trichloroacetic acid and, after readjusting the *p*H, were assayed for acetylcholine by the chemical method of Hestrin[5]. Measurements were made at 540 mμ using 1 cm. cuvettes in a Unicam Quartz Spectrophotometer. Nicotine did not interfere with the method.

Results are given in Table I.

TABLE I

EFFECT OF NICOTINE ON CHOLINE ACETYLASE

Experiment	Acetylcholine, μg./g. of dried brain/hour		Inhibition, per cent.
	—	Nicotine (0·015M)	
1	774	430	45
2	645	408	37
3	625	375	40
4	720	382	47

Effect of nicotine on sulphanilamide acetylation

In 3 experiments, nicotine (0·015M) had no effect on the rate of acetylation of sulphanilamide by extract of acetone-dried pigeon-liver as determined by the procedure of Johnson and Quastel[6].

DISCUSSION

The results of the present work show that nicotine, in a concentration which has comparatively little or no effect on most enzyme systems, inhibits appreciably the choline acetylating system of brain-tissue. Since nicotine does not inhibit sulphanilamide acetylation or the synthesis of citrate from acetate by baker's yeast[1], it appears that the drug is not a general inhibitor of all mechanisms in which active acetate is involved and that its action on choline acetylase is a specific one. While the effect on choline acetylase is not in itself sufficiently high to be of marked pharmacological significance, it must be realised that nicotine also inhibits the reaction whereby active acetate is produced from pyruvate and in all probability nerve-tissue depends, primarily if not exclusively, on pyruvate as the acetyl donor for choline acetylation. Since nicotine inhibits the initial step in pyruvate oxidation and, at the same time, stimulates glycolysis[7], the tendency for the local concentration of pyruvate to rise above the normal level would be an additional factor contributing towards the inhibition of acetylcholine synthesis. Nachmansohn and John[8] have shown that pyruvate, in common with other α-ketoacids, is a most powerful inhibitor of choline acetylase.

Evidence has also been obtained that nicotine interferes with coupled phosphorylation[9], thereby adding to the effects which follow from inhibition of the energy-yielding mechanism of carbohydrate oxidation. Under physiological conditions, therefore, the inhibitory action of nicotine on acetylcholine synthesis would be expected to be considerably greater than that due to direct action of the drug on choline acetylase. It has recently been shown, for example, that cocaine, which has no effect on choline acetylase, inhibits markedly the synthesis of acetylcholine in respiring fresh-brain homogenate[10]. In pharmacological opposition to the combined actions of nicotine which suppress acetylcholine synthesis is the ability of the drug to inhibit acetylcholine breakdown. Choline esterase of mammalian brain is inhibited by nicotine[11,12].

Summary

1. Nicotine inhibits the isolated choline acetylase system of brain but has no action on the sulphanilamide acetylating system of liver.

2. Various mechanisms whereby nicotine inhibits acetylcholine synthesis in nerve are discussed.

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