SOME EFFECTS OF NICOTINE ON VARIOUS DEHYDROGENASE SYSTEMS

By A. R. Fahmy and E. O'F. Walsh

From the Department of Physiology, Royal Free Hospital School of Medicine, London

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Nicotine, while inhibiting respiration, stimulates alcoholic fermentation in yeast[1] and anærobic glycolysis in brain[2,3]. Recent investigations[4] have shown that the depressant action of nicotine on the respiration of brain-tissue may be attributed to inhibition of the pyruvic oxidase system, in which pyruvic dehydrogenase is the enzyme most strongly affected. In view of recent observations that nicotine, while inhibiting some dehydrogenases, may activate others[5], it is now considered possible that the stimulant action of nicotine may be attributable to selective enzymic activation.

In the present work we have examined the effects of nicotine on a selection of dehydrogenase systems in the hope of discovering whether the action of nicotine is characteristic of the coenzyme or prosthetic group involved.

Experimental

Materials

Substrate solutions of the required *p*H were prepared freshly as required from pure crystalline materials. *iso*Citrate solution was prepared from the lactone as described by Krebs and Eggleton[6]. Diphosphopyridine nucelotide was prepared from fresh baker's yeast as described by Le Page[7]. Diaphorase was prepared as described by Straub[8]. Tissue homogenates were prepared in glass homogenisers of the Potter and Elvehjem type with external cooling. Krebs' phosphate-saline[9] was used in experiments with animal tissues. Clark and Lub's standard phosphate buffer was used in experiments with yeast.

Enzyme preparations and activity measurements

Dehydrogenase activities, in the presence and in the absence of nicotine, were measured either manometrically or colorimetrically by the triphenyltetrazolium bromide method[10].

1. *Colorimetric method*

Mixtures (4·0 ml.) containing buffer (2·0 ml.) $\pm$ nicotine, homogenate (approx. 100 mg. tissue), substrate (0·02M) and 0·5 per cent. w/v triphenyltetrazolium bromide solution (0·5 ml.) were incubated at *p*H 7·4 and 37° C. (20° C. in experiments with yeast) until suitable colours were developed (0·5 to 2 hours). Since, in order to determine the effect of an activator or an inhibitor on an enzymic system, it is essential that the duration of the experiment be restricted to the period before activity begins to fall off, suitable conditions for each system studied were determined by trial

experiments. The colours were extracted and measured by the method of Fahmy and Walsh[10]. When necessary, the values obtained were corrected for colour which developed in corresponding mixtures incubated without added substrate.

The colorimetric method was used to investigate the effects of nicotine (0·0075M to 0·03M) on the following systems.

Succinic dehydrogenase. Rat skeletal-muscle was homogenised, with cooling, and washed twice in the centrifuge with ice-cold phosphate-saline before preparing the final (1 in 10) suspension. Activities were measured in the presence of sodium succinate. No colour developed in the absence of added substrate.

Xanthine oxidase and Schardinger enzyme of milk. Whole, fresh, bovine milk was used. Activities were measured in the presence of (*a*) hypoxanthine and (*b*) acetaldehyde.

Lactic dehydrogenase of muscle. Rat-skeletal muscle homogenate was used with sodium lactate as substrate. Activities were measured in the presence of semicarbazide (0·033M) to bind the pyruvate formed[11]. No colour developed in the absence of added substrate.

Alcohol dehydrogenase. A washed suspension of *Saccharomyces cerevisiæ* was used. Cells, harvested from young, actively growing cultures were suspended in phosphate buffer and ærated for 2 hours at 30° C. in order to reduce dehydrogenase activity due to endogenous metabolites. Activities were measured with ethanol as substrate in the presence of semicarbazide (0·02M) which, by combining with the acetaldehyde formed, prevents the reverse reaction.

2. *Manometric method.*

Oxygen uptakes by mixtures (2 to 3·5 ml.) containing homogenate (approx. 100 mg. of tissue) and substrate (0·02M) in phosphate-saline, ± nicotine, were measured in the Warburg apparatus at 37° C. and *p*H 7·4. Gas phase, air. The centre wells contained 20 per cent. w/v potassium hydroxide (0·15 ml.).

The manometric method was used to investigate the effects of nicotine (0·0075M to 0·03M) on the following systems.

iso*Citric dehydrogenase.* Pigeon-liver homogenate was used with sodium *iso*citrate as substrate. Activities were measured in the presence of sodium arsenite (0·0033M) which, by inhibiting the metabolism of α-ketoglutarate[12], serves to restrict oxygen consumption to the reaction catalysed by *iso*-citric dehydrogenase.

Lactic dehydrogenase of brain. Rat-brain homogenate was used with sodium lactate as substrate. Activities were measured either in the presence of methylene blue and cyanide as used by Straub[13] or in the presence of semicarbazide (0·033M).

Malic dehydrogenase. Rat-kidney homogenate was used with sodium malate as substrate. Activities were measured in the presence of methylene blue and cyanide as for lactic dehydrogenase.

Glucose dehydrogenase. The partially purified enzyme was prepared from ox-liver by the method of Brunelli and Wainio[14]. Activities were

measured in a system reconstituted according to the procedure of Eichel and Wainio[15]. Each Warburg vessel contained enzyme (30 mg. of dried preparation), glucose (0·02M), potassium phosphate (0·05M), diphosphopyridine nucleotide (1 mg.), diaphorase (1 mg.), methylene blue (0·5 mg.), ± nicotine; total volume 3·5 ml.

TABLE I

EFFECT OF NICOTINE ON LACTIC DEHYDROGENASE IN BRAIN

Experiment	Oxygen, µl./100 mg. tissue/hour		Inhibition, per cent.
	–	Nicotine (0·0075M)	
1	90	89	1
2	102	102	0
3	88	89	−1
		Nicotine (0·015M)	
4	104	97	7
5	96	90	6
6	70	66	6
		Nicotine (0·03M)	
7	75	66	12
8	80	70	13
9	78	68	13

TABLE II

EFFECT OF NICOTINE ON GLUCOSE DEHYDROGENASE OF LIVER

Experiment	Diphosphopyridine nucleotide mg./vessel	Oxygen, µl./30 mg. enzyme/hour		Inhibition per cent.
		–	Nicotine (0·0075M)	
1	1	123	103	16
2	1	119	101	15
3	1	126	105	17
4	4	135	114	16
5	4	143	120	16
6	4	153	130	15

TABLE III

EFFECT OF NICOTINE ON ALCOHOL DEHYDROGENASE OF YEAST

Experiment	Activity/100 mg. moist weight of yeast/hour expressed as optical density × 1000		Activation per cent.
	–	(Nicotine (0·0075M)	
1	270	350	30
2	201	265	31
3	296	390	32
4	168	222	32
5	179	229	28
6	181	240	32

RESULTS

Nicotine (0·03M) had no effect on the activity of either *iso*citric dehydrogenase, xanthine oxidase or the Schardinger enzyme of milk. Succinic dehydrogenase was but slightly inhibited, nicotine (0·0075M) inhibiting by 4 to 5 per cent. (0·03M) by 8 to 9 per cent.

The activities of lactic dehydrogenase in brain and in muscle and of malic dehydrogenase in kidney were unaffected by nicotine in the lower concentration (0·0075M); in the higher concentration (0·03M), nicotine

inhibited these systems by about 12 per cent. The results of experiments with lactate, which are typical of this group, are given in Table I. The results obtained when methylene blue and cyanide were used were essentially the same as were those obtained when semicarbazide was used.

Nicotine (0·0075M) inhibited the glucose dehydrogenase system of liver by about 16 per cent. The results (Table II) show that increasing the concentration of diphosphopyridine nucleotide in this system does not modify the inhibitory action of nicotine.

Nicotine (0·0075M) activated the alcohol dehydrogenase system of yeast by about 30 per cent. (Table III).

Discussion

From the experiments with *iso*citrate, xanthine, acetaldehyde and succinate as substrates it may be inferred that nicotine does not interfere with the electron-transferring mechanism through the yellow enzymes and cytochrome system; the slight inhibition of succinic dehydrogenase as now measured by the colorimetric method being almost idential with that observed[4] when measured by oxygen consumption in a system dependent on the presence of cytochrome for activity. The experiments with lactate as substrate confirm this conclusion since oxygen consumption in brain homogenates are affected equally by nicotine whether methylene blue and cyanide or semicarbazide is used to isolate the system; and further, the degree of inhibition is equal to that obtained in a muscle preparation by the colorimetric method.

Since we have used methylene blue in some of our manometric experiments with nicotine, it is necessary to refer to reports[16,17] that aqueous nicotine and methylene blue take up oxygen when incubated together at 40° C. in the light. Under our experimental conditions, however, no oxygen was consumed when a buffered solution of nicotine and methylene blue was shaken in air for 3 hours at *p*H 7·4 and 37° C.

Of the enzymic systems studied those which are appreciably affected by nicotine, with the possible exception of choline acetylase[18], are the dehydrogenases which require a pyridine nucleotide as coenzyme. Nicotine also inhibits the enzyme for which diphosphopyridine nucleotide is the substrate[19].

Since nicotine, like the nicotinamide moiety of the coenzymes, contains a β-substituted pyridine ring, one might expect nicotine competitively to inhibit all the dehydrogenases which require one or other of the pyridine nucleotides. *iso*Citric dehydrogenase, however, is unaffected by nicotine while glucose-6-phosphate dehydrogenase is activated[5]. Both these enzymes require the triphosphopyridine nucleotide. On the other hand, lactic, malic, pyruvic[4] and glucose dehydrogenases, all of which require diphosphopyridine nucleotide, are inhibited by nicotine. From the experiments with glucose dehydrogenase, however, it would appear that inhibition is not competitive with regard to the coenzyme. Alcohol dehydrogenase, which also requires diphosphopyridine nucleotide, is strongly activated by nicotine to an extent which might well contribute to the stimulant effect of nicotine on alcoholic fermentation.

It must be concluded therefore that, while there is a tendency for nicotine to interfere with enzymic systems which involve the pyridine nucleotides, the nature of the effect is unpredictable and is characteristic of the individual dehydrogenase system rather than of the group to which it belongs.

Summary

1. The effects of nicotine on various dehydrogenase systems have been investigated by colorimetric and manometric methods and evidence is presented that nicotine does not interfere with electron-transferring mechanisms through the yellow enzymes and cytochrome systems.

2. Of the dehydrogenase systems which involve pyridine nucleotides, lactic, malic, pyruvic and glucose dehydrogenases are inhibited, *iso*citric dehydrogenase is unaffected and alcohol dehydrogenase is activated by nicotine.

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