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Induction of aldehyde dehydrogenase in a mitochondrial fraction

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SUMMARY

Mitochondrial membranes suffer irreversible structural and functional damage if exposed to aldehydes; membranes of other subcellular particles may be affected similarly. The need for an *in vivo* protective mechanism may be satisfied by aldehyde dehydrogenase (aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3) which is specifically induced in a subcellular fraction isolated from livers of rats administered ethanol orally.

By means of polarographic measurements, it was recently established[1] that an isolated rat liver mitochondrial fraction can metabolise both mono- and difunctional aldehydes. It has been suggested[2] that since the aldehydes are potentially harmful substances, the existence of an aldehyde dehydrogenase (aldehyde:NAD^+ oxidoreductase, EC 1.2.1.3) or oxidase activity in mitochondria or associated particles may constitute a protective mechanism which prevents the accumulation of potentially destructive concentrations of aldehydes. The need for such a mechanism has been demonstrated by treatment of mitochondria with malondialdehyde which modified both structure and function of the mitochondrial membranes[2]. Malondialdehyde is one of the many carbonyl compounds formed by the oxidation of polyunsaturated fatty acids and it has been postulated that oxidation of these lipids is a mechanism of disruption of biological membranes[3]. Mitochondrial membranes are particularly rich in unsaturated fatty acids and oxidative catalysts such as haemoproteins.

The aldehyde oxidising capacity of the rat liver mitochondrial fraction from normal rats is low and the existing enzyme levels would probably be insufficient to ensure the rapid metabolism of increased concentrations of aldehydes. If the aldehyde oxidising enzymes do play a protective role, and by analogy with alcohol dehydrogenase (alcohol:NAD^+ oxidoreductase, EC 1.1.1.1) which is induced when rats are fed ethanol[4], increased concentrations of alcohol or aldehydes should stimulate a rise in metabolic capacity for aldehydes.

This paper reports the induction of increased aldehyde dehydrogenase activity in a liver mitochondrial fraction isolated from rats administered ethanol orally. Investigation of the levels of several other mitochondrial enzymes in this fraction revealed that they were unaffected by this treatment. The specific activity of aldehyde dehydrogenase in a liver microsomal fraction isolated from the same animals was low compared with the specific activity in the mitochondrial fraction.

All rats used in experiments were 3-month-old females (see ref. 2) bred in this laboratory from an original Wistar strain and raised on the Oxoid pasteurised breeding diet. In addition, test animals were fed 15% (w/v) ethanol in the water supply for 3 weeks before killing; control animals were fed iso-caloric amounts of glucose. Rat liver mitochondrial fractions were isolated in 0.44 M sucrose containing 1 mM EDTA (pH 7.8) by the method of Packer *et al.*[5] and were ruptured by freezing and thawing prior to enzyme assay. Mitochondrial fractions from test and control animals were isolated simultaneously and subjected to identical procedures. Rat liver microsomal fractions were isolated according to the method of Sedgwick and Hübscher[6], and the protein content of both mitochondrial and microsomal fractions was assayed by the procedure of Lowry *et al.*[7]. Aldehyde dehydrogenase was assayed by the method of Racker[8] in the presence of sodium amytal (1 mM); succinate dehydrogenase (succinate:(acceptor) oxidoreductase,EC 1.3.99.1) was assayed by the procedure described by Shephard and Hübscher[9] and fumarate dehydratase (L-malate.hydro-lyase, EC 4.2.1.2) by the method of Hill and Bradshaw[10]. Individual details are given in the legend to Table I. NAD^+, sodium amytal and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride were obtained from Sigma (London) Chemical Company, London, S.W.6. Sodium succinate was purchased from Boehringer Corporation (London) Ltd., London. W.5., and bovine serum albumin was obtained from Armour Pharmaceutical Company Ltd., Eatbourne. All other chemicals used were of Analytical Reagent grade.

In the experiments reported here, ethanol was introduced into the animal in a controlled manner by inclusion in the water supply. According to Mistilis and Birchall[4] over 90% of the alcohol ingested is oxidised, presumably in the liver. It has been established that the rate of ethanol clearance is increased in alcoholic and normal humans and also in rats following prolonged alcohol ingestion presumably because of the increase in activity of alcohol dehydrogenase which is induced by increased levels of substrate[4]. The increased rate of ethanol oxidation must yield unusually high levels of acetaldehyde which the liver would have to metabolise rapidly to a potentially less harmful product. Since the normal levels of aldehyde oxidising enzymes in the liver mitochondrial fraction are low[2], and in view of the inducibility of alcohol dehydrogenase, it appeared likely that aldehyde dehydrogenase activity may be induced in response to increased levels of ethanol or aldehyde.

In the present investigation, liver mitochondrial fractions isolated from both test and control animals were assayed for three different enzymes and the results (Table I) show that aldehyde dehydrogenase activity was higher in the test than the control whilst no such difference was observed in succinate dehydrogenase and fumarate dehydratase activities. These results indicate that increased aldehyde dehydrogenase activity was induced by dietary intake of ethanol whilst the other enzymes examined were unaffected.

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TABLE I

ENZYMIC ACTIVITY OF RAT LIVER MITOCHONDRIAL FRACTION FROM TEST AND CONTROL ANIMALS

Reaction mixtures for enzyme assays: *(1) Aldehyde dehydrogenase:* The complete reaction mixture included in a final volume of 3.0 ml, rat liver mitochondrial fraction (0.3 mg protein) and the following (in μmoles): sodium pyrophosphate buffer (pH 9.3), 30; NAD^+, 0.32; acetaldehyde, 26.4; sodium amytal, 3.0. NADH was measured at 340 nm (20°). *(2) Succinate dehydrogenase:* The complete reaction mixture included in a final volume of 1.0 ml, rat liver mitochondrial fraction (0.1 mg protein); bovine serum albumin (0.1 mg) and the following (in μmoles): potassium phosphate buffer (pH 7.4), 50; sodium succinate (pH 7.4), 50; sucrose, 25; 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (pH 7.4), 1.98; ethylene diamine tetraacetate (pH 7.4), 2.0. The reaction mixture was incubated at 37° for 5 min and the reaction then stopped with 1.0 ml trichloroacetic acid and the formazan extracted with 4 ml ethyl acetate by shaking 50 times. The absorbance of 3 ml of the solvent layer *plus* 0.3 ml methanol was measured at 490 nm against a blank of ethyl acetate. No substrate controls were identical with the above reaction mixture except for the absence of succinate. *(3) Fumarate dehydratase:* The complete reaction mixture included in a final volume of 3.0 ml, rat liver mitochondrial fraction (0.1 mg protein) and the following (in μmoles): sodium phosphate buffer (pH 7.8), 140; L-malic acid (sodium salt, pH 7.8), 150. The reaction was followed by observing the increase in absorption at 250 nm and 25°.

Enzyme	*Unit of activity*	*Expt. No.*	*Test*	*Control*
Aldehyde dehydrogenase (EC 1.2.1.3)	μmoles NADH per mg per h	1	0.99 (± 0.12)	0.61 (± 0.09)
		2	1.16 (± 0.03)	0.82 (± 0.03)
		3	1.42 (± 0.15)	0.93 (± 0.02)
		4	1.81 (± 0.14)	0.98 (± 0.06)
		5	1.52 (± 0.18)	0.70 (± 0.11)
Succinate dehydrogenase (EC 1.3.99.1)	μmoles formazan per mg per h	1	10.39 (± 0.13)	11.21 (± 0.44)
		2	10.42 (± 0.05)	10.76 (± 0.03)
		3	13.47 (± 0.11)	13.73 (± 0.11)
		4	14.21 (± 0.29)	15.12 (± 0.13)
		5	13.31 (± 0.22)	16.91 (± 0.28)
Fumarate dehydratase (EC 4.2.1.2)	★Units/mg	1	39.2 (± 0.47)	38.20 (± 1.80)
		2	35.3 (± 1.99)	31.60 (± 2.99)
		3	51.4 (± 5.83)	49.30 (± 4.62)
		4	45.7 (± 2.68)	51.30 (± 0.50)
		5	60.5 (± 3.16)	63.30 (± 6.21)

Data are given as means of 3 experimental results. Figures in parentheses are standard deviations.
★Unit of activity is defined as the initial rate of change in absorbance/10 sec x 10^3. Specific activity is defined as the total number of units/mg of enzyme protein at 25°.

Measurements of the specific activity of aldehyde dehydrogenase in the liver microsomal fraction from test animals showed it to be low compared with that of the mitochondrial fraction which suggests that the induced enzyme is not of reticulo-endothelial origin. This means that the induced aldehyde dehydrogenase is in the mitochondrial fraction, which because of the isolation procedure probably contains peroxisomes and lysosomes as well as mitochondria. Aldehyde dehydrogenase has been shown to occur in mitochondria but so far has not been demonstrated in either peroxisomes or lysosomes. Thus whilst the available facts may favour the mitochondria as the location of the induced aldehyde dehydrogenase, the possibility remains that part of the enzymic activity may be either peroxisomal or lysosomal. Future studies involving careful particle separations will resolve this matter.

The reported inducibility of aldehyde dehydrogenase may constitute an important defence mechanism against the potentially destructive action of aldehydes not only of dietary origin but also those aldehydes resulting from metabolic reactions and from lipid peroxidation reactions and in this way may serve to protect the tissues from reactions which may contribute to premature ageing.

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