Rapid Papers

Rates of Induction of Mitochondrial Aldehyde Dehydrogenase in Rat Liver

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Aldehyde dehydrogenase activities in liver mitochondria isolated from rats given ethanol at hourly intervals by gastric intubation showed a brief lag period followed by a rapid increase in specific activities until a maximum was attained at about 3 h.

In view of the widespread human consumption of ethanol, the importance of a thorough understanding of the mammalian metabolic pathways in the oxidation of this substance needs little emphasis. Acetaldehyde, a product of the oxidation of ethanol, has been shown to have harmful effects on mitochondrial function that are similar in a number of ways to the effects of chronic ethanol consumption by rats (Cederbaum & Rubin, 1975). Previous studies in this laboratory (Horton, 1971) showed that ethanol administered to rats, by incorporation in drinking water, induced aldehyde dehydrogenase (aldehyde-NAD⁺ oxidoreductase, EC 1.2.1.3) activity in a mitochondrial fraction of liver. Marjanen (1972), Tottmar *et al.* (1973) and Horton & Barrett (1975) have reported the existence of two aldehyde dehydrogenases in rat liver. One of these enzymes, designated isoenzyme ALDH-I-NAD⁺, has an apparent K_m value for acetaldehyde of less than 5 μ M, and the other enzyme, designated isoenzyme ALDH-II-NAD⁺, has a corresponding K_m value of 1.5 mM. Of the total enzyme activity in the liver 80% was shown to reside in the mitochondrial fraction. In addition, Koivula & Koivusalo (1975) have reported the existence of two separable aldehyde dehydrogenase activities in a cytoplasmic fraction of liver as well as a microsomal enzyme with properties similar to the mitochondrial high- K_m enzyme.

Acetaldehyde has potentially harmful effects on mitochondrial function and generally throughout the cell, and a rapid rate of induction of at least one aldehyde dehydrogenase might be predicted to follow the administration of ethanol. This present paper reports on the rate of induction of the two aldehyde dehydrogenases in a mitochondrial fraction of rat liver. Deitrich *et al.* (1972) have reported a phenobarbital-inducible aldehyde dehydrogenase in the cytosolic fraction but not in the mitochondrial fraction. The effects of ethanol administration on the phenobarbital-inducible cytosolic aldehyde dehydrogenase (Deitrich *et al.*, 1972; Eriksson *et al.*, 1975) were not studied.

Experimental

All rats used in these experiments were 3-month-old females bred in this laboratory from an original Wistar strain and raised on the Oxoid pasteurized breeding diet. Ethanol (25%, w/v) was administered to unanaesthetized rats by gastric intubation (2 ml portions) at hourly intervals up to a maximum of five portions before the animals were killed. Control animals were supplied with isocaloric amounts of glucose by gastric intubation. Animals were deprived of food for 12 h before being killed by cervical fracture. Mitochondria were isolated by a modification of the method of Chappell & Hansford (1969), in which the isolation medium contained 0.25 M-sucrose and 5 mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)ethanesulphonic acid] adjusted to pH 7.6 with 1 M-NaOH. Protein was assayed by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The two aldehyde dehydrogenases (isoenzymes ALDH-I-NAD⁺ and ALDH-II-NAD⁺) were assayed by previously described methods (Horton & Barrett, 1975), in the presence of Amytal to inhibit mitochondrial oxidation of NADH.

Amytal (5-ethyl-5-isopentylbarbituric acid), sodium deoxycholate and NAD⁺ were obtained from Sigma (London) Chemical Co., Kingston-upon-Thames, Surrey, U.K. Acetaldehyde, supplied by Kodak Ltd., Kirkby, Lancs., U.K., was redistilled before use and was stable at -20°C in the form of an aqueous solution (500 mM) for several weeks. Hepes was purchased from Hopkin and Williams, Chadwell Heath, Essex, U.K. All other reagents were of A.R. grade.

Results and Discussion

Liver mitochondria, isolated from rats given ethanol by gastric intubation at hourly intervals for different periods of time, were assayed for isoenzyme ALDH-I-NAD⁺ and total aldehyde dehydrogenase (i.e. isoenzyme ALDH-I-NAD⁺ plus isoenzyme

Fig. 1. Rate of induction of total aldehyde dehydrogenase in a mitochondrial fraction

The complete reaction mixture for the assay of total aldehyde dehydrogenase included, in a final volume of 2ml: 50mM-sodium pyrophosphate buffer, pH8.8, 1mM-sodium Amytal (in ethanol, 0.25% of final volume), 0.5mM-NAD⁺, 5.0mM-acetaldehyde and mitochondria. Sodium deoxycholate (with the pH adjusted to that of the assay medium) at a concentration of 0.20mg/mg of protein was added to release latent activity and to disperse the particulate material to facilitate spectrophotometric measurements at 340nm. The reaction was started by addition of substrate. Results are means of five experiments $\pm$ s.d., which are indicated by error bars.

ALDH-II-NAD⁺) activities. The results of these experiments show that there is an initial lag period of nearly 1 h when little change in the specific activity of the total aldehyde dehydrogenase is observed, followed by a rapid increase in the specific activity for the following 2h, after which there is little or no change (Fig. 1). Isoenzyme ALDH-I-NAD⁺ also shows a stimulation of its specific activity in a corresponding series of phases that have a similar time-scale, but the rate of increase and the total change of the specific activity are approximately one-half of those of the total aldehyde dehydrogenase (Fig. 2a). In view of the observed changes in specific activities of the total aldehyde dehydrogenase and isoenzyme

Fig. 2. Rates of induction of the two aldehyde dehydrogenases that together constitute total aldehyde dehydrogenase activity

(a) Isoenzyme ALDH-I-NAD⁺. The reaction mixture was similar to that described in Fig. 1, with the following exceptions: the final concentrations of NAD⁺ and acetaldehyde were 0.5mM and 0.025mM respectively. Results are means of five experiments $\pm$ s.d., which are indicated by error bars. (b) Isoenzyme ALDH-II-NAD⁺. The activity of this enzyme is the difference between the total activity and the activity of isoenzyme ALDH-I-NAD⁺.

ALDH-I-NAD⁺, the specific activity of isoenzyme ALDH-II-NAD⁺ must show changes similar to those of isoenzyme ALDH-I-NAD⁺ (Fig. 2b).

Mitochondrial aldehyde dehydrogenases from rats supplied with a quantity of glucose that was isocaloric with the ethanol supplied to the experimental animals showed no change in specific activities. Further, in earlier experiments in this laboratory (Horton, 1971), it was observed that, when aldehyde dehydrogenase activity was increased as a result of the administration of ethanol, no corresponding changes were seen in the activities of succinate dehydrogenase [succinate-(acceptor) oxidoreductase,

EC 1.3.99.1] or fumarate hydratase (L-malate hydrolyase, EC 4.2.1.2). Thus the observed rapid increase in the specific activities of mitochondrial aldehyde dehydrogenases appears to be a specific response to the increased concentrations of acetaldehyde resulting from the higher rate of oxidation of ethanol, and probably constitutes a protective mechanism for the rapid removal of the potentially hazardous acetaldehyde.

In view of the rapid increase in the specific activities of the aldehyde dehydrogenases, similar changes in the activity of alcohol dehydrogenase (alcohol-NAD⁺ oxidoreductase, EC 1.1.1.1) might be expected, since this enzyme is generally acknowledged to be the most important enzyme in the metabolism of ethanol *in vivo* (Hawkins & Kalant, 1972; Lieber, 1973). However, it appears that there remains controversy on whether or not ethanol consumption affects activities of hepatic alcohol dehydrogenase, with increases, no change and even decreases reported (Lieber, 1973). From a consideration of the literature, it is clear that many experiments were conducted under very different conditions, and the apparent contradictions and conflicting interpretations probably result from the failure to consider sufficiently the effects of dose, manner and duration of ethanol administration. For example, many experiments subjected the animals to prolonged ethanol intake, and this may involve two processes affecting the activity of liver alcohol dehydrogenase: an adaptive process involving a specific induction and a non-specific decrease of enzyme concentration as a manifestation of depressed protein synthesis resulting from impairment of the liver. Experiments carried out over a short time-period (Mistilis & Birchall, 1969) showed a considerable increase in the specific activity of alcohol dehydrogenase after only 4 h from the time of ingestion of ethanol. Thus it appears that the rapid response of the liver to increased concentrations of ethanol is to quickly increase the activities of both alcohol dehydrogenase and aldehyde dehydrogenases to ensure the rapid metabolism of ethanol

and acetaldehyde to potentially less harmful substances.

Although some enzymes in the liver require much longer periods of time for induction, aldehyde dehydrogenases are not unique with respect to the speed at which they may be induced: tyrosine aminotransferase (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) undergoes an even more rapid increase in activity when the appropriate inducing stimulus is administered (Adelman, 1972).

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