Pharmacology Biochemistry & Behavior, Vol. 18, Suppl. 1, pp. 113–116, 1983.

The Role of Cytoplasmic Aldehyde Dehydrogenase in the Metabolism of N-*Tele*-Methylhistamine

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GITOMER, W. L. AND K. F. TIPTON. *The role of cytoplasmic aldehyde dehydrogenase in the metabolism of N-tele-methylhistamine.* PHARMACOL BIOCHEM BEHAV **18:** Suppl 1, 113–116, 1983.—The subcellular distributions of aldehyde dehydrogenase activities towards acetaldehyde have been compared with those toward N-*tele*-methylimidazole acetaldehyde, the aldehyde derived from the oxidation of N-*tele*-methylhistamine. At high concentrations of acetaldehyde (3.0 mM), significant aldehyde dehydrogenase activity can be found in the mitochondrial, light mitochondrial, microsomal and cytoplasmic fractions whereas, when the activity is determined with 15 μM acetaldehyde, the enzyme activity is enriched only in the mitochondrial fraction suggesting that this organelle will be the dominant site for the metabolism of acetaldehyde derived from ingested ethanol. The activity towards N-*tele*-methylimidazole acetaldehyde was determined by generating this compound in the assay by the oxidation of N-*tele*-methylhistamine in the presence of beef plasma amine oxidase. At the low steady-state aldehyde concentrations that will be present in such an assay, only the cytoplasmic form of aldehyde dehydrogenase showed activity towards this substrate.

Aldehyde dehydrogenase, subcellular localization N-*tele*-methylhistamine, metabolism
Histamine, metabolism N-*tele*-methylimidazide acetaldehyde, oxidation by aldehyde dehydrogenase
Acetaldehyde, oxidation by aldehyde dehydrogenase

THE catabolism of histamine, summarized in Fig. 1, involves either direct oxidation by an enzyme of the histaminase (amine oxidase (copper-containing) EC 1.4.3.6.) type, or methylation, in a reaction catalysed by histamine N-methyltransferase (EC 2.1.1.8) to produce N-*tele*-methylhistamine, followed by oxidation by the B-form of monoamine oxidase (amine oxidase (flavin-containing) EC 1.4.3.4.) [7]. The relative importance of these pathways may vary with the tissue involved and with metabolic circumstances, but in most tissues that involving methylation is the major route [2]. The immediate product of the oxidation of N-*tele*-methylhistamine will be the corresponding aldehyde, N-*tele*-methylimidazole acetaldehyde (MIA Ald) but due to the instability of this compound, there have been no studies on its further oxidation to its acid derivative, which is the major metabolite found [2,15].

The conversion of MIA Ald to N-*tele*-methylimidazole acetic acid has been assumed to involve the action of an aldehyde dehydrogenase [16] and thus there may be, either direct or indirect, competition between this process and the oxidation of ethanol [19–21]. In this paper the results of a study on the subcellular distribution of the aldehyde dehydrogenase activity catalyzing the oxidation of MIA Ald are compared with the localization of the enzymes oxidizing acetaldehyde.

METHOD

N-*tele*-methylhistamine was a gift from Smith, Kline and French Ltd., Welwyn Garden City, Hertfordshire, U.K. Beef plasma amine oxidase and rotenone were from Sigma Chemical Co., (London) Ltd., Poole, Dorset, U.K. and Pyrazole was from Eastman Kodak Ltd., Kirby, Liverpool, U.K. All other chemicals used were of analytical grade where this was available.

Beef liver was obtained within 1 hour of the animal's death and was transported to the laboratory on ice. Ten gram samples were homogenized and fractionated into mitochondrial (M), light-mitochondrial (L), microsomal (P) and cytoplasmic (S) fractions by the differential centrifugation procedure of de Duve *et al.* [4]. The degree of cross-contamination of the fractions was estimated by determining the concentration of RNA [6] and the activities of monoamine oxidase [13], glutamate dehydrogenase [11] and lactate dehydrogenase [1] in each of them.

Aldehyde dehydrogenase activity towards acetaldehyde was determined in each fraction by following the increase in absorbance at 340 nm in a mixture containing, in a total volume of 2.0 ml, 90 mM sodium phosphate buffer, pH 7.4, 720 μM NAD^+ and either 3.0 mM acetaldehyde to determine the total activity, or 15 μM acetaldehyde, to determine the activity of enzymes with low K_m values towards this sub-

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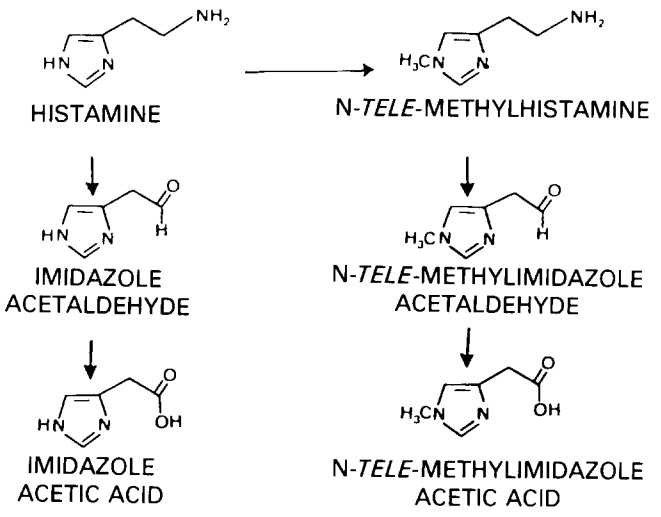

FIG. 1. Pathways of histamine catabolism.

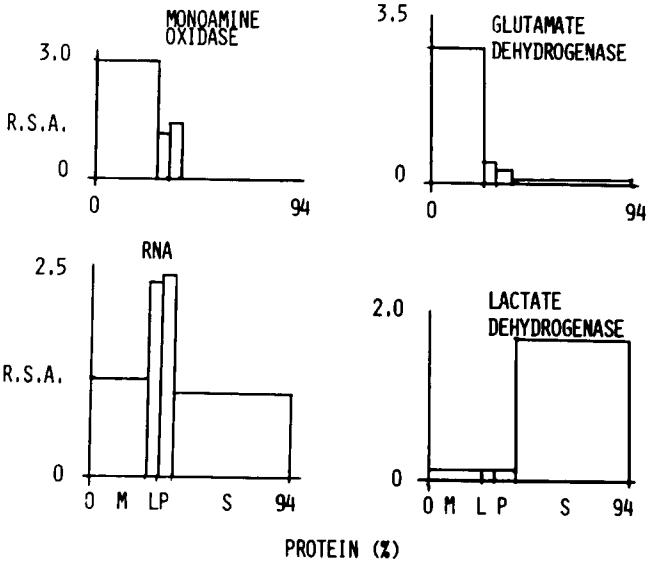

FIG. 2. Subcellular distribution of components in beef liver. The fractionation into mitochondrial (M), light mitochondrial (L), microsomal (P) and cytoplasmic (S) fractions and the determinations of RNA concentration and the activities of monoamine oxidase, glutamate dehydrogenase and lactate dehydrogenase were carried out as described in the text. The results shown are the mean of two separate determinations that differed by less than 5%.

strate [21]. The activity of aldehyde dehydrogenases with high K_m values towards this substrate was taken as the difference between these two values. Aldehyde dehydrogenase activity towards MIAA Ald was determined by generating this compound *in situ* by the oxidation of N-*tele*-methylhistamine catalysed by beef plasma amine oxidase. The reaction mixture contained, in a total volume of 1.8 ml, 90 mM sodium phosphate buffer, pH 7.4, 800 μM NAD^+, 1.4 μM rotenone, 1.1 mM pyrazole, 2.8 mM methylhistamine and 0.53 units of plasma amine oxidase. One unit of plasma amine oxidase activity is defined as the amount catalysing the oxidation of 1 μmol of benzylamine in 1 min at 30°C [24]. Prior to assay the subcellular fractions were solubilized by mixture with sodium deoxycholate to a concentration of 2.9 mg/ml. All enzyme assays were carried out at 30°C.

Protein concentrations was determined by the Lowry method [10] and the distribution of components in the subcellular fractions were expressed as relative specific activity (R.S.A.) values [4] where:

$$\text{R.S.A.} = \frac{\text{Percentage recovery of a component in the fraction}}{\text{Percentage recovery of protein in the fraction}}$$

RESULTS AND DISCUSSION

The distribution of the markers, lactate dehydrogenase (cytoplasm), RNA (microsomes), glutamate dehydrogenase (mitochondrial matrix) and monoamine oxidase (mitochondrial outer membrane), in the subcellular fractions from beef liver are shown in Fig. 2. R.S.A. values provide a measure of the enrichment of a component that has resulted from the preparation of the subcellular fraction. Thus an R.S.A. value of less than 1 indicates that the component is unlikely to be specifically associated with that fraction. These results show that there was considerable cross-contamination between the light-mitochondrial and microsomal fractions, but that the mitochondrial and cytoplasmic fractions were relatively free from contamination with other fractions.

Figure 3 shows that the subcellular distribution of aldehyde dehydrogenase activity. At a low concentration of acetaldehyde, 15 μM, only the activity associated with the mitochondria was found to be significant. This disagrees with an earlier report that there was considerable low-K_m aldehyde dehydrogenase activity in beef liver cytoplasm [18]. Similar results have been obtained with the aldehyde dehydrogenase activity in subcellular fractions from rat liver [19,21]. These results suggest that it is the mitochondrial enzyme that will play the dominant role in oxidizing the acetaldehyde formed from ingested ethanol. Fractionation of the mitochondrial fraction has shown that in both rat and beef liver this aldehyde dehydrogenase activity is associated with the mitochondrial matrix (Smith and Tipton, manuscript in preparation).

At a higher concentration of acetaldehyde (3.0 mM), aldehyde dehydrogenase activity could be found in all the subcellular fractions with the greatest enrichment being in the light mitochondrial and microsomal fractions. Enrichment of aldehyde dehydrogenase activity in the microsomal fraction has been reported with other species [3, 9, 22]. The high-K_m aldehyde dehydrogenase activity, calculated as the difference between the R.S.A. values determined at acetaldehyde concentrations of 3.0 mM and 15 μM showed enrichment in all fractions except the mitochondria, although the degree of enrichment in the cytoplasmic fraction was small. In contrast, when N-*tele*-methylimidazole acetaldehyde generated *in situ* was used as the substrate, only the aldehyde dehydrogenase associated with the cytoplasmic fraction showed significant activity. Similar experiments (not shown) indicated that it was the cytoplasmic aldehyde dehydrogenase in rat liver that showed appreciable activity towards this substrate.

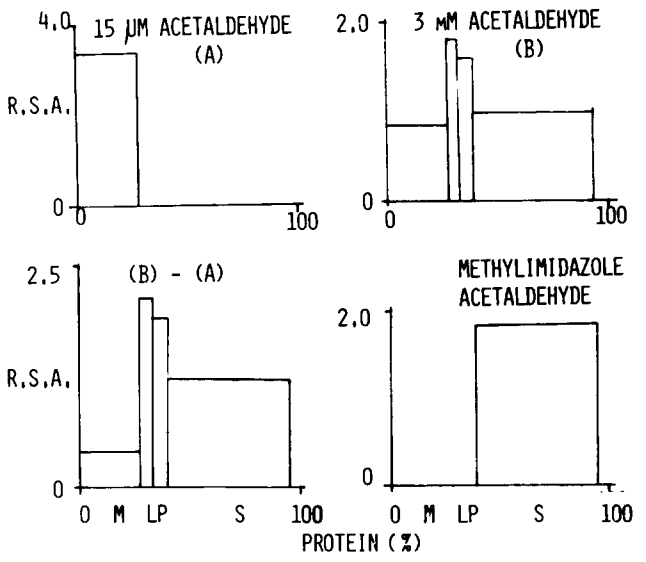

FIG. 3. Subcellualr distributions of aldehyde dehydrogenase activities in beef liver. The fractionation and assay procedures were as described in the text. Other details were as described in Fig. 2.

The use of plasma amine oxidase to catalyse the formation of N-*tele*-methylimidazole acetaldehyde in the assay mixture was necessitated by the instability of this aldehyde in solution. The K_m value of beef plasma amine oxidase towards N-*tele*-methylhistamine was found to be 260 μM under the conditions used in this study. Since the aldehyde is formed as an intermediate in the overall reaction, its steady-state concentration will be comparatively low, but this should approximate that occurring within the cell. The use of an assay system of this type results in a non-linear time-course for the reaction with an appreciable lag-period before the steady-state rate is approached [12]. Quantitation of the non-linear period of this progress curve allowed a value of 40 μM to be calculated for the K_m of ox liver cytoplasmic aldehyde dehydrogenase towards N-*tele*-methylimidazole acetaldehyde (Gitomer and Tipton, manuscript in preparation).

In rat liver it has been shown that the mitochondrial aldehyde dehydrogenase activity is responsible for the oxidation of the aldehydes derived from the oxidation of the biogenic amines 5-hydroxytryptamine [21] and dopamine [19] and the function of the cytoplasmic enzyme has not been clarified. The observations reported here suggest that one of its roles may be in the metabolism of histamine. The cytoplasmic aldehyde dehydrogenase from a number of species has been reported to be particularly sensitive to disulfiram (tetraethylthioperoxydicarbonic diamide) [5, 8, 9, 14, 23]. Since patients treated with this compound may experience drowsiness, fatigability and headache [17], it is possible that these symptoms could be due, in part, to interference with the pathway of histamine metabolism.

ACKNOWLEDGEMENTS

We are grateful to the Medical Research Council of Ireland and Trinity College, Dublin for the support of research grants.

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