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HETEROGENEITY OF ALCOHOL DEHYDROGENASE ENZYMES IN VARIOUS TISSUES*

V. GENE ERWIN and RICHARD A. DEITRICH†

University of Colorado School of Pharmacy, Boulder, Colo. 80302 and the Department of Pharmacology, University of Colorado School of Medicine, Denver, Colo. 80220, U.S.A.

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Abstract—The total aldehyde reducing capacity of various tissues, including liver, lung, brain, heart and testes, was determined with propionaldehyde or *m*-nitrobenzaldehyde as substrate. With each of these substrates, measurements of aldehyde reducing capacity were conducted with NADPH as well as NADH as the cofactors in the absence or presence of pyrazole. The capacity of heart, lung, brain and testes tissues to reduce *m*-nitrobenzaldehyde was 2- to 10-fold greater than the capacity to reduce propionaldehyde with either NADH or NADPH. However, aldehyde reductase activity in liver tissue was greater with propionaldehyde than with *m*-nitrobenzaldehyde as substrate. Propionaldehyde reducing capacity of various tissues was inhibited by pyrazole 98–100 per cent with NADH as cofactor, and 25–60 per cent with NADPH as cofactor, depending upon the tissue examined. On the other hand, pyrazole inhibited *m*-nitrobenzaldehyde reduction only 12–70 per cent with NADH as cofactor, and little inhibition was observed with NADPH as co-substrate. Separation and isolation of two aldehyde reductase fractions from rat liver, which were distinct from alcohol:NAD oxidoreductase (EC 1.1.1.1), was achieved. These fractions differed in substrate and cofactor specificities and in their sensitivity to inhibition by pyrazole and pentobarbital. Similar separations of aldehyde reducing activities were performed with bovine brain tissues, and two distinct enzyme fractions were isolated and compared with the fractions isolated from liver.

IT HAS long been known that alcohol dehydrogenase (alcohol:NAD oxidoreductase EC 1.1.1.1) is present in liver and kidney of various species.[1] In addition, the activity of aldehyde reductase (alcohol dehydrogenase) has been observed in various tissues, including intestine, prostate, lungs and testes.[2] Although several investigators have been unable to detect alcohol dehydrogenase activity in brain tissue,[2–4] Raskin and Sokoloff[5] have reported detection of this enzyme activity in brain. Tabakoff and Erwin[6] partially purified and characterized an aldehyde reductase (alcohol:NADP oxidoreductase; EC 1.1.1.2) from bovine brain. In these studies it was shown that: (1) this brain aldehyde reductase (alcohol dehydrogenase) was specific for NADPH; (2) the enzyme would not catalyze the reduction of short chain aliphatic aldehydes, but long chain aliphatic aldehydes and aromatic aldehydes were substrates; (3) the enzyme activity was not inhibited by pyrazole, but was markedly inhibited by pentobarbital.[7] In addition, it was found that bovine brain tissue also contains an NADH-linked aldehyde reductase (alcohol dehydrogenase). We have recently reported the

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presence of an NADH-dependent, pyrazole-insensitive aldehyde reductase enzyme in rat liver.[8]

Aldehyde reductase activities in kidney tissue capable of reducing lactaldehyde and aliphatic aldehydes have been described.[9,10] Also, partial purification and characterization of an aromatic aldehyde and ketone reductase from kidney have been effected,[11] and recently, the partial purification and characterization of an NADPH-linked aldehyde reductase from porcine kidney were reported.[12] It was of interest to compare the relative activities of the various aldehyde reductase enzymes in liver and other tissues. Further, we wished to separate physically and then proceed to classify these enzymes as to their substrate and inhibitor specificities, and to determine whether these enzyme-catalyzed reactions were reversible.

MATERIALS AND METHODS

Crystalline horse liver alcohol dehydrogenase, NADPH, NADH, NADP, NAD and calcium phosphate-gel were obtained from Sigma Chemical Company. Propionaldehyde (Aldrich Chemical Co.) was vacuum distilled before using and *m*-nitrobenzaldehyde and *p*-nitrobenzaldehyde were also purchased from Aldrich. All reagents were of the highest purity available. The concentration of various aldehydes in freshly prepared solutions was determined at 340 nm in the presence of excess aldehyde dehydrogenase and NAD.[13]

Various tissues were obtained from adult male Sprague–Dawley rats weighing 225–275 g. Tissues were removed at once after decapitation of the animal and were placed in ice-cold 0·1 M sodium phosphate, pH 7·4, blotted on filter paper and weighed. Each tissue was homogenized in sufficient 0·1 M sodium phosphate, pH 7·4, to make a 30 per cent suspension. The homogenates were centrifuged at 144,000 *g* for 1 hr and the resulting supernatant fluids were used for determination of enzyme activities or for ammonium sulfate fractionation. All procedures were carried out at 0–4°. Crystalline ammonium sulfate was added slowly to the bovine brain (bovine brains were homogenized and the supernatant fluid was obtained as described above)[7] and rat liver supernatant fluids with constant stirring until 0·4 saturation (24·3 g ammonium sulfate per 100 ml of solution) was attained; stirring was continued for an additional 30 min. The precipitation protein was removed by centrifugation at 27,000 *g* for 20 min, and sufficient ammonium sulfate was added to the resulting supernatant fluids to give 0·7 saturation (an additional 20·5 g ammonium sulfate per 100 ml of solution). After stirring for 30 min, the precipitate was separated by centrifugation, as above, and was suspended in 0·5 ml of 0·1 M sodium phosphate, pH 7·4, per 10 g wet wt, of brain or liver tissue. The suspensions were dialyzed against 10 l. of distilled water containing 0·5 mM mercaptoethanol and 0·05 mM EDTA for 4 and 14 hr successively. After centrifugation at 144,000 *g* for 1 hr to remove insoluble protein, the dialyzed fractions were placed on calcium phosphate-gel columns.

Calcium phosphate-gel cellulose columns were prepared according to the method of Massey.[14] Whatman standard grade cellulose powder was washed three times with 0·1 N NaOH, three times with 0·1 N HCl and three times with distilled water. Cellulose, 20 g, dry wt., was suspended in 200 ml of distilled water and mixed with 100 ml of a suspension of calcium phosphate-gel diluted to 30 g of solids per ml. A column, 3·5 × 18 cm, of calcium phosphate–gel cellulose was prepared and washed successively with

600 ml of 0·1 M sodium phosphate, pH 6·0, and with 1 l. of 0·01 M sodium phosphate, pH 6·0. All buffers contained 0·5 mM mercaptoethanol and 0·05 mM EDTA. Five ml of the dialyzed ammonium sulfate fraction, containing approximately 25 mg protein per ml, was placed on this column and non-enzyme protein was eluted with 50 ml of 0·01 M sodium phosphate, pH 6·0, followed by 50 ml of 0·05 M sodium phosphate, pH 6·0. Various fractions, representing peaks of enzyme activity, were eluted from the columns as described in the legends of the figures, and were pooled and concentrated in an Amicon model 50 apparatus with a filter of um 10 size porosity.

Routinely, enzyme assays were performed at 25° with 0·16 mM NADPH or NADH, 1·3 mM *m*-nitrobenzaldehyde or propionaldehyde, 0·05 M sodium phosphate, pH 7·0. The final volume was 3·0 ml. The rate of NADH or NADPH oxidation was observed by following the decrease in absorbance at 340 nm with a Gilford model 2400 spectrophotometer and recorder. All values were obtained by subtracting the rates of NADH or NADPH oxidation in a complete reaction mixture without added aldehyde substrate. Initial rates were determined within the first minute after initiating the reaction by adding cofactor in order to avoid interference with the assays by enzymes which might utilize NAD or NADP. Reaction rates were linear for at least 5 min in all assays.

Protein concentration in all crude preparations, such as supernatant fluids and ammonium sulfate fractions, was determined by the biuret method, but in further purified preparations, the more sensitive method of Murphy and Kies[15] was used. In all protein determinations, bovine serum albumin was employed as the primary standard.

RESULTS

Aldehyde reducing capacities of various tissues. Inasmuch as it was shown that brain,[7] liver[8] and kidney tissues[11,12] contain pyrazole-insensitive aldehyde reductases, the possibility that other tissues contain similar enzymes has been investigated with propionaldehyde and *m*-nitrobenzaldehyde as substrates. As shown in Fig. 1, with NADH as cofactor, propionaldehyde reducing capacity was greatest in the liver supernatant fluid followed by the kidney, testes, lung and heart extracts. No activity was observed in the supernatant fluid from brain tissue. With NADPH as cofactor, the greatest propionaldehyde reducing activity was observed in liver supernatant fluid followed by testes, kidney, heart, lung and brain. It is of interest to note that the ratios of activity with NADH and NADPH as cofactor varied greatly from tissue to tissue. Also, the per cent inhibition of enzyme activity by pyrazole with NADH as cofactor was greater than 90 per cent for all tissue extracts and for horse liver alcohol dehydrogenase. With NADPH as cofactor, the enzyme activity was inhibited by pyrazole approximately 70 per cent with rat liver supernantant fluid or horse liver alcohol dehydrogenase; however, with other tissue extracts, as little as 25 per cent inhibition was observed. The observation of different levels of alcohol dehydrogenase (aldehyde reductase) activity in various tissues is consistent with the results of others.[1,2,4] However, the wide variation in ratios of activity with NADH or NADPH and the marked tissue difference in sensitivity of the reaction to pyrzaole suggested the presence of more than one enzyme in these tissues.

Since some tissues, i.e. brain[6] and kidney,[11,12] contain aldehyde reductase enzymes which are inactive with short chain aliphatic aldehydes, studies similar to those shown

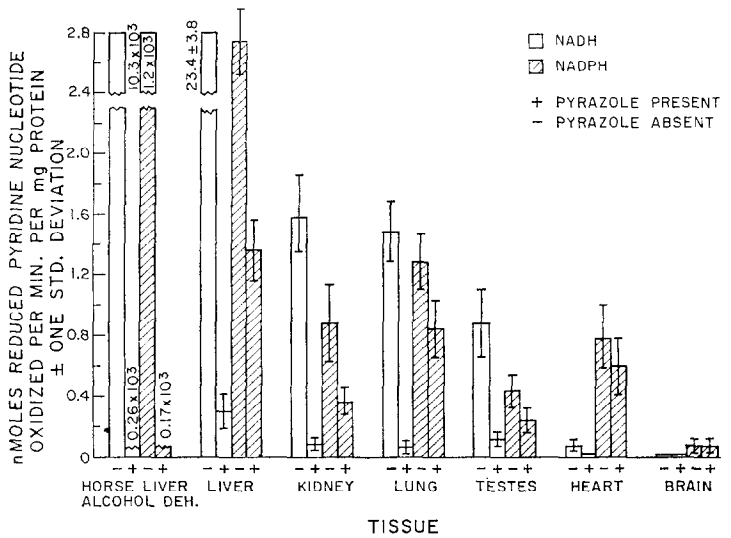

FIG. 1. Comparison of the effects of pyrazole on propionaldehyde reducing capacities of various tissues. The 144,000 *g* supernatant fluids from various tissues were obtained as descsibed in the text. Rates of NADH or NADPH oxidation with propionaldehyde were determined as described in the text at 25°. Values were corrected for rates of NADH or NADPH oxidation in the absence of added aldehyde. The reaction mixtures consisted of 0·05 M sodium phosphate, pH 7·0, 0·16 mM NADH or NADPH, 1·3 mM propionaldehyde, 10 mM pyrazole (final concentrations), and 0·1 ml of enzyme preparation (1–4·5 mg protein) in a total volume of 3·0 ml.

in Fig. 1 were conducted with *m*-nitrobenzaldehyde as substrate. As illustrated in Fig. 2, with NADPH as cofactor, *m*-nitrobenzaldehyde reducing capacity was greatest in the kidney followed by the liver, testes, heart, brain and lung. Of particular interest is

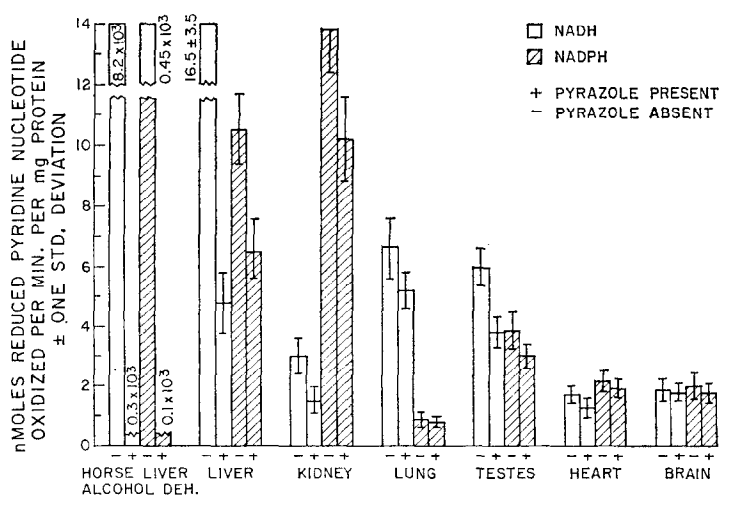

FIG. 2. Comparison of the effects of pyrazole on *m*-nitrobenzaldehyde reducing capacities of various tissues. Experimental conditions were as described in Fig. 1, except that rates of NADH or NADPH oxidation were determined with 1·3 mM *m*-nitrobenzaldehyde as substrate.

the observation that the reductase activity of heart, lung, brain and testes with *m*-nitrobenzaldehyde as substrate was 2- to 10-fold greater than when propionaldehyde was the substrate with either NADPH or NADH as cofactor. However, reductase activity of horse liver alcohol dehydrogenase and rat liver supernatant fluid was greater with propionaldehyde than with *m*-nitrobenzaldehyde with NADH as cofactor. Also, as shown in Fig. 2, pyrazole markedly inhibited reductase activity of horse liver alcohol dehydrogenase and rat liver supernatant with either cofactor, but little inhibition of *m*-nitrobenzaldehyde reductase activity of other tissues was observed. The data presented in Figs. 1 and 2 suggest that the various tissues studied have differing proportions of pyrazole-sensitive and pyrazole-insensitive alcohol dehydrogenase enzymes, which also differ in substrate and cofactor specificities.

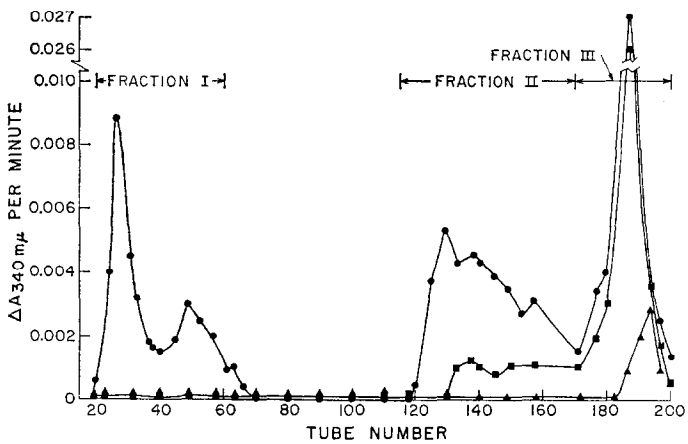

FIG. 3. A typical profile for various rat liver aldehyde reductase enzymes from calcium phosphate-gel cellulose columns. The 3·5 × 18 cm calcium phosphate-gel cellulose columns were prepared as described in the text and approximately 5 ml (ca. 150 mg protein) of the dialyzed ammonium sulfate fraction from rat liver was placed on the column. As described in the text, the columns were then washed with 50 ml of 0·01 M sodium phosphate, pH 6·0, and 50 ml of 0·05 M sodium phosphate, pH 6·0. Fractions containing enzyme activity were eluted with 200 ml of 0·1 M sodium phosphate, pH 6·0, 200 ml of 0·1 M sodium phosphate, pH 6·7, and 100 ml of 0·1 M sodium phosphate, pH 6·7, plus 0·5 M sodium chloride successively. Approximately 4 ml eluate was collected per tube and 0·1-ml aliquots of tubes indicated were assayed for aldehyde reductase activity as described in the text. Fractions I, II and III were obtained by pooling the eluates as indicated. Enzyme activities with NADPH and *m*-nitrobenzaldehyde (●——● NADH and *m*-nitrobenzaldehyde (■——■) and NADH and propionaldehyde (▲——▲) as substrates.

Separation of aldehyde reductase activities. When a dialyzed 40–70% saturated ammonium sulfate fraction from rat liver was eluted from a calcium phosphate-gel column, it was found that reductase activity eluted in three large peaks (Fig 3; i.e. fractions I, II, III). As shown in Table 1, the fractions differed in cofactor and substrate specificity. Fraction I was specific for NADPH (K_m 6·2 × 10^{-6} M) and propionaldehyde was not a substrate. Fractions II and III possessed reductase activity with either NADPH or NADH and *m*- or *p*-nitrobenzaldehyde as substrate. The K_m values for NADPH were 4·4 × 10^{-5} M and 2·6 × 10^{-5} M for fractions II and III respectively. Since these values are 4- to 7-fold greater than the K_m value obtained with fraction I, it is unlikely that the activities are due to the same enzyme protein. The ratio

of activities with NADH and NADPH in fractions II and III is markedly different, suggesting the presence of at least two and possibly three aldehyde reductase enzymes in liver which differ from the pyrazole-sensitive, "classical" alcohol dehydrogenase.

TABLE 1. COMPARISONS OF SUBSTRATE AND COFACTOR SPECIFICITIES OF LIVER ALDEHYDE REDUCTASE FRACTIONS OBTAINED BY CALCIUM PHOSPHATE-GEL CHROMATOGRAPHY*

Fraction	Varied substrate	Constant substrate	K_m $\times 10-5$ M	V_{max}†
I	NADPH	*m*-Nitrobenzaldehyde	0·6	39·8
	NADH	*m*-Nitrobenzaldehyde		0
	m-Nitrobenzaldehyde	NADPH	2·8	36·7
	p-Nitrobenzaldehyde	NADPH	3·1	33·8
	Propionaldehyde	NADPH		0
II	NADPH	*m*-Nitrobenzaldehyde	4·4	44·0
	NADH	*m*-Nitrobenzaldehyde	1·6	23·3
	p-Nitrobenzaldehyde	NADPH	1·7	21·3
	m-Nitrobenzaldehyde	NADPH	1·3	40·7
	m-Nitrobenzaldehyde	NADH	2·0	20·6
	Propionaldehyde	NADPH or NADH		0
III	NADPH	*m*-Nitrobenzaldehyde	2·6	27·4
	NADH	*m*-Nitrobenzaldehyde	7·6	25·9
	m-Nitrobenzaldehyde	NADH	2·6	22·2
	p-Nitrobenzaldehyde	NADPH	3·5	21·8
	Propionaldehyde	NADH		2·3‡

* Various fractions were collected from calcium phosphate-gel cellulose columns, as indicated in Fig. 3, and were concentrated as described in the text. Michaelis constants and maximal velocities were determined by the conventional Lineweaver–Burk double-reciprocal plot. The rate of NADPH or NADH utilization was measured at 340 nm at 25° as described in the text. Various concentrations of aldehydes were added to the reaction mixture containing 0·16 mM NADPH or NADH, enzyme, and 0·1 M sodium phosphate, pH 7·0, in a volume of 3·0 ml. For the determination of K_m for NADPH of NADH the reaction mixture contained 10^{-3} M *m*-nitrobenzaldehyde or 0·65 mM *p*-nitrobenzaldehyde while the concentration of NADPH or NADH was varied.

† Maximum velocity values represent nanomoles of NADH or NADPH oxidized per min per milligram of protein.

‡ After elution of fraction III from the column, activity with propionaldehyde decreased rapidly and was completely absent after 4–6 hr. Insufficient activity was present to allow for a kinetic determination of K_m or V_{max}, therefore, the value represents activity at a saturating concentration of substrates (see legend, Fig. 1).

The elution profile of a bovine brain ammonium sulfate fraction is shown in Fig. 4. Two fractions (I and II), somewhat comparable to fractions I and II from rat liver, were obtained. As shown in Table 2, these fractions from bovine brain were distinct and fraction I is the NADPH-dependent enzyme reported by Tabakoff and Erwin.[6] Fraction II has been characterized in detail and will be reported elsewhere. No fraction comparable to the liver fraction III was obtained from brain. These data show that there are at least two aldehyde reductase enzymes in bovine brain tissue.

In order to show that the reductase activities in the liver fractions were indeed alcohol dehydrogenase enzymes, the reversibility of the enzyme-catalyzed reactions was demonstrated. As shown in Table 3, with *m*-nitrobenzylalcohol as substrate, fraction I was reversible with NADP but not with NAD as cofactor and fractions II and III were reversible with either cofactor.

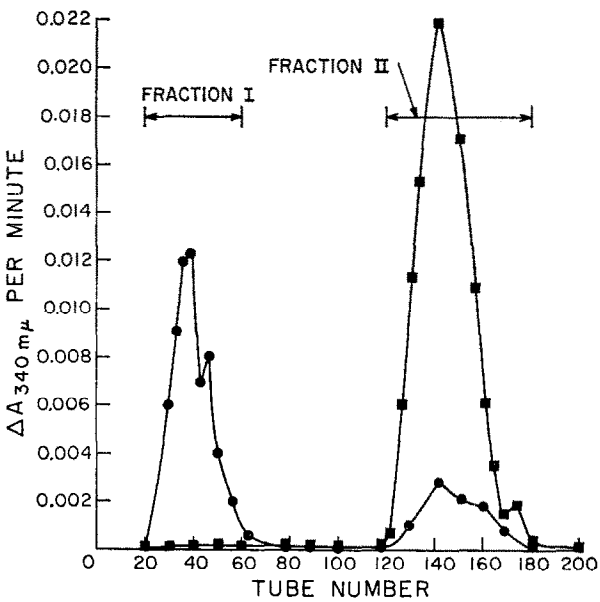

FIG. 4. Typical elution profiles for various bovine brain aldehyde reductase enzymes from calcium phosphate-gel cellulose columns. The elution profile of fraction I was obtained from calcium phosphate-gel cellulose column chromatography, as described in Fig. 3 and the text, of a dialyzed 40–55% saturated ammonium sulfate fraction from bovine brain, as described previously.[6] The elution profile for fraction II was obtained by similar chromatography of a 55–70% saturated ammonium sulfate fraction from bovine brain. Enzyme assay conditions were as described in Fig. 1. Enzyme activity when NADPH and *m*-nitrobenzaldehyde (●——●) and NADH and *m*-nitrobenzaldehyde (■——■) were substrates.

Effects of inhibitors on aldehyde reductases from liver. In order to further illustrate the differences between the rat liver alcohol dehydrogenase fractions, the sensitivity of the enzymes to inhibition by pyrazole and pentobarbital was investigated (Tables 4 and 5). When *m*-nitrobenzaldehyde was the substrate and NADPH was the cofactor,

TABLE 2. COMPARISONS OF SUBSTRATE AND COFACTOR SPECIFICITIES OF BRAIN ALDEHYDE REDUCTASE FRACTIONS OBTAINED BY CALCIUM PHOSPHATE-GEL CHROMATOGRAPHY*

Fraction	Varied substrate	Constant substrate	K_m × 10^{-5} M	V_{max}†
I	NADPH	*p*-Nitrobenzaldehyde	0·2	51·2
	NADH	*p*-Nitrobenzaldehyde		0
	p-Nitrobenzaldehyde	NADH	1·0	62·0
	Propionaldehyde	NADPH or NADH		0
II	NADPH	*m*-Nitrobenzaldehyde	3·0	23·0
	NADH	*m*-Nitrobenzaldehyde	1·0	284·1
	m-Nitrobenzaldehyde	NADH	50·0	284·0
III	No fraction comparable to liver fraction III obtained			

* Fractions I and II were obtained from pooled tubes as indicated in Fig. 4. Experimental conditions are as described in Table 1.

† Values represent nanomoles of NADH or NADPH oxidized per min per milligram of protein.

TABLE 3. REVERSIBILITY OF ALDEHYDE REDUCTION CATALYZED BY VARIOUS LIVER FRACTIONS WITH *m*-NITROBENZYLALCOHOL AS SUBSTRATE*

Fraction	Cofactor	NAD or NADP (nmoles/min/mg)†
I	NAD	0
	NADP	2·06
II	NAD	1·21
	NADP	2·45
III	NAD	1·45
	NADP	1·92

* Cofactor and substrate concentrations were 10^{-3} M. Experimental conditions were as described in the text.

† Values refer to the nanomoles of NAD or NADP reduced per min per milligram of protein.

pyrazole did not inhibit the reductase activity of any fraction, and with NADH as the cofactor, only the activity of fraction II was significantly inhibited (Table 4). Inhibition of activity of fraction III with propionaldehyde as substrate was the same as that obtained with horse liver alcohol dehydrogenase. As shown in Table 5, reductase activity of fraction I was inhibited 83 per cent by pentobarbital, whereas activity of fraction II was inhibited approximately 50 per cent with NADH as cofactor and only 10 per cent with NADPH as co-substrate. Activity of fraction III was not inhibited by pentobarbital.

TABLE 4. COMPARISONS OF PYRAZOLE INHIBITION OF ALDEHYDE REDUCTASE ACTIVITIES IN FRACTIONS FROM LIVER*

Fraction	Cofactor	Pyrazole†	NADH or NADPH (nmoles oxidized/min/mg)	Inhibition (%)
I	NADPH	−	57·87	
	NADPH	+	55·94	4
	NADH	−	0	
	NADH	+	0	0
II	NADH	−	15·43	
	NADH	+	6·75	56
	NADPH	−	19·29	
	NADPH	+	18·36	
III	NADH	−	48·23	
	NADH	+	44·85	8
	NADPH	−	47·81	
	NADPH	+	46·50	0
	NADH‡	−	3·61	
	NADH	+	0	100
	NADPH‡	−	1·83	
	NADPH	+	0·48	74

* Experimental conditions were as described in Fig. 1 with 0·16 mM NADPH or NADH and 1 mM *m*-nitrobenzaldehyde in the reaction mixture. The final concentration of pyrazole was 10^{-2} M.

† Symbols refer to the presence (+) or absence (−) of pyrazole.

‡ Values were obtained with propionaldehyde as substrate.

TABLE 5. COMPARISONS OF PENTOBARBITAL INHIBITION OF ALDEHYDE REDUCTASE FRACTIONS FROM LIVER*

Fraction	Cofactor	Pentobarbital†	NADH or NADPH oxidized (nmoles/min/mg)	Inhibition (%)
I	NADH	−	0	
	NADH	+	0	0
	NADPH	−	57·87	
	NADPH	+	9·64	83
II	NADH	−	15·43	
	NADH	+	8·19	47
	NADPH	−	19·29	
	NADPH	+	17·36	11
III	NADH	−	48·23	
	NADH	+	48·23	0
	NADPH	−	45·81	
	NADPH	+	45·81	0

* Experimental conditions were as described in Table 4 and Fig. 1 with 1 mM *m*-nitrobenzaldehyde as substrate. Pentobarbital final concentration was 10^{-3} M.

† Symbols refer to the presence (+) or absence (−) of pentobarbital.

DISCUSSION

It has long been reported that tissues other than liver and kidney contain little alcohol dehydrogenase or aldehyde reductase activity.[1–4] However, these studies have been performed only with short chain aliphatic aldehydes or alcohols as substrate, e.g. acetaldehyde or ethanol. It is of interest that Pugh and Quastel[16] suggested in 1937 that isoamylamine might be metabolized by brain tissue to the corresponding aldehyde and subsequently to the alcohol derivative. Only a few studies have been conducted using aromatic aldehydes or aliphatic aldehydes with aromatic substituents as substrates,[6,11,12] and these investigations have been limited to kidney and brain tissues. Elucidation of enzymes which will catalyze the reversible reduction of "aromatic" aldehydes, has become increasingly important, since it appears that these enzymes may be involved in the metabolism of aldehydes derived from the biogenic amines and as norepinephrine and serotonin.[17–20] The data presented in the present paper show that many tissues contain an *m*-nitrobenzaldehyde reductase in the high speed supernatant fluid. Whether the enzymes will reduce aldehydes derived from biogenic amines remains to be determined; however, it has been shown that brain tissue contains an *m*- or *p*-nitrobenzaldehyde reductase which rapidly catalyzes the reduction of indoleacetaldehyde, *p*-hydroxyphenylacetaldehyde and other aromatic aldehydes.[6,7]

It is of particular interest to note that *m*-nitrobenzaldehyde reductase activity in heart, lung, brain and testes was 2- to 10-fold greater than propionaldehyde reductase activity with either NADH or NADPH as cofactor. Conversely, reductase activity in rat liver supernatant fluid and horse liver alcohol dehydrogenase was greater with propionaldehyde than with *m*-nitrobenzaldehyde as substrate. These observations together with : (1) the wide variation in ratios of activity with NADH or NADPH when propionaldehyde was the substrate (Fig. 1); (2) the marked tissue differences in sensitivity of the aldehyde reductase reactions to inhibition by pyrazole (Figs. 1 and 2); (3) the separability of liver or brain fractions which show different substrate and

cofactor specificities as well as widely varying sensitivities to inhibition by pyrazole or pentobarbital; and (4) the reversibility of the reactions (Table 3); show that various tissues contain different proportions of alcohol dehydrogenase or aldehyde reductase enzymes. The data strongly indicate that rat liver and bovine brain contain at least two, and in the liver possibly three, alcohol dehydrogenases which differ from "classical" alcohol dehydrogenase. The previously investigated isozymes of alcohol: NAD oxidoreductase (EC 1.1.1.1)[21,22] were inhibited by pyrazole and these isozymes utilized short chain aliphatic alcohols or aldehydes as substrates. Therefore, the pyrazole-insensitive alcohol, NADP oxidoreductase, and the pyrazole-insensitive alcohol, NAD oxidoreductase, activities observed in the present paper probably do not represent isozymes of "classical" alcohol dehydrogenase, but may be entirely different enzyme proteins.

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