Biochemical Pharmacology, Vol. 25, pp. 317–323. Pergamon Press. 1976. Printed in Great Britain.

PARTIAL PURIFICATION AND CHARACTERIZATION OF A REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE PHOSPHATE-LINKED ALDEHYDE REDUCTASE FROM HEART*

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(*Received* 6 *January* 1975; *accepted* 9 *May* 1975)

Abstract—The presence of a nonspecific NADPH-linked aldehyde reductase (alcohol-NADP oxidoreductase, EC 1.1.1.2) from heart was observed in the soluble portion of tissue homogenates. The reductase activity was present in at least two forms. The enzyme which accounted for the major portion of activity was purified some 800-fold over the crude homogenate. The enzyme was capable of reducing a number of aromatic and medium chain length aldehydes in the presence of NADPH. No ketones were utilized as substrates and the enzyme was inactive with NADH. The enzyme was shown to have a pH optimum at 6·4 for the reduction of aldehydes. Oxidation of alcohols occurred optimally at pH 9·6 with NADP as cofactor, although the reaction proceeded at less than 10 per cent of the rate observed in the forward direction. A molecular weight of 29,000 was estimated by gel filtration on Sephadex G-75. Biogenic aldehydes prepared from β-hydroxylated phenylethylamines were actively reduced. The K_m values for 3,4-dihydroxyphenylglycolaldehyde, 4-hydroxy-3-methoxyphenylglycolaldehyde and 4-hydroxyphenylglycolaldehyde were: 0·53, 0·50 and 0·03 mM respectively. The aldehydes of non-β-hydroxylated phenylethylamines were not efficiently utilized as substrates. The K_m for NADPH was determined to be 0·013 mM in the presence of ρ-nitrobenzaldehyde. Inhibitor studies show the enzyme to be different from "classical" alcohol dehydrogenase. Various anticonvulsants and diuretics were inhibitory at concentrations of 0·01 mM. The enzyme is postulated to be responsible for the reduction of biogenic aldehydes in heart *in vivo*.

It has long been known that aldehydes occur in tissues as the result of oxidative deamination of amines by monoamine oxidase [1,2]. The aldehyde intermediate may then be oxidized to the corresponding acid or reduced to the alcoholic metabolite. Breese *et al.* [3] and Rutledge and Jonason [4] have suggested that the aldehyde intermediates of the phenylethylamines which are β-hydroxylated are preferentially reduced to glycols, while those which lack the β-hydroxyl group are primarily oxidized to the corresponding acid metabolites.

An NAD-dependent aldehyde dehydrogenase from brain has been described which can catalyze the oxidation of aldehydes to their acid metabolites [5]. Several reports describing enzymes capable of catalyzing the reduction of ketones to secondary alcohols have appeared [6–8] The reduction of aldehydes by brain enzyme preparations has been studied in some detail [9–12], but the enzymes responsible for the reduction of these compounds in a sympathetically innervated organ such as heart have not been elucidated. This study reports the properties of a highly purified aldehyde reductase from sheep heart.

MATERIALS AND METHODS

Materials. All reagents were of the highest grade commercially available. Aldehydes, ρ-nitrobenzyl alcohol and ρ-hydroxybenzyl alcohol were purchased from Aldrich Chemical Co. Chlorpromazine was furnished by Smith, Kline & French. Diphenylhydantoin was supplied by Parke, Davis. Hydralazine and guanethidine were furnished by Ciba. Propranolol was a gift from Ayerst. Procainamide was supplied by Squibb. Probenecid, ethacrynic acid, chlorothiazide and hydrochlorothiazide were furnished by Merck, Sharp & Dohme. Furosemide was a gift from Hoechst. Amobarbital was supplied by Lilly. Benzthiazide was furnishd by Robins. All other reagents were obtained from Sigma Chemical Co. All water used was double-distilled in quartz.

Standard assay of aldehyde reductase. Aldehyde reductase activity was determined spectrophotometrically in a reaction mixture consisting of protein, 0·5 mM ρ-nitrobenzaldehyde, 0·16 mM NADPH and 90 mM sodium phosphate, pH 7·4, in a final volume of 2·0 ml. All concentrations reported in this and the succeeding discussions are final concentrations employed in the reaction vessel. The reaction was routinely started by the addition of enzyme, although no differences were observed when the reaction was started by addition of aldehyde or NADPH. The rate of NADPH oxidation was measured by observing the decrease in absorbance at 340 nm at 30° with a Beckman model 25 double beam spectrophotometer. Addition of 0·2 ml (10% final concentration) of absolute methanol to the reaction mixture had no effect on the reaction; therefore, aldehydes were routinely dissolved in 10% methanol in water. Protein in crude homogenates and ammonium sulfate fractions was determined by the biuret method [13]. In more purified preparations the method of Murphy and Kies [14] was employed. Bovine serum albumin was used as a standard for both protein determinations.

* Supported in part by a grant from the Wyoming Heart Association.

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Preparation of monoamine oxidase and aldehyde dehydrogenase from rat liver. Monoamine oxidase (MAO) and aldehyde dehydrogenase were prepared from rat liver mitochondria by a modification of the method of Whittaker [15] as described by Erwin.* All procedures were carried out at 2°. Four adult WKY inbred strain rats of either sex were killed by decapitation, the livers were removed immediately, weighed and placed in sufficient ice-cold 0·25 M sucrose to yield a 20% suspension. The tissue was homogenized in a glass homogenizer with a tightly fitting Teflon pestle. The homogenate was centrifuged at 480 *g* for 20 min. The crude mitochondrial pellet was then resuspended in one-half the original volume of 0·25 M sucrose and again sedimented at 8000 *g* for 20 min. The washing step was repeated once more. The washed mitochondrial pellet was then resuspended in 20 ml of 0·25 M sucrose and was sonically disrupted using a Bronwill Biosonic III sonifier at maximm setting. Four sonifications of 30 sec each were performed at 1-min intervals while the suspension was immersed in an ice bath. The disrupted mitochondria were sedimented at 100,000 *g* for 60 min in a Beckman L265-B preparative ultracentrifuge. The supernatant contained crude mitochondrial aldehyde dehydrogenase which was frozen and used without further purification. The disrupted mitochondrial pellet was resuspended again in 20 ml of 0·25 M sucrose, sonified, and centrifuged as above. The pellet was suspended in 10 mM sodium phosphate, pH 7·4, and the mitochondrial MAO was used in the preparation of the biogenic aldehydes described below.

The presence of MAO was measured spectrophotometrically according to the method of Deitrich and Erwin [16]. The presence of aldehyde dehydrogenase was confirmed spectrophotometrically according to the method of Erwin and Deitrich [5].

Preparation of biogenic aldehydes. The corresponding aldehydes of dopamine, octopamine, tyramine, norepinephrine and normetanephrine were prepared according to the method of Renson *et al.* [17] as modified by Erwin.† The appropriate amine (2 mM) was incubated with MAO from rat liver mitochondria (10–20 mg protein) in 100 mM sodium phosphate, pH 7·4, in the dark at room temperature for 60–180 min with occasional mixing. Two mg ascorbic acid was added to prevent oxidation of the aldehydes. A blank was run using boiled MAO. The usual volume of the incubation mixture was 10 ml. The reaction was terminated by cooling to 2° and sedimenting the mitochondria at 45,000 *g* for 30 min in a Beckman J-21 refrigerated centrifuge.

Aldehydes were separated from the reaction mixture on small columns (0·5 × 3·5 cm) of AG50W-X8 (Bio Rad Laboratories) in the sodium form. The columns were washed with distilled water. Quantities (0·3 ml each) of the reaction mixture containing aldehyde were placed on the column and the effluent was discarded. Aldehyde was eluted from the resin by passing through the column an additional 1·7 ml of the reaction mixture containing aldehyde followed by 0·3 ml distilled water. Under these conditions unreacted amine was retained on the resin. The concentration of aldehyde in the effluent was assayed spectrophotometrically in a reaction mixture containing 0·2 ml rat liver aldehyde dehydrogenase (prepared as above), 0·5 mM NAD, 0·05 ml aldehyde from the column, and 90 mM sodium phosphate, pH 7·0, in a total volume of 2·0 ml. Reduction of NAD was monitored by observing the increase in absorbance at 340 nm at 37°. The reaction was allowed to go to completion and the concentration of aldehyde was calculated from the molar extinction coefficient of NADH ($6{\cdot}22 \times 10^3$ l cm^{-1} mole^{-1}). Since the stoichiometry of the reaction is 1 mole aldehyde oxidized for each mole of NAD reduced [5], the concentration of aldehyde was taken to be equal to the amount of NADH produced in the reaction. The final concentration of aldehyde was of the order of 0·1 to 0·5 mM.

The aldehydes prepared in this manner were used as substrates for aldehyde reductase. It was found that the amines themselves, or ascorbic acid, did not affect the kinetics, but they were routinely separated from the reaction mixture in order to provide a more homogeneous substrate preparation.

Subcellular distribution of aldehyde-reducing capacity. Heart mitochondria were prepared in a manner similar to that described previously for the preparation of MAO, except that the sonification was not performed. The intact mitochondria were resuspended in a small volume (10 ml/100 g of tissue) of 0·25 M sucrose and assayed for aldehyde reductase activity in the standard assay. Microsomes were separated from the 8000 *g* supernatant by centrifugation at 100,000 *g* for 60 min. The microsomes were resuspended in 0·5 ml of 0·25 M sucrose per 100 g of tissue and assayed for aldehyde reductase in the standard assay. Blanks containing no aldehyde substrate were employed to correct for the oxidation of NADPH by systems other than aldehyde reductase.

Estimation of molecular weight. The molecular weight of aldehyde reductase was estimated by gel filtration on a column (2·8 × 47 cm) of Sephadex G-75 at room temperature according to the method of Andrews [18]. The column was equilibrated with 100 mM sodium phosphate, pH 7·0, which had previously been degassed. Calibration proteins used and their molecular weights were: cytochrome *c* (12,400), α-chymotrypsin (22,500), ovalbumin (45,000), and bovine serum albumin (67,000). The column eluate was monitored by measurement of the absorbance at 280 nm with an ISCO UA-4 absorbance monitor. Aldehyde reductase was determined by its activity in the standard assay system.

Purification of aldehyde reductase. For the purification of heart aldehyde reductase all procedures were carried out at 0–4° except where noted. All buffers contained 0·5 mM 2-mercaptocthanol and 0·05 mM EDTA. Sheep hearts were generously provided by Dr. M. Riley of the Animal Science Department of the University of Wyoming and Monfort, Inc., of Greeley, Colo. Most of the fatty and connective tissue was removed and the hearts were coarsely ground in a meat grinder. Tissue that was not used immediately was stored at −20°. Storage was not found to be detrimental to recovery of enzyme activity. The

* V. G. Erwin, School of Pharmacy, University of Colorado, Boulder, Colo., personal communication.

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ground tissue was homogenized in a high-speed Waring blender for 60 sec in sufficient 75 mM sodium phosphate, pH 7·6, to give a 40% suspension. The homogenate was centrifuged at 17,000 *g* for 30 min in a Beckman J-21 instrument to remove large particulate material. The resulting supernatant was passed through a single layer of Miracloth (V. W. R. Scientific Co., Denver, Colo.) to remove solid lipid.

The resulting supernatant was then fractionated with crystalline ammonium sulfate. The fraction precipitating between 40 and 55% saturation was resuspended in 5 mM sodium phosphate, pH 6·0 (3–5 ml/100 g of tissue) and dialyzed extensively against the same buffer. The suspension was then centrifuged at 100,000 *g* for 60 min to remove insoluble protein. The resulting supernatant will be referred to as the ammonium sulfate fraction.

The ammonium sulfate fraction was diluted to 10 mg protein/ml with 5 mM sodium phosphate, pH 6·0. Calcium phosphate gel, 2 mg solids/mg of protein, was added to the solution and the suspension was stirred for 10 min. The suspension was then centrifuged at 3500 *g* for 5 min to remove the gel. The extraction was repeated twice more. Most of the aldehyde reductase activity was retained in the supernatant.

The protein in the thrice-extracted supernatant obtained from the above procedure was then concentrated by adding crystalline ammonium sulfate to 75% saturation. The precipitated protein was resuspended in 5 ml of 5 mM sodium phosphate, pH 7·7, and dialyzed against the same buffer.

DEAE-cellulose (Sigma, 0·92 mEq/g, coarse mesh) was washed three times with 0·1 N NaOH, three times with 0·1 N HCl, and extensively with distilled water. A column (2 × 27 cm) was prepared and washed extensively with 5 mM sodium phosphate, pH 7·7. Approximately 3 ml (10–15 mg protein) of the dialyzed calcium phosphate gel extract was placed on the column at room temperature. Enzyme activity was eluted from the column with 5 mM sodium phosphate, pH 7·7, just behind the breakthrough peak. Fractions containing the highest specific activity were pooled and used routinely for the studies outlined below. Enzyme activity would be retained at nearly the original level for at least 6 weeks when the enzyme was kept at 4°. The enzyme activity was quite stable over a pH range of 6·0 to 8·0, although storage at pH 6·0 resulted in best retention of activity. Freezing and thawing destroyed activity.

RESULTS

Purification of aldehyde reductase. With *p*-nitrobenzaldehyde as substrate, 95 per cent of the aldehyde reductase activity was found in the 17,000 *g* supernatant, with only negligible amounts in the mitochondria and microsomes. This is in agreement with results obtained for the enzymes isolated from brain and kidney cortex preparations.

The 17,000 *g* supernatant was capable of reducing a number of aldehydes in the presence of either NADPH or NADH. The ability to utilize NADH as a cofactor was lost in the ammonium sulfate precipitation which followed. Much of the NADPH-utilizing activity was recovered in the 40–55% ammonium sulfate fraction. Table 1 outlines a typical purification of aldehyde reductase. An 800-fold purification was obtained with a yield of about 3 per cent. This level of purification was routinely reached in each batch of enzyme prepared. During the course of purification, aldehyde dehydrogenase and alcohol dehydrogenase activities were monitored. After the calcium phosphate gel extraction procedure, these activities could no longer be detected. Since ethanol-oxidizing capacity can be separated from aldehyde-reducing activity, the point is made that the aldehyde reductase which we have succeeded in purifying is not a "classical" alcohol dehydrogenase.

In the course of preliminary experiments, utilizing enzyme prepared on calcium phosphate gel–cellulose columns prepared according to the method of Masahiko and Hamada [19], it was observed that the Lineweaver–Burk kinetic plots obtained were biphasic (Fig. 1). This situation can result from either the presence of more than one enzyme performing the same function and acting on the same range of substrates [20] or the presence of nonequivalent active sites on a single multivalent enzyme [21]. From the data reported below, the former alternative, the presence of two forms of aldehyde reductase, appears to be the case. The enzymes will be referred to as "high K_m" and "low K_m" based on their apparent Michaelis constants. Efforts were made to separate and purify

Table 1. Purification of aldehyde reductase*

Fraction	Total protein (mg)	Specific activity (nmoles NADPH oxidized/min/mg protein)	Total activity (nmoles NADPH oxidized/min)	Fold purification	Recovery (%)
17,000 *g* Supernatant	34,300	2·1	72,000		100
Ammonium sulfate precipitate	2220	12·8	28,400	6·1	39·5
Calcium phosphate gel extract	23·4	190	4450	90·5	6·2
DEAE-cellulose column	1·3	1750	2170	835	3·0

* Aliquots of the various fractions were assayed by monitoring the decrease in absorbance at 340 nm in a reaction mixture containing 0·16 mM NADPH, 0·5 mM *p*-nitrobenzaldehyde and 90 mM sodium phosphate, pH 7·4, in a total volume of 2·0 ml.

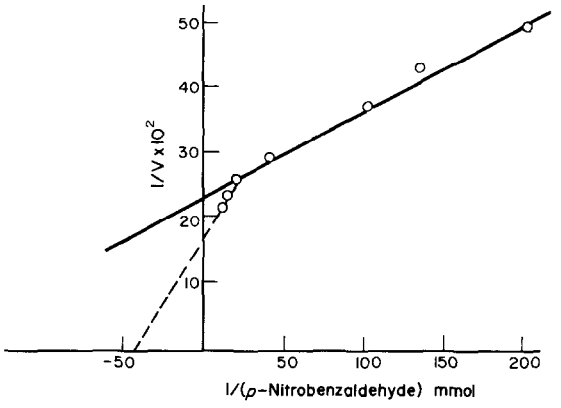

Fig. 1. Illustration of the biphasic nature of the conventional Lineweaver–Burk double-reciprocal plot in a 14-fold purified preparation of aldehyde reductase. Conditions were: 0·16 mM NADPH, 0·2 mg protein, 90 mM sodium phosphate, pH 7·4, varying the concentration of ρ-nitrobenzaldehyde. The ordinate gives the reciprocal of the velocity (nmoles NADPH oxidized/min/mg of protein).

the enzymes, but to date only the high K_m enzyme has been successfully purified (Table 1); the low K_m enzyme appears to be quite labile and has evaded purification. Efforts are continuing to purify the low K_m enzyme, and the properties of this enzyme will hopefully be the subject of a forthcoming communication. The highly purified enzyme from the DEAE-cellulose step was utilized in generating all data concerning the high K_m enzyme. Unless specifically indicated, the discussion which follows concerns the high K_m enzyme.

Substrate specificity. Aldehyde reductase was seen to catalyze the reduction of a large number of aromatic and medium chain length aliphatic aldehydes in the presence of NADPH (Table 2). Benzaldehydes with substituents which could induce a partial positive charge on the carbonyl carbon were the most active substrates. The reaction when an equimolar concentration of NADH was substituted for NADPH proceeded at no more than 1 per cent of the rate observed with NADPH. It is interesting to note that the maximal velocities reported for hexanal and dodecanal, while low, occurred at concentrations of 7·5 and 5·0 μM respectively. Accurate determinations of the K_m values of these compounds were not possible due to limited rates of reaction and substrate inhibition at concentrations exceeding 10 μM. Several ketones including acetophenone, ρ-nitroacetophenone, propiophenone and cyclohexanone were tested as substrates. No reduction was observed with either NADPH or NADH as cofactor.

The kinetic constants for the high K_m enzyme were calculated from the usual Lineweaver–Burk double-reciprocal plots (Fig. 2). As can be seen, no curvature is apparent, indicating that the low K_m enzyme activity has been removed (compare to Fig. 1). During the course of purification, it was found that high and low K_m enzyme activities were separated after the calcium phosphate gel extraction procedure. The Michaelis constants reported for the low K_m enzyme have been estimated from the curved plots obtained from crude preparations. It is not possible to extract the kinetic constants from these plots directly, since the curve obtained is a mixture (sums and products) of the K_m values and maximal velocities of the two enzymes [20,21]. We have made the assumption that at low substrate concentrations the contribution of the high K_m enzyme to the total activity observed is minimal. As can be seen from Fig. 1, at low substrate concentrations the curve becomes approximately linear so that the slope of the asymptote, when extrapolated back to the vertical axis, may be used to approximate the apparent K_m for the low K_m enzyme. The K_m values estimated in this manner cannot, of course, be considered to be exact, but from an order of magnitude consideration, at least, they can be used as reasonable indicators of the affinity of the enzyme for its substrate. The data of Table 2 then indicate that aldehyde reductase exists in at least two forms which have significantly different affinities for aldehyde substrates. V_{max} values have been corrected for an average specific activity of 500 nmoles NADPH oxidized/min/mg of protein with ρ-nitrobenzaldehyde as substrate.

pH optimum. As shown in Fig. 3, the pH optimum for the reaction of aldehyde reductase with ρ-nitrobenzaldehyde as substrate occurred at 6·4. At pH 7·4, the reaction proceeded at about 90 per cent of the rate observed when run at 6·4. Kinetics were run at pH 7·4, since this approximates the pH the enzyme would presumably encounter in cytoplasm *in vivo*. The reverse reaction with ρ-hydroxybenzyl alcohol as substrate and 0·16 mM NADP had a pH optimum at 9·6. The reverse reaction at optimal pH proceeded at less than 10 per cent of the rate observed for the forward reaction. The K_m of ρ-hydroxybenzyl alcohol in the reverse reaction was determined to be 5 mM at pH 9·6 in the presence of 0·16 mM NADP. No activity was observed when an equimolar amount of NAD was substituted for NADP. The K_m for NADP in the reverse reaction with 2·5 mM ρ-hydroxybenzyl alcohol as substrate could not be accurately determined because of the poor activity. It was estimated to be of the order of 75–100 μM. Thus it appears that the enzyme functions as an aldehyde reductase rather than an alcohol dehydrogenase.

Estimation of molecular weight. The molecular weight of aldehyde reductase was estimated to be 29,900 by gel filtration on Sephadex G-75. The enzyme activity was eluted in a single symmetrical peak from the column. Crude preparations gave essentially the same results, indicating that both forms of aldehyde reductase have similar elution characteristics on gel filtration. Storing the enzyme at 4° for up to 6 weeks did not change the position of elution of NADPH-linked aldehyde reductase activity. The estimate of the molecular weight is in good agreement with the molecular weights of the pig brain [22] and kidney enzymes [7], although Ris and von Wartburg [12] and Bronaugh and Erwin [11] have reported somewhat higher values for human and monkey brain aldehyde reductases respectively.

Effect of inhibitors. Table 3 lists the effects of some inhibitors on the reaction of aldehyde reductase with ρ-nitrobenzaldehyde and NADPH. Pyrazole, a very potent inhibitor of alcohol dehydrogenase, inhibited aldehyde reductase only 20 per cent at a concentration of 1 mM. The reductases isolated from pig and

Table 2. Michaelis constants and maximal velocities for various substrates*

Substrate	High K_m ($\times 10^5$ M)	High V_m (nmoles NADPH oxidized/min/mg protein)	Low K_m ($\times 10^5$ M)
3.4-Dihydroxyphenyl-glycolaldehyde	52·8	405	
4-Hydroxy-3-methoxy-phenylglycolaldehyde	50·0	340	
4-Hydroxyphenylglycol-aldehyde	2·9	450	
3.4-Dihydroxyphenyl-acetaldehyde	25·0	118	
4-Hydroxyphenylacet-aldehyde		40	
p-Carboxybenzaldehyde	1·9	473	0·14
p-Cyanobenzaldehyde	2·1	680	
p-Nitrobenzaldehyde	10·3	672	5·6
m-Nitrobenzaldehyde	120·0	375	3·4
p-Chlorobenzaldehyde	357·0	394	
m-Chlorobenzaldehyde	38·2	415	
p-Tolualdehyde	257·0	85·5	
2-Pyridine-carboxaldehyde	1580·0	345	5·1
3-Pyridine-carboxaldehyde	58·0	440	1·3
4-Pyridine-carboxaldehyde	46·1	555	10·0
m-Anisaldehyde	667·0	136	
p-Anisaldehyde		25·2	
Octanal	20·5	125	
Decanal	47·0	210	
Hexanal		19	
Dodecanal		43	
Palmitaldehyde		0	
Phenylacetaldehyde		0	
Acetaldehyde		24	
Cyclohexanone		0	
Indole-3-aldehyde		0	
NADPH	1·3		0·5

* Kinetic constants were determined by conventional Lineweaver–Burk double-reciprocal plots. Various concentrations of substrates were added to a reaction mixture containing 0·16 mM NADPH, enzyme and 90 mM sodium phosphate, pH 7·4, in a total volume of 2·0 ml. For the determination of K_m for NADPH, the reaction mixture contained 0·5 mM *p*-nitrobenzaldehyde while the concentration of NADPH was varied. V_{max} values have been corrected for an average sp. act. of 500 nmoles NADPH oxidized/min/mg of protein with 0·5 mM *p*-nitrobenzaldehyde as substrate.

bovine brain were not inhibited at all by concentrations of pyrazole as high as 10 mM [9,23]. The enzymes purified from human and rat brain were inhibited to about the same extent as reported here [12].

Chlorpromazine has been shown to be an efficient inhibitor of aldehyde reductases from bovine [9,22], pig [10] and human and rat [12] brain. The aldehyde reductase of sheep heart reported here was not appreciably inhibited by 0·1 mM chlorpromazine. Chlorpromazine free-radicals were generated by u.v. irradiation according to the method of Akera and Brody [24]. The inhibition of enzymatic activity was not enhanced by this procedure. Propranolol was shown to inhibit both alcohol and aldehyde dehydrogenases [25]. No inhibition was observed in the course of these experiments, even at concentrations of 1 mM.

All of the diuretics tested including furosemide, ethacrynic acid and the benzothiadiazines were effective in inhibiting aldehyde reductase. The effects of these drugs on the enzymatic activity of brain and kidney preparations have not been reported. Another interesting observation is that of all the anti-arrhythmial

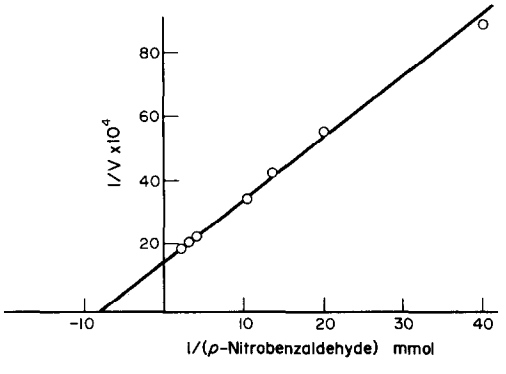

Fig. 2. Illustration of the Lineweaver–Burk double-reciprocal plot for *p*-nitrobenzaldehyde in a purified preparation of aldehyde reductase from DEAE-cellulose chromatography. The plot is linear, indicating the presence of a single isozyme. The conditions are the same as listed in the legend of Fig. 1.

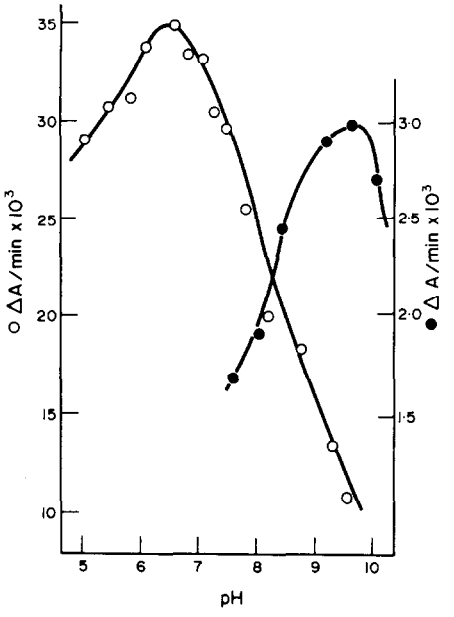

Fig. 3. pH optimum for the reaction of aldehyde reductase (○). Conditions were: 0·16 mM NADPH, 0·5 mM *p*-nitrobenzaldehyde and enzyme (17 μg) in a total volume of 2·0 ml. Sodium phosphate (90 mM) was used for the pH range from 5·0 to 8·2. Sodium pyrrophosphate (90 mM) was used for the pH range above 8·2. pH curve for the reverse reaction utilizing 0·16 mM NADP and 2·5 mM *p*-hydroxybenzyl alcohol (●).

agents tested (procainamide, quinidine, lidocaine and diphenylhydantoin) diphenylhydantoin was by far the most effective inhibitor of aldehyde reductase. The level of inhibition (77 per cent at 10 μM) was the highest observed of all compounds tested. Diphenylhydantoin, along with other anticonvulsant compounds, has been shown to inhibit bovine brain aldehyde reductase [26]. The concentration of diphenylhydantoin required to inhibit brain aldehyde reductase was some 100 times higher than that which yielded a similar level of inhibition of the heart enzyme activity; 40 per cent inhibition at 100 μM for the brain enzyme vs 40 per cent inhibition at only 1 μM for the heart enzyme (not shown in Table 3).

As with the aldehyde reductases mentioned above, the barbiturates were very potent inhibitors of heart aldehyde reductase. The only exceptions have been an NADH-linked reductase from bovine brain [27] and two of the reductases isolated from human brain [12], which have been shown to be barbiturate insensitive.

Disulfiram, a potent inhibitor of aldehyde dehydrogenase, was effective in inhibiting heart aldehyde reductase. This may be due to the presence of a necessary sulfhydryl group on the enzyme [28], since *p*-hydroxymercuribenzoate (PMB) was also efficient in inhibiting aldehyde reductase. The inhibition of enzyme activity by PMB could be prevented by prior incubation with NADPH or reduced glutathione, and partially reversed by the addition of excess reduced glutathione. Prior incubation with aldehyde was not effective in preventing PMB inhibition. This implies that a free sulfhydryl group is necessary for activity, most probably at the binding site of the pyridine nucleotide. Brain enzyme preparations have been reported to be insensitive to inhibition by disulfiram [10,12].

DISCUSSION

The evidence presented here suggests that sheep heart contains an NADPH-linked aldehyde reductase which is present in at least two forms. The enzymes are rather nonspecific, catalyzing the reduction of a number of aromatic and aliphatic aldehydes. Heart aldehyde reductase catalyzed the reduction of 3,4-di-

Table 3. Effect of inhibitors on heart aldehyde reductase*

Inhibitor	Inhibitor concentration (mM)		
	1·0 (% inhibition)	0·1 (% inhibition)	0·01 (% inhibition)
Chlorpromazine		13·5	6·0
Disulfiram		58·8	57·8
Pyrazole	21·6	8·0	1·5
Procainamide	14·5	10·0	1·5
Propranolol		12·0	2·5
Quinidine		14·5	14·5
Diphenylhydantoin		100·0	77·2
Guanethedine	20·5	17·0	13·5
Hydralazine		18·7	5·7
Probenecid	96·7	46·5	26·0
Ethacrynic acid	100·0	72·0	23·5
Furosemide		64·0	25·0
Chlorothiazide	100·0	63·0	19·5
Hydrochlorothiazide		21·0	10·5
Benzthiazide		100·0	52·0
Amobarbital	100·0	63·2	15·5

* Various inhibitors were added to a reaction mixture consisting of 0·16 mM NADPH, 0·5 mM *p*-nitrobenzaldehyde, enzyme, and 90 mM sodium phosphate, pH 7·4, immediately before the reaction was started by the addition of enzyme. The total volume of the reaction mixture was 2·0 ml. Inhibitor concentrations reported in the table are the final concentrations.

hydroxyphenylglycolaldehyde, 4-hydroxyphenylglycolaldehyde and 4-hydroxy-3-methoxyphenylglycolaldehyde at greater rates than 3,4-dihydroxyphenylacetaldehyde and 4-hydroxyphenylacetaldehyde. Thus, it has been shown that the aldehydes derived from β-hydroxylated phenylethylamines are better substrates for aldehyde reductase than are those which lack the β-hydroxyl group. This is in good agreement with results reported for partially purified brain preparations [9,11,23,29].

Bosron and Prarie [7] have suggested that aldehyde reductase activity isolated from several tissues and species may be attributable to a single enzyme. This speculation, while a desirable one, is difficult to support since several differences in the various enzymes can be seen. Most noticeable is the observation that aldehyde reductase from kidney cortex [6,7] and from erythrocytes and liver [8] are capable of reducing ketones, while brain [9–12] and heart preparations utilize ketones as substrates only poorly, if at all. The enzymes from pig and ox brain [10], human and rat brain [12], as well as the heart preparation reported here, have been reported to exist in multiple forms. No such claim has been made for the kidney cortex enzymes. The molecular weights of the enzymes have ranged from 29,000 [10] to as high as 70,000 [11], thus the possibility of active subunit aggregation cannot be completely obviated.

Erwin *et al.* [30] and Erwin and Dietrich [26] have postulated that the pharmacological action of anticonvulsant compounds including diphenylhydantoin and the central nervous system depressant effects of the barbiturates may be mediated, in part, by an increase in the steady state levels of brain biogenic aldehydes due to the inhibition of aldehyde reductase. In the present study it has been demonstrated that the benzothiadiazines, which are effective antihypertensive agents, are inhibitors of aldehyde reductase, as is the potent anti-arrhythmic agent, diphenylhydantoin. It is tempting to speculate that some of the pharmacological activity of these agents on the cardiovascular system may be the result of a shift in amine metabolism brought about by the inhibition of aldehyde reductase.

The properties of the partially purified enzyme in this study differ significantly from those of alcohol dehydrogenase. Alcohol dehydrogenases generally utilize NADH more efficiently than NADPH and are active with short and medium chain aliphatic aldehydes. Aldehyde reductase from heart is unable to utilize NAD or NADH to any appreciable extent and is inactive with short chain aldehydes. Pyrazole sensitivity is a characteristic of alcohol dehydrogenases; aldehyde reductases isolated from a number of species and tissues have all been shown to be remarkably pyrazole insensitive. Barbiturates inhibit reductase, but alcohol dehydrogenase resists inhibition by these compounds. These data are consistent with the conclusion that a probable physiological role of aldehyde reductase in heart is the reduction of β-hydroxylated biogenic amines to their glycolic metabolites.

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