MOLECULAR PHARMACOLOGY, 9, 428–437

Enzymatic Reduction of "Biogenic" Aldehydes in Brain

BORIS TABAKOFF, ROBERT ANDERSON, AND SPYRIDON G. A. ALIVISATOS

Department of Biochemistry, The Chicago Medical School, Chicago, Illinois 60612

(Received January 12, 1973)

SUMMARY

TABAKOFF, BORIS, ANDERSON, ROBERT, AND ALIVISATOS, SPYRIDON, G. A.: Enzymatic reduction of "biogenic" aldehydes in brain. *Mol. Pharmacol.* 9, 428–437 (1973).

Aldehyde derivatives of the biogenic amines (e.g., serotonin, dopamine) were prepared enzymatically, purified, and used as substrates for aldehyde reductase (EC 1.1.1.2), an enzyme capable of reducing these deaminated derivatives. The enzyme was partially purified from rat brain tissue. Aldehyde reductase derived from brain tissue differed from alcohol dehydrogenase (EC 1.1.1.1) in cofactor specificity and was inhibited by low concentrations of barbiturates (i.e., pentobarbital). The presence of various functional groups on the parent phenylacetaldehyde or indoleacetaldehyde structures was shown to significantly affect the Michaelis constants (K_m, V_{max}) for aldehyde reductase. The relationship between Michaelis constants determined for aldehyde reductase and the final product of biogenic amine metabolism in rat brain is discussed.

INTRODUCTION

Biogenic amines, such as dopamine or serotonin, are deaminated by monoamine oxidase (EC 1.4.3.4) to their aldehyde analogues (1). The aldehydes may be further metabolized in the central nervous system to their acid (2) or alcohol (3) excretion products. Previous investigators have shown that certain biogenic amines are metabolized primarily to either their acid or alcohol derivatives (Table 1). The enzymes responsible for transforming the aldehydes to the corresponding acids are the NAD^+-dependent aldehyde dehydrogenases (7). The enzymes catalyzing the production of the alcohol derivatives of the "biogenic" aldehydes in the central nervous system, on the other hand, have not been identified thus far. Although an enzyme resembling liver alcohol dehydrogenase with respect to cofactor requirement (NAD^+) and inhibitor (pyrazole) sensitivity has been noted in brain tissue (8), the activity of this alcohol dehydrogenase was shown to be quite low (9). Previous reports also indicate that the production of the reduced metabolites of certain amines in brain tissue depends on the presence of NADPH rather than NADH (4, 10). Recently Tabakoff and Erwin (11) reported the presence of an NADPH-dependent, aldehyde-reducing enzyme in brain tissue which was capable of converting aromatic and aliphatic aldehydes to their corresponding alcohol derivatives. It was therefore of interest to examine the substrate specificity of this enzyme by utilizing the aldehyde derivatives of certain biogenic amines.

This work was partly supported by grants from the National Institute of Mental Health (MH-20758 and MH15410), the National Science Foundation (GB-30295), the State of Illinois Department of Mental Health (101-13-RD), William S. Deree and Licensed Beverage Industries, Inc.

MATERIALS AND METHODS

Materials. All chemicals were of the highest commercially available quality. Methanol (histological grade, Fisher) was refluxed

TABLE 1

Deaminated final products of various amines found in rat brain, expressed as percentage of deaminated amines

Amine	Acid product	Neutral products, presumably alcohols
	%	%
Serotonin[a]	27–30	14
Tyramine[b]	75	5
Octopamine[b]	6	78
Dopamine[c]	90	1
3-Methoxytyramine[c]	78	22
Normetanephrine[c]	8	88

[a] Values derived from data of Feldstein and Williamson (4).

[b] Values derived from data of Breese *et al.* (5).

[c] Values derived from data of Breese *et al.* (6).

with 2,4-dinitrophenylhydrazine (Matheson, Coleman, and Bell) and distilled before use. Phenylacetaldehyde and *p*-nitrobenzaldehyde were purchased from Aldrich Chemical Company. Indoleacetaldehyde sodium bisulfite, the various salts of the "biogenic" amines, and the pyridine nucleotide cofactors were supplied by Sigma Chemical Company. Radioactive compounds were obtained from New England Nuclear Corporation.

Preparation of liver enzymes. Male Sprague-Dawley rats were decapitated, and their livers were quickly removed and placed in chilled 0.25 M sucrose. All procedures for the preparation of enzymes were performed in the cold (0–4°).

Monoamine oxidase was prepared from rat liver mitochondria which were isolated by the method of Whittaker (12). The mitochondrial pellet was washed twice with 0.32 M sucrose and then suspended in 0.01 M sodium phosphate (pH 8.0). The mitochondria were sonically disrupted using a Branson Sonifier. Six sonications of 1 min each, at a setting of 4 on the Sonifier, were performed at 2-min intervals while the suspension was immersed in an acetone-ice bath. The resultant suspension was centrifuged at 126,000 × *g* for 60 min. The pellet was resuspended in 0.01 M sodium phosphate (pH 8.0) and used for determination of monoamine oxidase activity. The presence of monoamine oxidase activity was determined using a modified spectrophotometric assay as described by Tabakoff and Alivisatos (13).

Rat liver mitochondrial aldehyde dehydrogenase was also obtained from mitochondria. After sonication and centrifugation at 126,000 × *g*, as described above, the supernatant fluid was recovered, dialyzed against 0.025 M sodium phosphate (pH 7.0) containing 0.05 mM EDTA and 0.5 mM mercaptoethanol, and used as crude aldehyde dehydrogenase. The presence of aldehyde dehydrogenase was demonstrated by monitoring the conversion of added NAD^+ to NADH, spectrophotometrically at 340 nm, in the presence of propionaldehyde as the substrate. Diamine oxidase was obtained from rat liver microsomes (14). Microsomes were isolated from the supernatant fluid after the removal of cellular debris. nuclei, and mitochondria (12). The supernatant fluid was centrifuged at 126,000 × *g* for 90 min, the resultant microsomal pellet was suspended in 0.32 M sucrose, and this material was used for determination of diamine oxidase activity.

Preparation of aldehydes. Free indoleacetaldehyde was prepared from indolacetaldehyde sodium bisulfite in aqueous solution as described previously (15).

Other aldehydes listed in Table 2, with the exclusion of 3,4,5-trimethoxyphenylacetaldehyde, were prepared by incubation of the appropriate amine (1 mM) with rat liver monoamine oxidase prepared as described above. In certain instances ^{14}C- or ^{3}H-labeled amines of previously determined specific activity were utilized in the incubation mixtures. The incubation mixtures consisted of an amine, monoamine oxidase (50–100 mg of protein), and 0.05 M sodium phosphate (pH 8.0) in a total volume of 50 ml. Sodium phosphate (0.05 M, pH 7.4) was utilized for incubation mixtures containing dihydroxyphenylethylamine. The aldehyde derivative of mescaline, 3,4,5-trimethoxyphenylacetaldehyde, was prepared by incubation of mescaline (1 mM) with rat liver microsomes (see above), in a solution of sodium phosphate (0.01 M, pH 8.2). Incubations were performed at 30°. The length of incubation (45–120 min) was adjusted for maximum recov-

ery of aldehyde (see RESULTS). The reactions were terminated by the addition of 3 N HCl in ice. The major portion of precipitated protein was removed by centrifugation at 15,000 × *g* for 20 min, and the remainder of the protein was removed by filtration of the supernatant fluid through an Amicon UM-10 porosity filter under nitrogen. The filtrate was collected, mixed with an equal volume of ether, and further extracted three times with equal volumes of ether. The pooled ether fraction was washed in succession with 0.1 N HCl, 0.1 M sodium phosphate (pH 7.4), and water, The ether, which contained the aldehyde, was evaporated over water under reduced pressure (5 mm Hg), and the resultant water solution of the aldehyde was purged with nitrogen, filtered, and used immediately.

Identification and determination of aldehydes. The concentration of aldehyde in solution was determined by reaction with 2,4-dinitrophenylhydrazine as described by Lappin and Clark (16), using an extinction coefficient of 27,200 at 480 nm for the hydrazine derivatives. Extinction coefficients were also determined by the use of *p*-nitrobenzaldehyde and phenylacetaldehyde as standards (see Table 3). The concentration of aldehyde in aqueous solutions was also determined by incubating an aliquot of the solution with rat liver mitochondrial aldehyde dehydrogenase, NAD^+ (1 mM), and sodium bicarbonate (0.1 M, pH 9.2). The total aldehyde-dependent conversion of NAD^+ to NADH was determined spectrophotometrically. It was assumed that this conversion was quantitative and was taken to equal the amount of aldehyde added to the incubation mixture (see Table 3). When the incubation mixtures contained ^{14}C- or ^{3}H-labeled amines of known specific activity, the concentration of aldehyde recovered in the ether extract and final water solution was estimated by liquid scintillation counting. The radioactive and nonradioactive aldehydes were chromatographed on silica gel plates (Merck, Darmstadt), using *sec*-butyl alcohol–formic acid–water (15:3:2) as a solvent system. The parent amines were chromatographed in a similar system. After chromatography the plates on which radioactive materials were applied were examined under an ultraviolet lamp (265 nm) and areas of absorption or fluorescence were noted. The plates were divided into rectangular sections, each section was scraped into scintillation vials, Triton X-100-containing fluor (17) was added, and the radioactivity was determined by liquid scintillation counting. In other cases both aldehyde and amine were spotted in duplicate on the same silica gel plate. After chromatography the plate was separated into two sections. Each section contained one of the chromatographed amines and its corresponding aldehydes. One section was sprayed with 2,4-dinitrophenylhydrazine (1%) in methanol, followed by 10% potassium hydroxide in methanol–water (4:1), while the other half of the plate was sprayed with diazotized sulfanilic acid reagent (18).

Preparation of aldehyde reductase from rat brain. Rat brain tissue (10%, w/v) was homogenized in 0.32 M sucrose containing 5 mM sodium phosphate (pH 7.4) and 0.05 mM EDTA. The homogenate was centrifuged at 1000 × *g* for 15 min, and the supernatant fluid was recentrifuged at 27,000 × *g* for 30 min.

Ammonium sulfate (27.7 g/100 ml) was slowly added to the supernatant fluid with stirring, and the suspension was stirred for an additional 30 min. Precipitated protein was removed by centrifugation at 27,000 × *g* for 30 min. The supernatant fluid was recovered, and ammonium sulfate (9.9 g/100 ml) was added as above. After centrifugation the precipitated protein was resuspended in 0.025 M sodium phosphate buffer (pH 7.0) containing 0.05 mM EDTA and 0.5 mM mercaptoethanol. This suspension was dialyzed against 100–200 volumes of 0.025 M sodium phosphate (pH 7.0) containing 0.05 mM EDTA and 0.5 mM mercaptoethanol for 20 hr, with the dialysis medium being changed once during this period. After dialysis,[1] undissolved protein was removed by centrifugation at 126,000 × *g* for 90 min, and an aliquot of the ammonium sulfate fraction was assayed for the presence of aldehyde reductase activity by methods described below. This fraction, which will be referred to

[1] The concentration of ammonium sulfate in the preparations after dialysis was determined to be below 10 nM, using 1 M barium chloride as a titrant.

as the 45–60% ammonium sulfate fraction, was used for the determination of Michaelis and inhibitor constants and pH optima. Protein concentrations were determined by the procedure of Lowry *et al.* (19).

Assay procedures. The assay mixture for monitoring aldehyde reductase activity (standard assay) consisted of sodium phosphate (0.1 M, pH 7.0), NADPH (170 μM), and enzyme protein (0.1–1 mg), with *p*-nitrobenzaldehyde (600 μM) as substrate, in a total volume of 1 ml. Utilization of NADPH was monitored at 340 nm at ambient temperature (approximately 23°) in a Gilford model 2400 recording spectrophotometer.[2]

The optimal pH for aldehyde reductase activity was determined utilizing 0.05 M sodium phosphate to obtain pH values from 5.5 to 8.0. Sodium pyrophosphate (0.05 M) was utilized for pH values of 8.0–9.0, and sodium bicarbonate (0.05 M) was used for pH values of 9.0 and above.

Michaelis constants (K_m) for NADPH and aldehyde reductase were determined in mixtures containing 0.05 M sodium phosphate (pH 7.0), using *p*-nitrobenzaldehyde (600 μM) as the substrate.

Values of K_m and V_{max} for the various aldehydes and inhibitor constants for pentobarbital were determined in mixtures containing 0.1–0.3 mg of enzyme protein, 170 μM NADPH, and 0.1 M sodium phosphate (pH 7.0). At least five or six different concentrations were used for each determination of K_m, and the concentration of aldehyde substrate was varied within approximately a 10–15-fold range. The data were plotted graphically in double-reciprocal form as described by Lineweaver and Burk (20). The line of best fit was determined by the least-squares method, and correlation analysis was performed with the aid of a programmed Olivetti 101 calculator. Each kinetic constant was determined with at least two separate preparations of the enzyme, and the reported values are means of two to four determinations. The reported V_{max} values were scaled to compensate for variation in activity of different preparations of the enzymes. The scaling was accomplished by multiplying the observed V_{max} by a value (Z).[3] The coefficients of determination (r^2) (21) for each plot of the reported kinetic data, with the exception of Michaelis constants derived for 3,4-dihydroxyphenylacetaldehyde (see DISCUSSION), were determined to be 0.95 or greater (Fig. 1).

RESULTS

Table 2 lists the R_F values for the amines and the corresponding aldehydes used in these studies. The aldehydes were usually visualized either under an ultraviolet lamp or after spraying with 2,4-dinitrophenylhydrazine, in which case a reddish brown spot was noted. The corresponding amines did not exhibit a similar color reaction. However, the amines did react, producing a pink or red spot, upon spraying of the plate with diazotized sulfanilic acid. Aldehydes, whose structures contain phenolic groups, were also visualized as pink spots after treatment of the plate with diazotized sulfanilic acid. One could thus ascertain the position of the aldehyde and, in an adjacent system, ascertain the relative mobility of the amine. Although in two instances (3,4,5-trimethoxyphenylacetaldehyde and 4-hydroxyphenylglycolaldehyde) other minor spots were visualized after chromatography of the aldehyde preparations, the other aldehydes migrated as a single spot. A major and a minor spot also appeared when the commercial phenylacetaldehyde was chromatographed. The R_F values reported in Table 2 are those of the major spots reacted with 2,4-dinitrophenylhydrazine. When radioactive amines were utilized for biosynthesis of the aldehydes, the major portion of radioactivity was recovered from an area with an R_F value similar to that in which the material reacting with dinitrophenylhydrazine was found. In most instances the recovery of the aldehyde derivatives was low, ranging from 0.5 to

[2] We would like to thank Dr. Paul Gordon, of the Department of Pharmacology of The Chicago Medical School, for the use of the Gilford model 2400 spectrophotometer. The recording was performed routinely on a 0.1 optical density full-scale expansion.

[3] Z is a factor by which the activity of the various preparations would be adjusted to bring them to a common specific activity of 20 nmoles of cofactor utilized per minute per milligram of protein in a standard assay system with *p*-nitrobenzaldehyde as the substrate.

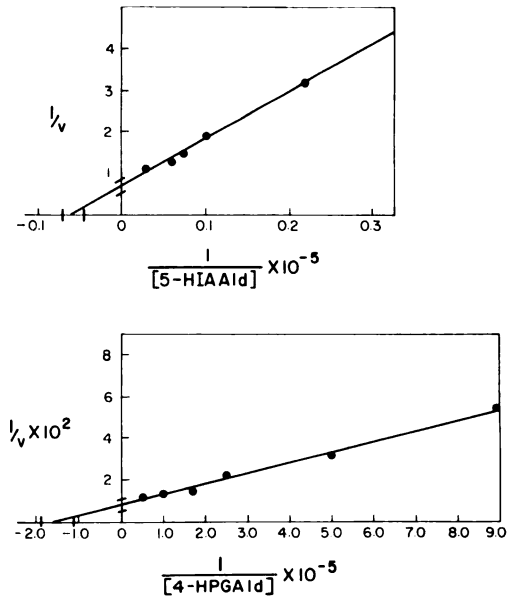

FIG. 1. *Graphical representation of kinetic data with statistically derived limits for Michaelis constants*

5-Hydroxyindoleacetaldehyde (5-HIAAld) and 4-hydroxyphenylglycolaldehyde (4-HPGAld) were the variable substrates, and assays for activity were performed as described in the text. Several similar determinations were used to obtain the values in Table 4. The coefficients of determination for the regression line data obtained with aldehyde reductase and 5-hydroxyindoleacetaldehyde were calculated to be $r^2 = 0.980$ and $r^2 = 0.960$ for 4-hydroxyphenylglycolaldehyde. The cross-hatching along the ordinate and abscissa on either side of each intercept indicates the upper and lower limits of the derived Michaelis constants at the 90% confidence limit ($p = 0.1$). The calculated V_{max} values were scaled to compensate for the fluctuation in enzyme activities as described in the text.

5.0%. However, sufficient quantities were recovered for the determination of kinetic constants. In Table 3 a comparison among three methods for determining the concentration of aldehyde recovered by our procedure reveals that the colorimetric estimate of the amount of carbonyl groups present in a solution containing 3,4-dihydroxyphenylacetaldehyde was appreciably higher than the estimate of reactive aldehyde groups obtained by using the enzymatic assay. In the other cases examined the two types of assays gave similar results. Aldehyde concentration was therefore routinely estimated utilizing the liver aldehyde dehydrogenase assay.

The major portion of aldehyde-reducing capacity utilizing NADPH as a cofactor in brain has been previously shown (11) to be recovered in the supernatant fluid after centrifugation of homogenates at 27,000 × *g*. In the present study, although oxidation of NADPH was evident in the presence of the 27,000 × *g* supernatant fluid when *p*-nitrobenzaldehyde or 5-hydroxyindoleacetaldehyde was the substrate, no oxidation of NADH above that of control mixtures containing no substrate was demonstrable with either of these aldehydes. The total NADPH-oxidizing capacity of the 27,000 × *g* supernatant fluid from rat brain was calculated to be 109 nmoles oxidized per minute per gram of brain with *p*-nitrobenzaldehyde as the substrate. The major portion of the NADPH-utilizing enzyme (aldehyde reductase) activity was recovered with protein precipitating between 45% and 60% saturation of the supernatant fluid with ammonium sulfate. This activity accounted for approximately 35–44% of the activity in the 27,000 × *g* supernatant fluid, using *p*-nitrobenzaldehyde as substrate. Oxidation of NADH was evident after ammonium sulfate fractionation in the presence of *p*-nitrobenzaldehyde and 5-hydroxyindoleacetaldehyde. However, the major portion of this activity appeared in the protein which remained in solution after 60% saturation with ammonium sulfate. Utilization of NADH with 5-hydroxyindoleacetaldehyde or *p*-nitrobenzaldehyde as substrate with protein precipitating between 45% and 60% saturation with ammonium sulfate was approximately 8% of the rates obtained with NADPH as the cofactor.

The pH optimum for aldehyde reductase activity was determined to be 6.8 with *p*-nitrobenzaldehyde as the substrate.

The K_m value for NADPH with aldehyde reductase was found to be 1.2 μM. Thus the K_m values for various aldehyde substrates were obtained at a cofactor concentration well above K_m values for this cofactor. The representation of kinetic data by the methods of Lineweaver and Burk (20) resulted in linear graphs for the aldehydes listed in

TABLE 2

Preparation of aldehydes; chromatography on silica gel

Amines and corresponding aldehydes, prepared by the methods described in the text, were chromatographed on silica gel plates using *sec*-butyl alcohol–formic acid–water (15:3:2) as the solvent system. Methods used for visualization and identification are listed in the text.

Amine	R_F	Aldehyde	R_F
Phenylethylamine	0.38	Phenylacetaldehyde	0.77
Tyramine	0.39	4-Hydroxyphenylacetaldehyde	0.85
Dopamine	0.36	3,4-Dihydroxyphenylacetaldehyde	0.70
3-Methoxy-4-hydroxyphenylethylamine	0.31	3-Methoxy-4-hydroxyphenylacetaldehyde	0.74
3,4-Dimethoxyphenylethylamine	0.53	3,4-Dimethoxyphenylacetaldehyde	0.79
Normetanephrine	0.22	3-Methoxy-4-hydroxyphenylglycolaldehyde	0.78
Mescaline	0.40	3,4,5-Trimethoxyphenylacetaldehyde	0.80
Octopamine	0.43	4-Hydroxyphenylglycolaldehyde	0.81
Tryptamine	0.50	Indoleacetaldehyde	0.51
Serotonin	0.36	5-Hydroxyindoleacetaldehyde	0.72

Table 4, throughout the concentration range employed.

The Michaelis constants (K_m, V_{max}) determined for aldehyde reductase were found to depend on the structure of the "biogenic" aldehyde. The presence of an α-hydroxyl group on the aldehyde-containing side chain had a profound effect in lowering the K_m value and increasing the velocity of the reduction of such aldehydes in the presence of aldehyde reductase (i.e., 4-hydroxyphenylacetaldehyde vs. 4-hydroxyphenylglycolaldehyde, Table 4). On the other hand, the rate of utilization of 3,4-dihydroxyphenylacetaldehyde was found to be quite low with aldehyde reductase (Table 4). Because of the low activity of the enzyme when 3,4-dihydroxyphenylacetaldehyde was the substrate, the reported K_m value should be considered an estimate. If a methoxy group was present at position 3 of the phenylacetaldehyde instead of a hydroxyl group, the aldehyde was found to be a good substrate for aldehyde reductase (compare 3,4-dihydroxyphenylacetaldehyde and 3-methoxy-4-hydroxyphenylacetaldehyde, Table 4). The presence of an α-hydroxyl group, as in 3-methoxy-4-hydroxyphenylglycolaldehyde, further increases the rate of reduction of this substrate by aldehyde reductase (see Table 4). The aldehyde derivative of mescaline, 3,4,5-trimethoxyphenylacetaldehyde, proved to be a substrate for aldehyde reductase derived from rat brain. Thus one might expect a certain amount of 3,4,5-trimethoxyphenylethanol to be formed in brain tissue.

Table 5 and Fig. 2 illustrate the effects of two characteristic inhibitors on the activity of the reductase derived from rat brain. The inhibition of aldehyde reductase derived from rat brain by sodium pentobarbital (Fig. 2) was similar to the inhibition demonstrated with reductase purified from bovine brain (15). The inhibitor constant (K_i) was determined to be approximately 36 μM. Pyrazole, which has been shown to be a potent inhibitor of the metabolism of either *p*-nitrobenzaldehyde or propionaldehyde, utilizing NADH or NADPH, by rat liver alcohol dehydrogenase (22, 23) and rat brain (8, 9) alcohol dehydrogenase, by forming a ternary complex with the enzyme and the NAD^+

TABLE 3

Comparison of methods used to determine aldehyde concentration

Aldehyde	Method		
	Radioisotopic[a]	Colorimetric[b]	Enzymatic[c]
	μM	μM	μM
p-Nitrobenzaldehyde[d]		16 (16)[d]	13
Phenylacetaldehyde[e]		19 (59)[e]	37
5-Hydroxyindoleacetaldehyde	38	22	19
3,4-Dihydroxyphenylacetaldehyde	130	330	150
4-Hydroxyphenylacetaldehyde	290	200	240

[a] Labeled amines of known specific activity were used for the preparation of aldehyde (see the text). The molarity of the aldehyde in the final water solution was estimated by subjecting an aliquot of the solution to scintillation counting.

[b] Colorimetric determinations of concentration were based on the absorbance at 480 nm of the hydrazone derivative (16) of the aldehyde prepared as described in the text, using E_{480} = 27,200. When the extinction coefficient (see below) calculated from known concentrations of commercial samples was used to determine concentrations, the values in parentheses were obtained.

[c] Enzymatic estimation of aldehyde concentration was performed utilizing rat liver aldehyde dehydrogenase under standard assay conditions described in the text. The total conversion of NAD^+ to NADH, measured at 340 nm in the presence of the aldehyde, was taken to equal the aldehyde concentration.

[d] Theoretical concentration by weight equals 16.5 μM. The extinction coefficient at 480 nm was calculated to be 25,600 for the hydrazone derivative.

[e] Theoretical concentration by weight equals 59 μM. The extinction coefficient at 480 nm was calculated to be 8700 (see DISCUSSION).

(24, 25), was found to have no effect on brain aldehyde reductase activity (Table 5).

DISCUSSION

The results of studies utilizing the various inhibitors (Table 5 and Fig. 2) indicate that the enzyme activity which utilizes NADPH to metabolize "biogenic" aldehyde substrates differs from the classic liver alcohol dehydrogenase activity (25, 26). Enzymatic activity similar to the type found in liver (i.e., utilizing NAD^+ and inhibited by pyrazole) has been described in rat brain tissue (27). However, the insensitivity to pyrazole of the enzyme activity we have studied, its sensitivity to inhibition by pentobarbital, and the preferential utilization of NADPH as a cofactor identify the activity as similar to aldehyde reductase (EC 1.1.1.2) (11, 28), which has been shown by Tabakoff and Erwin (11) to catalyze the conversion of aldehydes to their alcohol derivatives in bovine brain tissue. Similarly, Deitrich *et al.* (29) found that the administration of pyrazole *in vivo* to rats had no effect on brain reduction of *p*-nitrobenzaldehyde.

Since the determination of Michaelis constants depends on a knowledge of substrate concentration in the reaction mixture, our results reflect the assay system used to determine the concentration of aldehyde present (see Table 3). Although in most cases (Table 3) the various assay methods produced similar results (within the tolerance for experimental error for K_m determinations, Fig. 1), the differences between the assayed concentrations of the commercially obtained phenylacetaldehyde and the concentration calculated on the basis of weight, might reflect the presence of both impurities and nonreactive polymers, which might, in turn, affect the calculation of kinetic constants.

When dopamine was incubated with rat brain slices, Rutledge and Jonason (30) found the major product to be dihydroxyphenylacetic acid. Similarly, the predominant product of tyramine metabolism in rat brain *in vivo* was found to be the acid derivative (4-hydroxyphenylacetic acid) (5). It can be seen from Table 4 that the aldehyde derivatives of these amines (dopamine and tyramine) are poor substrates for aldehyde reductase compared to those substrates having a hydroxyl group on the α-carbon of the aliphatic side chain (4-hydroxyphenylglycolaldehyde and 3-methoxy-4-hydroxyphenylglycoaldehyde, Table 4). Thus it is of interest

TABLE 4

Effect of substrate on aldehyde reductase activity

Kinetic constants were calculated from graphic representations of data by the method of Lineweaver and Burk (20). Velocities of aldehyde utilization were determined at various concentrations of the aldehyde in assay systems described in the text. Values are the averages of two to four separate determinations.

Aldehyde	K_m (range)	V_{max} (range)[a]
	M	
Phenylacetaldehyde	8.7×10^{-4}	36.3
	($5.5\text{–}11.8 \times 10^{-4}$)	(21–41)
4-Hydroxyphenylacetaldehyde	1.3×10^{-4}	8.5
	($0.6\text{–}3.0 \times 10^{-4}$)	(3–12)
4-Hydroxyphenylglycolaldehyde	6.4×10^{-6}	142.0
	($4.7\text{–}10.1 \times 10^{-6}$)	(105–240)
3,4-Dihydroxyphenylacetaldehyde	1.8×10^{-5}	1.1
3-Methoxy-4-hydroxyphenylacetaldehyde	2.3×10^{-5}	26.0
	($2.0\text{–}2.6 \times 10^{-5}$)	(17–35)
3-Methoxy-4-hydroxyphenylglycolaldehyde	4.0×10^{-5}	98.4
	($1.6\text{–}6.5 \times 10^{-5}$)	(60–212)
3,4,5-Trimethoxyphenylacetaldehyde	2.2×10^{-5}	7.1
	($1.2\text{–}6.0 \times 10^{-5}$)	(3–18)
Indoleacetaldehyde	2.0×10^{-4}	21.5
	($0.9\text{–}2.5 \times 10^{-4}$)	(11–43)
5-Hydroxyindoleacetaldehyde	1.7×10^{-4}	7.2
	($1.5\text{–}2.2 \times 10^{-4}$)	(6–8)

[a] Velocities are expressed as nanomoles of NADPH oxidized per minute per milligram of protein. All velocities have been adjusted to a standard enzyme activity of 20 nmoles of cofactor utilized per minute per milligram, using *p*-nitrobenzaldehyde as substrate (see the text).

that the major metabolite of octopamine in rat brain (5) was found to be the alcohol derivative. Similarly, normetanephrine was metabolized primarily to its alcohol derivative in rat brain (6). The presence of a methoxy substituent in place of a hydroxyl on position 3 of the catechol also increases the reduction of the aldehyde by aldehyde reductase (compare 3,4-dihydroxyphenylacetaldehyde with 3-methoxy-4-hydroxyphenylacetaldehyde, Table 4). The substrate specificity, in terms of Michaelis constants (K_m, V_{max}) of the aldehyde reductase, might therefore be of value in predicting the metabolic route by which the deaminated derivatives of the biogenic amines will be metabolized. However, the intracellular pH and the availability of cofactors (31) would also play an important role in determining the pathway of metabolism for the "biogenic" aldehydes.

Friedhoff and Goldstein (32) and Harley-Mason (33) have postulated that a deaminated product of mescaline (i.e., the aldehyde or the alcohol derivative) may be the psychogenically active form of this hallucinogen. The capacity of aldehyde reductase to metabolize 3,4,5-trimethoxyphenylacetaldehyde might result in the production of the alcohol derivative of mescaline in brain. In addition, since the reaction catalyzed by aldehyde reductase has been shown to be reversible (11, 22), administered 3,4,5-trimethoxyphenylethanol (32) could be converted to the aldehyde derivative. Although the reported alcohol dehydrogenase in brain tissue (9, 27) would probably be capable of reducing certain "biogenic" aldehydes, its ability to utilize the "biogenic" aldehydes, particularly those possessing an α-hydroxyl

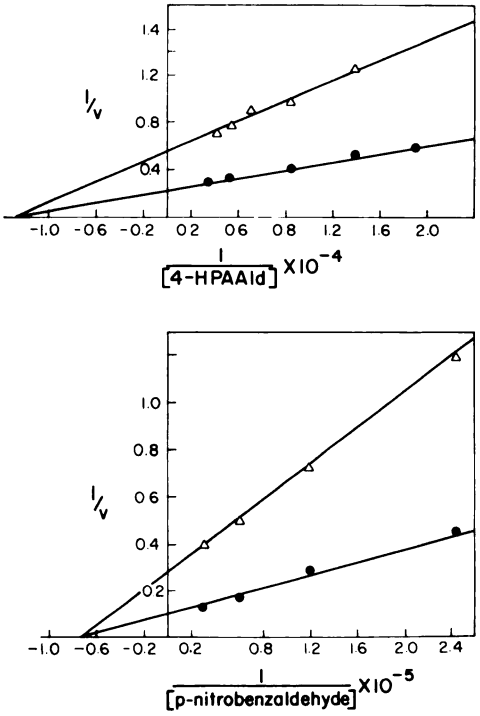

FIG. 2. *Inhibition of aldehyde reductase activity by pentobarbital*

Aldehyde reductase activity utilizing various concentrations of 4-hydroxyphenylacetaldehyde (4-HPAAld) or *p*-nitrobenzaldehyde (K_m = 16 μM) was determined as described in the text. Velocity is expressed as nanomoles of NADPH oxidized per minute per milligram of protein in the presence of substrate. In certain reaction mixtures (△) pentobarbital sodium (100 μM) was incubated with enzyme for approximately 2 min before the addition of substrate to start the reaction. Data were plotted by the method of Lineweaver and Burk (20). Noncompetitive inhibition of activity was demonstrated; K_i = 34 μM was calculated for pentobarbital when 4-hydroxyphenylacetaldehyde was the substrate, and K_i = 36 μM was determined with *p*-nitrobenzaldehyde as the substrate.

group[4] on the carbonyl-containing side chain, is yet to be demonstrated. On the other hand, the ability of aldehyde reductase to metabolize particular aldehydes is an indicator of the preferred pathways for metabolism of many biogenic amines in brain tissue.

[4] Dihydroxyphenylglycol (Regis Chemical Company) was found not to be a substrate for horse liver alcohol dehydrogenase (Sigma Chemical Company) (B. Tabakoff, R. Anderson, and C. Vugrincic, unpublished observations).

TABLE 5

Effects of pyrazole and pentobarbital on aldehyde reductase activity

Inhibition of enzyme activity was determined after incubating the inhibitor with enzyme for approximately 2 min. Aldehyde reductase activity was determined as described in the text, using *p*-nitrobenzaldehyde (600 μM) as substrate. Horse liver alcohol dehydrogenase activity was determined in reaction mixtures containing protein (0.025 mg), NADH or NADPH (160 μM), and *p*-nitrobenzaldehyde (600 μM) in 0.05 M sodium phosphate, pH 7.0.

Inhibitor	Inhibition	
	Aldehyde reductase	Horse liver alcohol dehydrogenase[a]
	%	%
Sodium pentobarbital, 1.0 mM	78[b]	1[b]
		3[c]
Pyrazole, 10 mM	0[b]	65[b]
		96[c]

[a] Horse liver alcohol dehydrogenase was purchased from Sigma Chemical Company.
[b] NADPH was used as a cofactor.
[c] NADH was used as a cofactor.

ACKNOWLEDGMENTS

The authors wish to thank Miss MaryLu Prendergast and Mr. Terry Bercovitz for their fine technical assistance.

REFERENCES

1. S. Udenfriend, E. Titus, H. Weissbach, and R. E. Peterson, *J. Biol. Chem.* **219**, 335–344 (1956).
2. H. Weissbach, B. G. Redfield, and S. Udenfriend, *J. Biol. Chem.* **229**, 953–963 (1957).
3. S. Kveder, S. Iskrić, and D. Keglević, *Biochem. J.* **85**, 447–449 (1962).
4. A. Feldstein and O. Williamson, *Brit. J. Pharmacol. Chemother.* **34**, 38–42 (1968).
5. G. R. Breese, T. N. Chase, and I. J. Kopin, *Biochem. Pharmacol.* **18**, 863–869 (1969).
6. G. R. Breese, T. N. Chase, and I. J. Kopin, *J. Pharmacol. Exp. Ther.* **165**, 9–13 (1969).
7. R. A. Deitrich, *Biochem. Pharmacol.* **15**, 1911–1922 (1966).

8. N. H. Raskin and L. Sokoloff, *Science* **162**, 131–132 (1968).
9. N. H. Raskin and L. Sokoloff, *J. Neurochem.* **19**, 273–282 (1972).
10. D. Eccleson, A. T. Moir, H. W. Reading, and I. M. Ritchie, *Brit. J. Pharmacol. Chemother.* **28**, 367–377 (1966).
11. B. Tabakoff and V. G. Erwin, *J. Biol. Chem.* **245**, 3263–3268 (1970).
12. V. P. Whittaker, *Biochem. J.* **72**, 694–706 (1959).
13. B. Tabakoff and S. G. A. Alivisatos, *Anal. Chem.* **44**, 427–428 (1972).
14. E. A. Zeller, J. Barsky, E. R. Berman, M. S. Cherkas, and J. R. Fouts, *J. Pharmacol. Exp. Ther.* **124**, 282–289 (1958).
15. V. G. Erwin, B. Tabakoff, and R. Bronaugh, *Mol. Pharmacol.* **7**, 169–176 (1971).
16. G. R. Lappin and L. C. Clark, *Anal. Chem.* **23**, 541–542 (1951).
17. A. Liakopoulou and S. G. A. Alivisatos, *J. Biol. Chem.* **240**, 3110–3115 (1965).
18. K. N. F. Shaw, A. McMillan, and M. D. Armstrong, *J. Biol. Chem.* **226**, 255–266 (1957).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.* **193**, 265–275 (1951).
20. H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.* **56**, 658–666 (1934).
21. C. M. Wolf, *in* "Principles of Biometry," pp. 213–216. Van Nostrand, Princeton, N. J., 1968.
22. V. G. Erwin and R. A. Deitrich, *Biochem. Pharmacol.* **21**, 2915–2924 (1972).
23. B. Tabakoff, Purification and characterization of a reduced nicotinamide adenine dinucleotide phosphate-linked aldehyde from brain. Ph.D. thesis, University of Colorado, 1970.
24. H. Theorell and T. Yonetani, *Biochem. Z.* **338**, 537–553 (1963).
25. H. Sund and H. Theorell, *in* "The Enzymes" (P. D. Boyer, H. Lardy, and K. Myrbäck, eds.), Vol. 7, pp. 25–83. Academic Press, New York, 1963.
26. K. Dalziel and F. M. Dickinson, *Biochem. J.* **95**, 311–320 (1965).
27. N. H. Raskin and L. Sokoloff, *J. Neurochem.* **17**, 1677–1687 (1970).
28. A. J. Turner and K. F. Tipton, *Eur. J. Biochem.* **30**, 361–368 (1972).
29. R. A. Deitrich, A. C. Collins, and V. G. Erwin, *Biochem. Pharmacol.* **20**, 2663–2669 (1971).
30. C. O. Rutledge and J. Jonason, *J. Pharmacol. Exp. Ther.* **157**, 493–502 (1967).
31. B. Tabakoff, F. Ungar, and S. G. A. Alivisatos, *Nature New Biol.* **238**, 126–128 (1972).
32. A. J. Friedhoff and M. Goldstein, *Ann. N. Y. Acad. Sci.* **96**, 5–13 (1961).
33. J. Harley-Mason, *Confin. Neurol.* **18**, 152–155 (1958).