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ETHANOL-INDUCED ALTERATION OF DOPAMINE METABOLISM IN RAT LIVER *

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Abstract—Ethanol alters the metabolism of dopamine such that the final product is no longer predominantly the acid, 3,4-dihydroxyphenylacetic acid (DOPAC), but is a mixture of the acid and the alcohol derivative, 3,4-dihydroxyphenylethanol (DOPET). The ratio of DOPAC/DOPET produced in rat liver slices incubationed with [ethylamine-2-^{14}C]dopamine hydrochloride in the absence of ethanol is *ca.* 10, while in the presence of ethanol it is 0.25. Addition of alcohol dehydrogenase (ADH) inhibitors prevents the alteration in metabolism. Changing the NAD/NADH ratio of the liver cytosol by adding lactate to the incubation medium does not cause an alteration in the metabolism of dopamine. Acetaldehyde addition in the presence or absence of ADH inhibitors does not enhance the production of the alcohol derivative, though there was a small decrease in DOPAC levels. Thus, neither the decreased liver cytosol NAD/NADH ratio nor the preferential oxidation of acetaldehyde over 3,4-dihydroxyphenyl acetaldehyde (DOPAL) can explain the ethanol-induced alteration in dopamine metabolism. 3-Etiocholan-3β-ol-17-one, an alternative substrate for ADH, whose product of oxidation is neither a substrate nor an inhibitor of aldehyde dehydrogenase, mimics the effect of ethanol such that in its presence the metabolism of dopamine to its alcohol derivative is enhanced. An increased reduction of DOPAL by the NADPH-dependent aldehyde reductase cannot explain the dramatic enhancement of DOPET formation observed in the presence of ethanol or the sterol because the NADPH/NADP ratio is normally very high in the liver. Due to the unique enzyme mechanism of ADH, in which the rate-limiting step of the reaction is the release of NADH from the enzyme, a finite concentration of the enzyme–NADH complex will exist during alcohol metabolism. We propose that the biogenic aldehyde binds to this form of ADH and is reduced.

Aldehyde intermediates produced by the monoamine oxidase (monoamine: O_2 oxidoreductase, EC 1.4.3.2, MAO) catalyzed oxidative deamination of the biogenic amines, norepinephrine, serotonin and dopamine, are rapidly metabolized to either an alcohol or an acid. The aldehyde oxidation is catalyzed by non-specific NAD(P)-dependent aldehyde dehydrogenases (aldehyde:NAD oxidoreductase, EC 1.2.1.3, ALDH), which are located in most, if not all, mammalian organs [1]. The reduction of these aldehydes is thought to be catalyzed by the NAD-dependent alcohol dehydrogenase (alcohol:NAD oxidoreductase, EC 1.1.1.1, ADH) localized primarily in liver [2], but since the discovery of an NADP-dependent aldehyde reductase (alcohol:NADP oxidoreductase, EC 1.1.1.2, ALRed) in liver and brain [3,4], it is not known with certainty which enzyme is responsible for the reduction in tissues containing both enzyme systems.

Since the oxidation or reduction of aldehydes requires a pyridine nucleotide coenzyme system, an alteration in the cellular ratio of NAD/NADH may cause a change in the normal metabolism of an aldehyde. The oxidation of ethanol is known to alter the hepatic NAD/NADH ratio [5,6]. Following the ingestion of ethanol, a change is seen in the levels of the excreted acid and alcohol products of the biogenic amines. For example, the major urinary products in man derived from serotonin and norepinephrine are normally 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylvanillyl mandelic acid, respectively, but during ethanol consumption the excretion of the reduced metabolites, 5-hydroxytryptophol and 3-methoxy-4-hydroxyphenylglycol, is increased [7,8]. Davis *et al.* [9] have shown that the metabolism of dopamine in liver homogenates is altered in the same manner if ethanol is present; the formation of 3,4-dihydroxyphenylethanol (DOPET) is enhanced at the expense of 3,4-dihydroxyphenylacetic acid (DOPAC). These alterations in the metabolism of the biogenic amines may be due to the decreased cellular NAD/NADH ratio in the liver produced by ethanol oxidation.

A second hypothesis for the ethanol-induced alteration in biogenic amine metabolism is that acetaldehyde, a product of ethanol oxidation, acts as an alternative substrate for ALDH, causing the biogenic aldehyde level to increase. This aldehyde can then become a substrate for aldehyde reductase. Davis *et al.* [9] have investigated the effect of acetaldehyde on dopamine metabolism in rat liver homogenates and found approximately a 50 per cent inhibition of DOPAC formation and a 3-fold increase in DOPET levels. It was found that in brain tissue the oxidation of 5-hydroxyindole acetaldehyde, which is derived from serotonin, is competitively inhibited by acetaldehyde [10]. However, high levels of acetaldehyde (0.5 to 4 mM) were employed in these studies.

We undertook an investigation to determine if the alteration in dopamine metabolism in liver slices is due to the change in NAD/NADH or to acetaldehyde acting as an inhibitor of DOPAL oxidation. The incubation experiments are performed with liver slices

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rather than homogenates in order to better simulate *in vivo* conditions. A preliminary report of these findings has been presented at a National Council on Alcoholism meeting [11].

EXPERIMENTAL PROCEDURES

Materials. Epinephrine, dopamine, norepinephrine and the available metabolites derived from these amines, as well as various steroids, were obtained from the Sigma Chemical Co. (St. Louis, MO). The syntheses of the unavailable metabolites will be described below. 3,4-Dihydroxphenylethanol was at times available from the Regis Chemical Co. (Morton Grove, IL). Tetrahydropapaveroline (THP) was a gift from Dr. A. Collins, School of Pharmacy, University of Colorado (Boulder, CO). Phenol reagent was obtained from Anderson Laboratories, Inc. (Fort Worth, TX). Triton X-100, diphenyloxazole and 1,4-bis[2(4-methyl-5-phenyloxazolyl)]benzene were obtained from the Sigma Chemical Co. [Ethylamine-2-^{14}C]dopamine hydrochloride and [methylene-^{14}C]noradrenaline-DL-bitartrate were purchased from the Amersham–Searle Co. (Chicago, IL). The radioactive compounds were dissolved in distilled water with sufficient non-labeled amine to prepare a solution of the desired specific activity. The solution was adjusted to pH 5 and stored at −20°. Unless otherwise specified, all chemicals were of the highest purity commercially available and were used without further purification.

Animals. Adult male rats, weighing from 350 to 400 g, were employed. All rats were of an original Wistar strain and were bred in the facilities of the Biochemistry Department at Purdue University.

Chemical synthesis of metabolites. The synthesis of 3,4-dihydroxphenylacetaldehyde (DOPAL) was performed as described by Robbins [12] or by the enzymatic deamination of dopamine using rat liver monoamine oxidase. Either synthesis produced low yields of only partially pure aldehyde. The chemical synthesis, however, yielded sufficient quantities of aldehyde for the enzymatic assays. The contaminant reacted with the Folin–Ciocalten phenol reagent, but did not react with 2,4-dinitrophenylhydrazine, a reagent which normally yields a yellow–orange product after reaction with an aldehyde. Thus, the contaminant was a non-aldehyde phenolic compound and was not characterized further. It consituted approximately 10–15 per cent of the final product. When separated by electrophoresis (see below), the contaminant was found to have no effect on the catalytic activity of aldehyde or alcohol dehydrogenase or of aldehyde reductase.

The enzymatic procedure for the synthesis of the aldehyde intermediate entailed incubating partially purified monoamine oxidase with dopamine. Monoamine oxidase was isolated from the outer mitochondrial membrane as described by Greenawalt [13]. This preparation was found to be void of alcohol and aldehyde dehydrogenase, as well as aldehyde reductase, activities. Dopamine (4 mM) was incubated with the membrane preparation for 2–4 hr at 37° in 2 ml of 100 mM sodium phosphate buffer, pH 7.4. The reaction was terminated by the addition of five drops of 6 N HCl. The product was separated from the aqueous medium by ether extraction. The aldehyde was stable in solution or as an oil for 24–48 hr when kept at −20° under nitrogen. The product was shown to be an aldehyde by mass spectroscopic identification. The details of the analysis are presented elsewhere [14].

Synthesis of DOPET was accomplished by reducing an aliquot of the corresponding aldehyde dissolved in 100 mM sodium phosphate buffer (pH 7.4), and reacting it with 5–10 mg sodium borohydride. The reaction was allowed to proceed at room temperature for 30 min, and then the excess borohydride was destroyed by the addition of 2 N HCl. The alcohol was extracted into ether and the ether was removed by evaporation. The alcohol was either stored as an oil or dissolved in buffer and stored frozen under nitrogen. Its chromatographic properties were identical to those of the commercially available alcohol.

Liver slice incubations with dopamine. All slice incubations, except those involving the addition of acetaldehyde, were performed as described here. Rats were killed by cervical dislocation, and the livers were rapidly excised and placed on ice. Liver slices were obtained by the use of a Stadie–Riggs tissue slicer. The slices weighed *ca.* 50 mg and were *ca.* 0.3 mm thick. The slices were placed in polyethlene centrifuge tubes containing Krebs–Ringer phosphate buffer, pH 7.4 [15], and 1 mg/ml ascorbic acid. Ascorbic acid is present to prevent autoxidation of the catechols. The tubes were shaken for 5 min in a water bath at 37°, after which [^{14}C]dopamine (2 μmoles, 0.2 μCi) was added to initiate the reaction. Additions of 40 mM ethanol, 20 mM lactate, 1 mM etiocholan-3β-ol-17-one (dissolved in redistilled methanol), or alcohol dehydrogenase inhibitors were made 5 min prior to the addition of dopamine. The total volume was 1.02 ml. The incubations were carried out for 2 hr at 37° under an oxygen atmosphere and were terminated by placing the tubes on ice and adding two drops of 0.2 N HCl. The slices were removed after centrifugation at 40,000 *g* for 10 min, and 25 μl of the supernatant fraction were subjected to two-dimensional chromatography-electrophoresis.

In selected experiments, the sedimented slice was weighed, suspended in 1 ml of 3 N perchloric acid, and homogenized with a Potter–Elvehjem tissue homogenizer. The homogenate was centrifuged for 15 min at 40,000 *g*; the precipitate was then washed twice with 3 N perchloric acid. The radioactivity in both the supernatant fraction and the precipitate was determined by liquid scintillation counting. In certain experiments, this supernatant fraction was analyzed further for dopamine metabolites. The total recovery of added radioactivity was between 80 and 90 per cent.

Liver slice incubations in the presence of acetaldehyde. Liver slices were obtained as described and placed in 5 ml glass reaction vials fitted with Teflon-coated septum caps. The vials were kept in ice and contained 0.5 ml of Krebs–Ringer phosphate buffer, pH 7.4. Additions of [^{14}C]dopamine (1 μmole, 0.2 μCi), 20 μmoles ethanol and alcohol dehydrogenase inhibitors were made if required. The vials were transferred to a 37° water bath and specific concentrations of acetaldehyde were injected into the vials with a Hamilton syringe through the septum cap. Since acetaldehyde is oxidized very rapidly by the liver cell, sequential additions of acetaldehyde were administered to differ-

ent vials in one of the following ways: 25 nmoles every 2 min; 100 nmoles every 4 min; or 500 nmoles every 10 min. The rates of these additions were based on the observation that the rate of acetaldehyde oxidation was *ca.* 15 nmoles/min in a 50 mg liver slice [14]. Controls (incubations performed in the absence of a liver slice) showed that there was no loss of acetaldehyde from the vials. Reactions were terminated as described and product analysis was performed on the incubation mixtures.

Product separation by two-dimensional paper chromatography-electrophoresis. A separation of dopamine and its deaminated metabolites was achieved by a paper chromatographic–electrophoretic technique. Ten to fifty μl of material were spotted in the lower left-hand corner of a strip of Whatman 3MM paper (9 × 2.5 in). The spotted material was subjected to ascending chromatography in the 2.5 inch direction using ethyl acetate–methanol–acetic acid (19:6:0.3) as the solvent system. When the chromatography was completed, the paper was allowed to dry and was lightly sprayed with 50 mM sodium borate buffer, pH 9.5. Paper electrophoresis in the same borate buffer [16] was performed at a 90° angle to the direction of the chromatography for 3 hr at 150 V in a 4° cold room. The metabolites were visualized by spraying the dried paper with 50% aqueous phenol reagent, followed by 20% Na_2CO_3. The spots were cut out and placed in scintillation vials. Radioactivity was determined with a Beckman LS-100 C liquid scintillation counter. The scintillation mixture consisted of 700 ml toluene, 300 ml Triton X-100, 4 g diphenyloxazole, and 100 mg of 1,4-bis[2(4-methyl-5-phenyloxazolyl)]benzene. All samples were counted to a 3 per cent error. The radioactivity was quenched by a factor of 25 per cent when counting was performed directly off the paper strips. The counting efficiency of an unquenched ^{14}C-standard in this mixture was 70 per cent.

Miscellaneous assays. Acetaldehyde concentrations in liver slice incubations were determined by the gas chromatographic method described by Sippel [17]. Pyruvate levels were determined by following the decrease in absorption of NADH at 340 nm in assay mixtures containing 2 μg lactate dehydrogenase, 0.2 mM NADH, and an aliquot of sample. The dehydrogenases were assayed by following the increase or decrease in NAD(P)H fluorescence upon addition of substrate and pyridine nucleotide to an aliquot of sample containing the enzyme.

Presentation of data. The level of each dopamine metabolite is presented in the tables as a percentage of the total deaminated metabolites recovered from the supernatant fraction obtained after centrifugation of the liver slice. The percentage value for each product relative to total deaminated products except THP was calculated as illustrated for DOPAC:

$$\%\ \text{DOPAC} = 100 \times \left[\frac{\text{cpm}_{\text{DOPAC}}}{(\text{Total recovered cpm in supernatant}) - (\text{cpm}_{\text{Dopamine}} + \text{cpm}_{\text{THP}}/2)}\right]$$

Since THP is a condensation product of dopamine and DOPAL, its specific activity was twice that of the other metabolites; therefore, half its value was added to that of dopamine, while the other half was used for calculating its percentage. No radioactivity corresponding to the 3-methoxy-4-hydroxy amine or alcohol derivatives was found; hence, these potential metabolites are not listed in the tables.

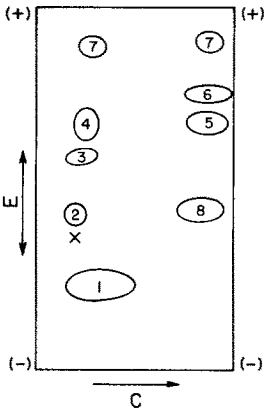

Fig. 1. Separation of dopamine and its metabolites by a two-dimensional paper chromatographic–electrophoretic technique. "X" denotes the site at which the sample is applied. The arrows indicate the direction of chromatography (C) and electrophoresis (E). The symbol (+) represents the anode. The spots represent the following compounds (1) 3-0-methyltyramine; (2) dopamine; (3) tetrahydropapaveroline; (4) 3,4-dihydroxyphenylacetaldehyde; (5) 3,4-dihydroxyphenylethanol; (6) homovanillic acid; (7) 3,4-dihydroxyphenylacetic acid; and (8) 3-methoxy-4-hydroxyphenylethanol. Solvents used for the separation are described in the experimental procedures. The area between the two 3,4-dihydroxyphenylacetic acid spots (number 7) was included with that acid when analyzing for radioactive metabolites.

RESULTS

Product separation. In Fig. 1 a representative tracing of the separation of the metabolites derived from [^{14}C]dopamine is presented. Eighty to ninety per cent of the radioactivity placed on the paper was recovered in the regions described in the figure. Insignificant amounts of radioactivity were found on the chromatograph corresponding to the *0*-methylated derivaties of the dopamine metabolites. DOPAC was found in the two regions, as indicated in Fig. 1. The two spots observed in the tracing were obtained using commercial DOPAC as well as that formed during liver slice incubation with dopamine. It is possible that the portion not migrating during chromatography is the ionized form.

Recovery of radioactivity from tissue incubations. The bulk of the radioactivity was recovered in the supernatant fraction of the centrifuged incubation mixture at the completion of the incubation (Table 1). Inhibiting MAO with pargyline or the inclusion of ethanol did not grossly alter the recovery of radioactivity nor the distribution of radioactivity between the pelleted liver slice and the supernatant fraction. Furthermore, when the pelleted slice was homogenized in perchloric acid and the homogenate centrifuged, essentially the same distribution of metabolites as determined by chromatography-electrophoresis was found in the resulting supernatant (acid-extractable fraction in Table 1) as that found in the original slice supernatant

Table 1. Percent of recovered radioactivity found in the supernatant and pellet fraction after centrifugation of the incubation mixtures.*

Additions	Percent recovery	Recovered cpm	Percent of recovered radioactivity		
				Pellet	
			Supernatant	Acid-extractable fraction	Acid-non-extractable fraction
Pargyline† (10 mM)	90.3 ± 7.7	271,000 ± 23,000	94.8 ± 6.8	4.6 ± 1.6	0.6 ± 0.1
None	81.3 ± 8.7	244,000 ± 29,500	90.6 ± 7.8	6.4 ± 3.1	2.8 ± 1.0‡
Ethanol (40 mM)	91.0 ± 10.0	273,000 ± 29,900	92.0 ± 7.4	5.5 ± 2.6	2.5 ± 1.0§

* The acid treatment of the pellet was described in Experimental Procedures. The percent recovery was based on the cpm supplied to the incubation mixtures (300,000 cpm). The results are presented as the means ± S.D. obtained from four determinations. Statistical significance was determined by Student's *t*-test. The values which are significantly different from the pargyline-treated control values are designated with a superscript and the level of significance is presented in the last two footnotes.

† A monoamine oxidase inhibitor.

‡ $P < 0.01$.

§ $P < 0.02$.

fraction. Therefore, in routine experiments only radioactivity found in the supernatant fraction of the centrifuged slice was subjected to chromatography-electrophoresis, and these results are presented in Tables 2–4.

After perchloric acid treatement, homogenization, and centrifugation of the pelleted slice, a portion of the radioactivity still remained bound to the sedimented material (acid-nonextractable fraction in Table 1). In the presence of pargyline, however, the radioactivity found in this fraction was abolished almost completely. Presumably, this acid-nonextractable radioactivity was aldehyde bound to protein, as suggested by Walsh *et al.* [18]. The presence of ethanol did not affect the level of this "bound radioactivity". Since it represents only 2 per cent of the total radioactivity employed, omitting this fraction from the data presented in the tables did not alter the conclusions.

Metabolism of dopamine in liver slices. The fraction of the various metabolites derived from dopamine after incubation with liver slices are presented in Table 2. The rate of deamination was found to be linear over the 2 hr incubation period. Even though the distribution of products was basically similar at all times during the incubation period, the percent of the acid product increased at the expense of the alcohol product as the incubation time increased. Routinely, a 2 hr incubation point was employed in order to obtain a large concentration of metabolites. Even though the exact distribution of metabolites isolated differed between individual animals, the major metabolic product derived from dopamine in all animals was the acid derivative DOPAC. Homovanillic acid was found in low concentrations. Significant levels of DOPET, DOPAL and THP were also recovered. These results are in reasonable agreement with those reported by Davis *et al.* [9] who employed rat liver homogenates containing added NAD.

Effect of ethanol on dopamine metabolism. The dramatic alteration in dopamine metabolism in the presence of ethanol is summarized in Table 2. DOPAC production was decreased to one-fifth that of controls, while DOPET formation was increased by 6-fold. A

Table 2. Percentages of deaminated products formed from metabolism of dopamine in rat liver slices incubated in the presence and absence of ethanol or lactate*

Additions	N	Per cent of deaminated products				
		THP	DOPAL	DOPET	DOPAC	Per cent deamination
None	6	11.9 ± 6.0	21.5 ± 5.0	6.6 ± 3.1	59.9 ± 8.7	64.2 ± 16.1
Ethanol (40 mM)	6	8.7 ± 2.1	39.1 ± 8.8†	40.3 ± 11.0	11.8 ± 2.4†	57.0 ± 12.9
ADH inhibitors‡	6	11.8 ± 6.5	18.5 ± 8.3	5.9 ± 2.6	63.8 ± 14.7	62.0 ± 20.8
Ethanol (40 mM) + ADH inhibitors	6	18.0 ± 9.2	22.1 ± 4.8	8.4 ± 4.0	51.5 ± 12.5	43.0 ± 21.5
Lactate (20 mM)	6	10.7 ± 3.1	27.0 ± 9.9	5.7 ± 3.9	56.6 ± 12.3	54.4 ± 12.3

* The experiments were performed and the percentages calculated as described in Experimental Procedures. The results are presented as the means ± S.D. obtained from the percent of products formed in liver slices from three different rats. N = the number of slice incubations employed for each set of data. Statistical significance was determined by Student's *t*-test, and values which are statistically different than controls are designated with superscripts.

‡ The alcohol dehydrogenase inhibitors, pyrazole (10 mM) and isobutyramide (10 mM), were used in combination in these incubations.

† $P < 0.01$.

statistically significant increase in the level of DOPAL was observed, while no alteration in the level of THP was seen. To determine whether the mere presence of ethanol or the metabolism of ethanol was responsible for this alteration, the incubations were performed in the combined presence of the ADH inhibitors, pyrazole and isobutyramide [19]. In the absence of ethanol, the inhibitors did not alter the metabolism of dopamine; they did though, prevent the ethanol-induced alteration in the metabolism. Thus, the presence of ethanol itself did not cause the change in metabolism.

Effect of lactate on dopamine metabolism. In order to mimic the alteration in the NAD/NADH ratio which occurs during the metabolism of ethanol [5,6], slices were incubated with 20 mM lactate. Rat liver homogenates contain *ca.* forty times more lactic dehydrogenase activity than ADH activity,* suggesting that lactate is oxidized much faster then alcohol in liver slices. After a 2 hr incubation with lactate, a 7-fold increase in pyruvate levels was found in the slices, indicating that the NAD/NADH ratio was changed since it has been shown that the lactate–pyruvate system is at equilibrium [20]. However, no change, in the metabolism of dopamine was observed in the presence of lactate (Table 2). Lactate did not prevent the ethanol-induced alterations in dopamine metabolism from occuring.

Effect of acetaldehyde on dopamine metabolism. Due to the volatility and rapid metabolism of acetaldehyde, the incubations were carried out for only 30 min. The results of these experiments are presented in Table 3. The results of control incubations were similar but not identical to those presented in Table 2; the differences in the DOPAC/DOPET ratio in the two tables could be attributed to the different times of incubation.

We have employed three concentrations of acetaldehyde in these studies. However, the acetaldehyde was rapidly metabolized by the liver slices (*ca.* 15 nmoles/min upon the addition of 1 mM acetaldehyde to a 50 mg liver slice); thus, its concentration was not constant during the entire incubation period. We attempted to circumvent this problem by sequentially adding acetaldehyde at a rate such that ALDH was always saturated with substrate (as occurs during the oxidation of ethanol in the liver). The peak concentrations of acetaldehyde varied from 50 μM to 1 mM. Acetaldehyde added at an initial concentration of 50 μM had no effect on the metabolism of dopamine, though during the oxidation of ethanol in these slices the concentration of acetaldehyde was between 10 and 25 μM.* At a higher concentration (0.2 mM) of acetaldehyde there was a decrease in DOPAC levels and a small but statistically significant increase in DOPET levels. In the presence of 1 mM acetaldehyde the alteration in dopamine metabolism was not greater than that seen in slices incubated in the presence of 0.2 mM acetaldehyde.

In order to determine if the lack of effect of low concentrations of acetaldehyde is due to its being reduced to ethanol, incubations were performed in the presence of ADH inhibitors. Though no statistical differences were found when ADH inhibitors were added, individual animals did show an unexplained increase, not decrease, in DOPAC formation in the presence of acetaldehyde.

Effect of an alternative substrate for ADH on dopamine metabolism. The steroid, etiocholan-3β-ol-17-one, is a substrate for rat liver ADH, as it is for horse liver ADH [21]. Neither the product of the oxidation (the dione) nor the sterol itself is an inhibitor of

* A. W. Tank and H. Weiner, unpublished observations.

Table 3. Percentages of deaminated products formed from metabolism of dopamine in rat liver slices incubated in the presence of varying concentrations of acetaldehyde*

Additions	N	Per cent deaminated products				Per cent deamination
		THP	DOPAL	DOPET	DOPAC	
None	8	13.8 ± 4.2+,‡	19.9 ± 4.6	20.9 ± 4.5§,‖,¶	45.3 ± 5.6**,††,§§	16.5 ± 5.0
Ethanol (40 mM)	8	7.5 ± 1.7+	20.3 ± 6.8	52.4 ± 8.4§	19.8 ± 3.7**	15.6 ± 5.0
Acetaldehyde (50 μM)	7	11.3 ± 4.4	22.4 ± 5.3	24.4 ± 4.5	41.9 ± 7.5	24.4 ± 4.2
Acetaldehyde (50 μM) and ADH inhibitors‡‡	7	8.9 ± 2.0‡	20.4 ± 6.0	25.6 ± 3.9	45.1 ± 6.3	21.3 ± 6.1
Acetaldehyde (0.2 mM)	7	12.9 ± 3.5	24.0 ± 5.5	27.5 ± 4.0‖	35.7 ± 5.6††	18.3 ± 2.6
Acetaldehyde (0.2 mM) and ADH inhibitors	8	10.9 ± 1.8	23.0 ± 8.3	26.1 ± 5.4	40.0 ± 9.3	17.4 ± 3.5
Acetaldehyde (1 mM)	8	11.2 ± 3.2	26.7 ± 8.1	28.3 ± 5.1¶	33.8 ± 4.8§§	17.6 ± 3.5

* The experiments were performed and the percentages calculated as described in Experimental Procedures. The results are presented as the means ± S.D. obtained from the percent of products formed in incubations of liver slices from four different rats. N = the number of slice incubations employed for each set of data. Statistical significance was determined by Student's *t*-test and the statistically compared values are designated with identical superscripts.

‡‡ The alcohol dehydrogenase inhibitors, pyrazole (10 mM) and isobutyramide (10 mM), were used in combination.

+ $P < 0.01$.
‡ $P < 0.05$.
§ $P < 0.01$.
‖ $P < 0.05$.
¶ $P < 0.05$.
** $P < 0.01$.
†† $P < 0.01$.
§§ $P < 0.01$.

ALDH.* The metabolism of dopamine was investigated in the presence of 1 mM sterol dissolved in methanol, and the results are presented in Table 4. The presence of methanol in the incubations produced a small but statistically significant ($P < 0.02$) increase in DOPET formation (compare lines 1 of Tables 2 and 4). The effect of ethanol on the metabolism of dopamine was also slightly altered in the presence of methanol in that the shift in the metabolism of DOPAL was not as great as that seen in the absence of methanol. However, the general metabolic distribution and the qualitative effect of ethanol were not affected by the presence of methanol.

When compared to controls run in the presence of methanol, the addition of the sterol to the incubation mixtures caused a large decrease in DOPAC levels and a 2-fold increase in DOPET levels. This alteration in dopamine metabolism was not as great as that observed in the presence of ethanol. Due to the low solubility of the sterol, it was not possible to determine if this was the maximum degree of alteration obtainable. ADH inhibitors completely prevented the effect of the sterol, as they did of ethanol in runs previously discussed. This blockade of the effect of the sterol by the ADH inhibitors indicated that this effect is mediated by ADH.

Alcohol dehydrogenase and aldehyde reductase activities. At pH 7.4 there was *ca.* ten times more ADH-reducing activity than ALRed activity when assayed at V_{max} conditions using *p*-nitrobenzaldehyde as a substrate.* The velocity of the ADH reaction, however, was dependent on the NAD/NADH ratio, as indicated from the data in Fig. 2, while the activity of ALRed was almost insensitive to changes in coenzyme ratios [22]. Thus, under physiological conditions where the NAD/NADH ratio is *ca.* 500:1 [20], ADH acts as an oxidizing enzyme, as was mathematically predicted by Purich and Fromm [23]. The fact that ADH inhibitors do not reduce the small levels of DOPET formed in the liver slice incubations is a verification that, in liver, aldehyde reductase is responsible for reducing biogenic aldehyde.

* A. W. Tank and H. Weiner, unpublished observations.

DISCUSSION

In liver slices dopamine metabolism is altered in the presence of ethanol confirming the results observed with liver homogenates [9]. The two major hypothesis presented in the literature purporting to explain this alteration in dopamine metabolism have been tested. Our results do not support either hypothesis. Neither a decrease in the NAD/NADH ratio in the cytosol of liver slices nor the presence of acetaldehyde at physiological concentrations in the incubations medium mimics the large increase in DOPET and the corresponding large decrease in DOPAC observed in the presence of ethanol.

The first hypothesis is predicated upon the fact that during the metabolism of ethanol the cytosolic NADH level increases at the expense of NAD, favoring the reduction of carbonyl-containing compounds. It has been shown that both the cytosol lactate-pyruvate and the mitochondrial β-hydroxybutyrate-acetoacetate equilibrium systems [24] are shifted toward the reduced products in the presence of ethanol in rat liver [5,6] and to a lesser degree in mouse brain [25]. Feldstein and Williamson [26] have shown that disulfi-

Table 4. Percentages of deaminated products formed from metabolism of dopamine in rat liver slices incubated in the absence and presenc etiocholan-3β-ol-17-one*

Additions	N	Percent of deaminated products				Per cent deamination	DOPET/DOP
		THP	DOPAL	DOPET	DOPAC		
None	6	11.5 ± 3.4	19.4 ± 7.0	13.6 ± 4.6+‡	55.5 ± 10.8§,‖	28.3 ± 8.2	0.26 ± 0.12¶,
Ethanol (40 mM)	6	11.1 ± 3.2	32.7 ± 16.9	34.2 ± 9.9+	21.9 ± 14.2§	27.1 ± 4.7	2.2 ± 1.2¶
Etiocholan-3β-ol-17-one 1 mM)	6	9.9 ± 3.6	25.6 ± 9.9	28.6 ± 8.6‡,††	35.9 ± 7.6‖	26.9 ± 4.2	0.84 ± 0.36**
Etiocholan-3β-ol-17-one (1 mM) + ADH inhibitors§§	6	16.0 ± 5.5	20.1 ± 6.4	17.8 ± 5.7††	46.0 ± 9.8	22.6 ± 4.5	0.40 ± 0.15‡‡

* The experiments were performed and the percentages calculated as described in Experimental Procedures. Etiocholan-3β-ol-17- dissolved in redistilled methanol (20 μl) was added to the appropriate incubation mixtures. Methanol (20μl) was added to the mixtures that not contain the sterol. The results are presented as the means ± S.D. obtained from the percent of products formed in incubations of liver sl from three different rats. N = the number of slice incubations employed for each set of data. Statistical significance was determined by Stude *t*-test, and the statistically compared values are designated by identical superscripts.

+ $P < 0.01$.
‡ $P < 0.01$.
§ $P < 0.01$
‖ $P < 0.01$
¶ $P < 0.01$
** $P < 0.01$.
†† $P < 0.05$.
‡‡ $P < 0.05$.
§§ The alcohol dehydrogenase inhibitors, pyrazole (10 mM) and isobutyramide (10 mM), were used in combination.

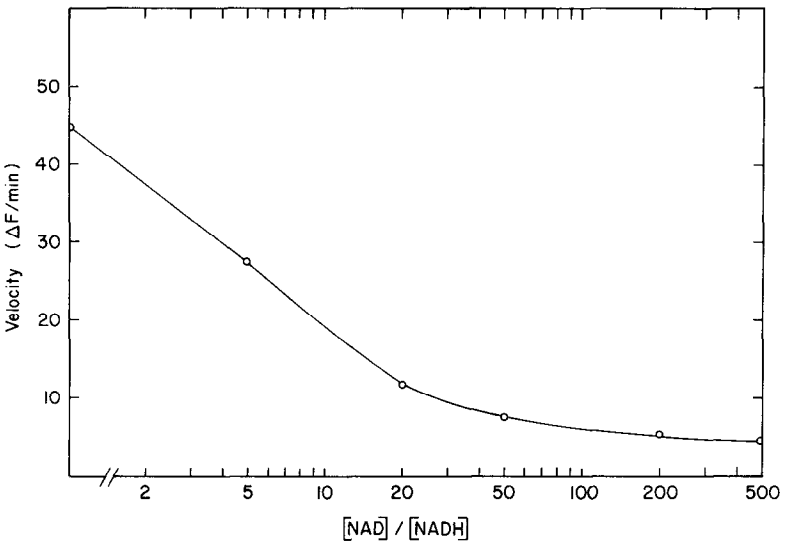

Fig. 2. Relative reducing velocity of alcohol dehydrogenase in the presence of various NAD/NADH ratios. Assays were performed by adding a small aliquot of rat liver homogenate to a cuvette containing 100 mM sodium phosphate, pH 7.4, 0.1 mM *p*-nitrobenzaldehyde and coenzyme at 25°. The NADH concentration was kept at 4 μM, and NAD concentrations ranging from 0 to 2 mM were added. The assay solution also contained 0.2 mM disulfiram (to inhibit aldehyde dehydrogenase) and 2.5 mM phenobarbitol (to inhibit aldehyde reductase). It was found that the K_m for NADH in the crude homogenate was 2 μM and that the concentration of *p*-nitrobenzaldehyde was sufficient to saturate the enzyme. The change in fluorescence (ΔF) was monitered, and the velocity is reported as an arbitrary fluorescence change per minute. The residual activity may be due to some other NADH-dependent reaction because in the presence of pyrazol and isobutyramide, inhibitors of alcohol dehydrogenase, a velocity of 3–5 ΔF/min is obtained. Thus, the reduction reaction of alcohol dehydrogenase is essentially zero at high NAD/NADH ratios. Essentially identical results were obtained if alcohol dehydrogenase was first separated from the homogenate by isoelectric focusing and assayed in the presence of different NAD/NADH ratios (unpublished observations).

ram, an irreversible inhibitor of aldehyde dehydrogenase [27], inhibits 5-hydroxyindoleacetic acid formation in rat liver homogenates and causes a large build-up of 5-hydroxyindoleacetaldehyde. Only when they supply NADH to the incubation medium is the aldehyde reduced. These authors argue that the increased NADH concentration in the liver which is produced during ethanol oxidation is needed to reduce any accumulating biogenic aldehyde. If this hypothesis were correct, then DOPET levels should rise when the NAD/NADH ratio is decreased by means other than ethanol oxidation.

A 7-fold increase in pyruvate levels is detected in the medium after the addition of lactate to liver slices. Thus, the NAD/NADH ratio is decreased, since the lactate–pyruvate system is at equilibrium [24]. However, no increase in DOPET formation is observed in incubations containing lactate, suggesting that the metabolic fate of DOPAL is not dependent upon a decrease in the NAD/NADH ratio which occurs during ethanol metabolism.

The second hypothesis discussed in the literature proposes that DOPAL oxidation is prevented by the preferred use of acetaldehyde as a substrate for ALDH [7–10]. This inhibition of DOPAL oxidation should cause an increase in its levels and a concomitant increase in its reduction to DOPET by ALRed. Several workers have presented evidence supporting this hypothesis from experiments on the metabolism of serotonin and norepinephrine [10,28]. Davis *et al.* [9] have investigated the effect of acetaldehyde on dopamine metabolism in rat liver homogenates. They found a 50 per cent inhibition of DOPAC formation and a 3-fold increase in DOPET formation in the presence of 0.5 to 4 mM acetaldehyde and exogenously supplied NAD. Blood levels of acetaldehyde rarely reach 50 μM upon ethanol ingestion [29,30]. The concentration of acetaldehyde in the liver has been reported to be as high as 0.2 mM after an intraperitoneal injection of 1.5 g/kg of ethanol or during liver perfusions [31,32]. Whether this high concentration (relative to that found in the blood) represents totally free acetaldehyde accessible to oxidation by ALDH or the sum of acetaldehyde bound to cellular material plus that which is free had not been established. Only 10–25 μM acetaldehyde accumulates in the incubation medium during the oxidation of ethanol by liver slices under the conditions employed in the present study.

We have studied the effects of acetaldehyde on dopamine metabolism in liver slices using concentrations of acetaldehyde varying from 50 μM to 1 mM. High initial levels of acetaldehyde (0.2 and 1 mM) inhibit DOPAC formation by about 25 per cent and cause a slight elevation in the levels of DOPET. However, these metabolic changes are not as great quantitatively as those observed in the presence of ethanol. Lower initial levels of acetaldehyde (50 μM) do not affect dopamine metabolism at all. Thus, even though high concentrations of acetaldehyde (such as those reported by Eriksson [31]) can inhibit DOPAL oxidation in rat liver slices, the alteration does not quantitatively mimic that produced by ethanol. Most significantly, the presence of acetaldehyde does not cause a large production of DOPET. These results are not in agreement with those

of Davis *et al.* [9] who found a large increase in DOPET formation in rat liver homogenates when acetaldehyde was present. This discrepancy may be due to the fact that in homogenates the mitochondrial matrix ALDH is no longer linked to the electron transport system. Since this matrix enzyme is responsible for acetaldehyde oxidation in the liver [33–36], the NADH produced from this oxidation in liver slices is reoxidized to NAD by the electron transport system. However, in liver homogenates this acetaldehyde-produced NADH is added to the rest of the NADH pool and may change the NAD/NADH ratio enough to allow ADH to contribute to the reduction of DOPAL. Alternatively, the enzyme involved in the oxidation of DOPAL may be localized in a different subcellular compartment than the enzyme involved in the oxidation of acetaldehyde, in which case acetaldehyde would have little effect on the metabolism of DOPAL in liver slices where subcellular compartmentalization is maintained. However, with the liver homogenates employed by Davis *et al.* [9] this aspect of cellular control is lost.

The results obtained from the experiments with lactate and acetaldehyde suggest that neither of the previously proposed postulates can explain the effect of ethanol on dopamine metabolism. Even if the large decrease in DOPAL formation in the presence of ethanol could be accounted for by acetaldehyde inhibition of DOPAL oxidation, it is still not possible to explain the increased reduction of DOPAL in the presence of ethanol.

The two enzymes capable of reducing DOPAL are ADH and ALRed. From thermodynamic considerations it would not be expected that ADH would function as a reducing enzyme because the NAD/NADH ratio is *ca.* 500:1, but ALRed could function since the NADP/NADPH ratio is 1:100 [20,24]. Hence, the enzyme responsible for DOPAL reduction under normal physiological coenzyme ratios must be the NADPH-dependent ALRed. Any increase in the concentration of reduced pyridine nucleotides in the cytosol due to the oxidation of ethanol, or for that matter lactate, should not increase the ALRed activity because of the existing high NADPH/NADP ratio.

The fact that the addition of the incubation mixtures of an oxidizable substrate of ADH such as ethanol or the sterol, etiocholan-3β-ol-17-one (whose product is neither a substrate nor an inhibitor of ALDH), causes enhanced reduction of DOPAL, suggests that the mechanism for this enhanced reduction must directly involve ADH. Based on the thermodynamic arguments presented above and the results obtained with lactate, the involvement of ADH cannot simply be enzyme binding more NADH with the increased concentration of free NADH. However, since the rate-limiting step in the overall reaction of ADH is the release of NADH from the enzyme [37], aldehydes theoretically can bind to this E–NADH complex, which would be present at a finite concentration only during alcohol oxidation, and be reduced prior to the dissociation of NADH from ADH. This sequence of events, which is based on kinetic not thermodynamic arguments, is illustrated in Fig. 3. It has been suggested previously [2] that such a mechanism may indeed explain the large production of

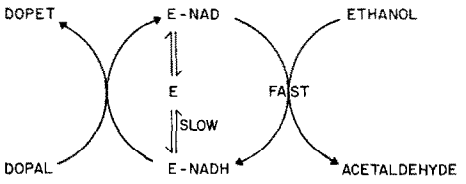

Fig. 3. A schematic representation of the model for the ethanol-induced alteration of dopamine metabolism. Alcohol dehydrogenase (E) normally exists as a binary complex with NAD. The rate-limiting step for the enzyme is the release of NADH from the enzyme [38]. If DOPAL binds to the enzyme complex prior to the release of NADH, it can be reduced to DOPET. Only during the oxidation of an alcohol substrate is alcohol dehydrogenase in this reducing complex.

DOPET by liver slices when ethanol or another oxidizable substrate of ADH is present. This mechanism has been invoked to explain the enhanced rate of reduction of chloralhydrate in the presence of ethanol [38]. Recently, the direct transfer of deuterium from deuterated ethanol to chloralhydrate to produce [^{2}H]-1-trichloroethanol has been demonstrated *in vivo* in the rat, proving that the mechanism proposed occurs [22,39]. This phenomenon has been demonstrated with a number of other aldehydes *in vitro* in the presence of ethanol and ADH [40], and has been shown to occur in liver perfusions [41,42].

The mechanism presented in Fig. 3 demonstrates why acetaldehyde does not cause an increase in the level of DOPET. However, it is suprising to find that DOPAL does not accumulate in the presence of acetaldehyde, since ALDH has a low K_m for acetaldehyde. It is possible that different isozymes of ALDH are responsible for the oxidation of the two aldehydes. The subcellular localization of DOPAL oxidation and the isozymes responsible for its oxidation will be discussed in a subsequent article.*

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