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EFFECTS OF INDUCTION OF RAT LIVER CYTOSOLIC ALDEHYDE DEHYDROGENASE ON THE OXIDATION OF BIOGENIC ALDEHYDES*

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Abstract—Phenobarbital and tetrachlorodibenzo-*p*-dioxin (TCDD) induce two different forms of aldehyde dehydrogenase (EC 1.2.1.3, ALDH), designated ϕ and τ respectively, in the rat liver cytosol. The physiological substrates for these enzymes are as yet unknown. In this study we investigated whether the induction of these enzymes forms affected the metabolism of dopamine and norepinephrine in rat liver slices. A 10-fold increase in ϕ-ALDH produced by phenobarbital treatment resulted in small increases in the formation of 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxymandelic acid from the biogenic amines. The 50- to 100-fold elevation of the τ-isozyme did not alter the rate of formation of the acids. When liver slices were incubated with 40 mM ethanol, the formation of the reduced products of dopamine and norepinephrine, 3,4-dihydroxyphenylethanol and 3,4-dihydroxyphenylglycol, respectively, was favored. Under these conditions, the induction of the ϕ-isoenzyme again produced only a small increase in the formation of the acid products, whereas the induction of the τ-isoenzyme had no effect on acid production from biogenic amine metabolism. The results suggest that neither the ϕ- nor the τ-forms of ALDH are involved in the hepatic metabolism of dopamine or norepinephrine and support the conclusion that the oxidation of the aldehyde derived from dopamine occurs in mitochondria [A. W. Tank, H. Weiner and J. Thurman, *Biochem. Pharmac.* **30**, 3265 (1981)].

Aldehyde dehydrogenase (EC 1.2.1.3, ALDH) is localized in various subcellular organelles of rat liver; in addition, there are many isozymes of the enzyme [1, 2]. It has been shown, using various techniques, that acetaldehyde [3, 4] and the biogenic aldehyde derived from dopamine [2] are primarily oxidized by the rat liver mitochondrial enzyme. The role of cytoplasmic enzymes is not known.

It is possible to increase the levels of different cytosolic ALDHs. Phenobarbital and tetrachlorodibenzo-*p*-dioxin (TCDD) induce two different forms of ALDH, designated ϕ and τ, respectively, which differ in their molecular weights, K_m values for acetaldehyde, and several physical properties [5–12]. The phenobarbital induction of the ϕ isoenzyme is genetically controlled by a single, autosomal, codominant gene [6]. This genetically controlled induction is not related to the induction of the microsomal mixed-function oxidase system of the liver elicited by phenobarbital treatment. Selective breeding has established two lines of rats; one line which shows a 10- to 30-fold increase in cytosol ALDH activity after phenobarbital treatment is designated RR, and a second line which shows less than a 2-fold increase in activity after phenobarbital treatment is designated rr. Mitochondrial and microsomal ALDH activities are not affected by phenobarbital treatment. In addition, phenobarbital acts as an inhibitor of isolated aldehyde reductase (EC 1.1.1.2, ALR), an enzyme involved in the reduction of biogenic aldehydes [13, 14].

The τ form of ALDH does not exist in non-TCDD-treated animals [11]. The chemical will cause induction to occur in both RR and rr rats [5]. Small increases in mitochondrial and microsomal ALDH activity occur in livers removed from TCDD-treated rats; however, these increases may be due to cytosolic contamination.

The physiological roles of the ϕ- and τ-isoenzymes, as with other cytosolic ALDHs, are not known. The K_m values for acetaldehyde, the first oxidation product of ethanol, are 0.2 and 12 mM for the ϕ and τ forms, respectively, when activity is measured at pH 7.4; the K_m values for a number of other aldehydes have also been determined [12]. Petersen *et al.* [15] have shown that, after a 2.5 g/kg (i.p.) dose of ethanol, blood acetaldehyde levels are lower in RR rats treated with phenobarbital than in those not treated with phenobarbital. The induction of the τ-ALDH by TCDD did not lower the blood acetaldehyde levels after the animals were given a 2.5 g/kg dose of ethanol. Thus, ϕ-ALDH but not τ-ALDH may play some role in acetaldehyde oxidation when either blood alcohol levels and, hence, blood acetaldehyde levels are high, or when blood acetaldehyde levels are elevated as a result of inhibition of mitochondrial ALDH [9].

Aldehydes of another class, designated biogenic aldehydes, are derived from amines such as norepinephrine, dopamine and serotonin. The biogenic

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aldehydes are either oxidized to their respective acids by ALDH or reduced to their respective alcohols by NADPH-dependent aldehyde reductase [1]. In rats, the aldehyde derived from norepinephrine (NE) by the action of monoamine oxidase (EC 1.4.3.4, MAO) is primarily reduced to 3,4-dihydroxyphenylglycol (DHPG), whereas the aldehydes derived from serotonin and dopamine (DA) are primarily oxidized to 5-hydroxyindolacetic acid and 3,4-dihydroxyphenylacetic acid respectively [16]. It has been shown that an ALDH present in the liver mitochondrial matrix is primarily responsible for oxidizing dihydroxyphenylacetaldehyde [1, 2]; however, the form(s) of ALDH responsible for liver oxidation of 3,4-dihydroxyphenylglycoaldehyde (NORAL) and 5-hydroxyindole acetaldehyde is still unknown. It has been shown that, 3,4-dihydroxyphenylacetaldehyde (DOPAL) can diffuse into the cytosol where it can be reduced to 3,4-dihydroxyphenylethanol (DOPET) by ALR or, if ethanol is present, by alcohol dehydrogenase (EC 1.1.1.1, ADH) [2]. In this study, cytosolic ALDH activity was induced, and the metabolism of biogenic amines was investigated. The purpose was to determine the role these isoenzymes may perform in biogenic aldehyde oxidation.

METHODS

Administration of drugs. Injections of phenobarbital into Long–Evans rats, bred at the University of Colorado, were made over 3 days as described in Ref. 6, at which time enzyme induction had reached a maximum. The induced level of ALDH was maintained for a month by including phenobarbital (0.5 mg/ml) in the drinking water of the animals. The TCDD-treated rats were injected once with TCDD (75 μg/kg) in dioxane and killed 1–2 weeks later.

Materials. Epinephrine, dopamine and norepinephrine and the available metabolites were obtained from the Sigma Chemical Co. Tetrahydropapaveroline (THP) was a gift from Prof. A. C. Collins, University of Colorado. [Ethylamine-2-^{14}C]Dopamine hydrochloride and D,L-[methylene-^{14}C]noradrenaline-D,L-bitartrate were purchased from the Amersham-Searle Co. The radioactive compounds were dissolved in water with sufficient unlabeled amine to prepare a solution at the desired specific activity (2 μCi/ml). The solution was adjusted to pH 5 and stored at −20°.

Synthesis of DOPAL and NORAL. 3,4-Dihydroxyphenylacetaldehyde was synthesized by one of two methods: (1) a pinecol–pinacolone type of rearrangement of epinephrine as described by Robbins [17]; and (2) an enzymatic oxidative deamination of dopamine using MAO partially purified from rat liver mitochondria [18] or from beef liver mitochondria [19]. The concentration of dopamine used in the enzymatic synthesis was no more than 4 mM, for above that tetrahydropapaveroline formation increases with increasing dopamine concentration [20]. Thus, 4 mM dopamine was incubated with the MAO preparation for 2–4 hr at 37° in 0.1 M sodium phosphate buffer, pH 7.4. The reaction was terminated by the addition of 6 N HCl, and the aldehyde was extracted with ether. Both methods of synthesis produced poor yields, but were used for product identification in the chromatography–electrophoresis separation technique described below. 3,4-Dihydroxyphenylglycoaldehyde was synthesized from norepinephrine by the enzymatic synthesis described above.

Liver slice incubations. Rats were killed by cervical dislocation, and their 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.* 3 mm thick. The slices were placed in polyethylene centrifuge tubes containing Krebs–Ringer phosphate buffer (pH 7.4) [21] and 1 mg/ml ascorbic acid. The tubes were placed for 5 min in a shaking water bath set at 37°, at which time either [ethylamine-2-^{14}C]dopamine hydrochloride (2 μmoles, 0.2 μCi) or D,L-[methylene-^{14}C]noradrenaline D,L-bitartrate was added to initiate the reaction as described previously [22]. When appropriate, 40 μmoles ethanol was added 5 min before the addition of dopamine or norepinephrine. The total volume was maintained at 1.02 ml. The incubations were carried out at 37° under an oxygen atmosphere and were terminated by placing the tubes on ice and adding 2 drops of 0.2 N HCl. Samples containing dopamine were incubated for 1 hr, whereas those containing norepinephrine were incubated for 2 hr. The slices were removed after centrifugation at 10,000 *g* for 10 min, and 25 μl of the supernatant fraction was subjected to either two-dimensional paper chromatography–electrophoresis as previously described for the analysis of dopamine and its metabolites [22] or to paper electrophoresis for the analysis of norepinephrine and its metabolites [23]. Visualization and liquid scintillation counting of the separated metabolites were achieved as described previously [22].

Quantitation. The level of each metabolite is presented in the tables as a percent of the total deaminated metabolites recovered from the supernatant fraction after separation and corresponded to the radioactivity isolated at the R_f of the known standard metabolite as we did before [22].

Subcellular fractionation of rat liver. Rats were killed by cervical dislocation, and the livers were rapidly removed, placed on ice, and minced with a tissue grinder. A 20% (w/v) suspension of the liver in 0.25 M sucrose–10 mM Tris–HCl buffer (pH 7.4) (referred to as the isolation medium) was homogenized at 340 rpm on a Potter–Elvehjem homogenizer. Centrifugation of the homogenate at 900 *g* for 15 min was performed, and the pellet, which represented the nuclear fraction, was discarded. The mitochondria were isolated by centrifugation at 9000 *g* for 10 min; this pellet was designated the mitochondrial pellet. Centrifugation at 12,000 *g* for 15 min was performed on the supernatant fraction of the first 9000 *g* centrifugation; the pellet was designated the lysosomal pellet. The supernatant fraction was submitted to centrifugation at 104,000 *g* for 60 min; this final pellet was rinsed twice with isolation medium and designated the microsomal pellet. An aliquot of the final supernatant fraction was passed through a Sephadex G-25 column (1.2 × 38 cm) that had been equilibrated previously with isolation

Table 1. Percentage of the deaminated products formed from the metabolism of dopamine in incubations of liver slices from drug-treated and control rats

Rat genotype	Drug treatment	N	Percent of deaminated products					Percent deaminated
			THP	DOPAL	DOPET	DOPAC	HVA	Unknown	
rr		(5)	11.1 ± 1.0	29.7 ± 1.6	9.8 ± 1.2*	41.1 ± 1.4†	2.2 ± 0.4	6.2 ± 0.6	62.5 ± 4.7
RR		(4)	7.4 ± 1.0	26.7 ± 3.2	12.3 ± 0.7	44.2 ± 4.9	2.8 ± 0.6	6.5 ± 0.6	56.1 ± 6.0
rr	Phenobarbital	(4)	11.4 ± 1.5	32.1 ± 1.1	18.8 ± 0.6*	27.2 ± 1.5†‡	4.1 ± 1.1	5.5 ± 0.6	64.8 ± 3.9
RR	Phenobarbital	(5)	7.5 ± 0.7	27.2 ± 1.0	15.6 ± 2.3	40.7 ± 3.0‡	3.1 ± 0.5	5.9 ± 0.4	50.1 ± 6.3
rr	TCDD	(4)	13.9 ± 0.5	34.0 ± 1.8	11.1 ± 1.0	31.1 ± 3.5	2.9 ± 0.5	7.0 ± 1.1	46.9 ± 2.4

Liver slices were obtained from RR or rr rats treated with either phenobarbital or TCDD. Control slices were obtained from untreated RR or rr rats. Radioactivity isolated after paper chromatography–electrophoresis was found at the R_f values for THP, DOPAL, DOPET, DOPAC, homovanillic acid (HVA) and at an R_f which represents an unknown metabolite. The results are expressed as the mean ± SEM; the number of rats is shown in parentheses.
*–‡ Statistical significance was determined by Student's *t*-test; values that are significantly different from each other have the same superscript: *$P < 0.01$, †$P < 0.001$, and ‡$P < 0.01$.

medium. The fractions that contained 90–95% of the ALDH, ADH and ALR activities, which were placed on the column, were pooled and designated the cytosol fraction.

RESULTS

Incubations of liver slices with dopamine. Dopamine metabolism in the slices isolated from liver of rats not treated with phenobarbital or TCDD was the same in both the rr and RR rats (Table 1, lines 1 and 2). This metabolism was very similar to that seen in the Wistar strain of rats used in previous studies [22, 24]. A decrease in DOPAC production and an increase in DOPET production was found in the liver slices (line 3) from rr rats treated with phenobarbital. This observation cannot be explained by the small induction of ALDH activity produced by phenobarbital treatment in the rr rats [6], nor by a possible inhibition of ALR by phenobarbital, because both these effects should have produced the opposite change in metabolism (an increase in DOPAC formation and a decrease in DOPET formation). The mechanism behind this observation must be due to an unknown action of phenobarbital.

Phenobarbital has been shown to induce ϕ-ALDH in RR rats by more than 10-fold [6]; yet, no change in dopamine metabolism was observed in slices from phenobarbital-treated RR rats when compared with slices from untreated RR rats (lines 2 vs 4). There was a significant difference in DOPAC formation in liver slices from phenobarbital-treated RR rats compared to that observed in slices from phenobarbital-treated rr rats. Production of DOPAC in liver slices obtained from rr rats treated with TCDD was similar to that observed in slices from untreated rr rats (line 1 vs line 5).

Incubations of liver slices with dopamine and ethanol. Ethanol causes marked alterations in dopamine metabolism in liver slices obtained from Wistar rats [22, 24]. These alterations were observed in both RR and rr Long-Evans rats (Table 2). There was a dramatic decrease in DOPAC formation and a marked increase in DOPET formation in the presence of 40 mM ethanol (compare lines 1 and 2 at Tables 2 and 1). DOPAL levels appeared to increase, but the increase was not statistically significant. There were no statistically significant differences in the two genotypes with respect to the products derived from dopamine in the incubations containing ethanol, although the RR rats tended to produce less THP than the rr rats.

If the ϕ- and τ-isoenzymes were involved in the metabolism of dopamine either by virtue of a high V_{max} or a low K_m for the biogenic aldehydes, then the induction of these enzymes should alleviate the drastic alteration in dopamine metabolism that occurs in the presence of ethanol. Slight increases in DOPAC formation were observed in both RR and rr rats treated with phenobarbital; however, the 10-fold induction of the enzyme in the RR rats treated with phenobarbital only slightly overcame the ethanol-induced inhibition of DOPAC formation (line 2 vs line 4, Table 2). Induction of the τ-isoenzyme by TCDD also failed to overcome the alter-

Table 2. Effect of 40 mM ethanol in incubations of liver slices from drug-treated and control rate on percentages of the deaminated products formed from the metabolism of dopamine

Rat genotype	Drug treatment	N	Percent of deaminated products						Percent deaminated
			THP	DOPAL	DOPET	DOPAC	HVA	Unknown	
rr		(4)	10.8 ± 0.8*	40.6 ± 5.8	31.2 ± 4.7	7.7 ± 1.3‡	5.3 ± 0.6	4.4 ± 0.3	51.3 ± 3.6
RR		(3)	4.6 ± 0.7*	38.5 ± 4.6	39.9 ± 1.6	7.1 ± 2.1§	3.9 ± 1.4	6.2 ± 1.5	51.7 ± 4.8
rr	Phenobarbital	(4)	10.3 ± 1.3†	32.0 ± 1.9	38.2 ± 3.5	11.6 ± 0.5‡	5.0 ± 0.2	4.1 ± 0.2	52.8 ± 5.2
RR	Phenobarbital	(3)	5.3 ± 1.0†	31.5 ± 2.4	32.5 ± 9.5	19.3 ± 4.2§	5.9 ± 1.8	5.5 ± 1.2	43.8 ± 8.9
rr	TCDD	(2)	11.8	43.1	26.2	8.3	4.8	5.7	46.3

Liver slices were obtained from RR or rr rats treated with either phenobarbital or TCDD. Control slices were obtained from untreated RR or rr rats. All the radioactivity isolated from the incubations after the paper chromatography–electrophoresis was found at the R_f values for THP, DOPAL, DOPET, DOPAC, HVA and at an R_f which represents an unknown metabolite. The results are expressed as the mean ± SEM; the number of rats is shown in parentheses.

*–§ Statistical significance was determined by Student's t-test; values that are significantly different from each other have the same superscript: *$P < 0.01$, †$P < 0.02$, ‡$P < 0.05$, and §$P < 0.01$.

Table 3. Percentages of the deaminated products formed from the metabolism of dopamine in incubations of liver supernatant fractions

Additions	Rat genotype	Drug treatment	N	Percent of deaminated products						Percent deaminated
				THP	DOPAL	DOPET	DOPAC	HVA	Unknown	
MAO			(2)	8.5	71.3	6.9	5.9	3.0	4.4	32.2
Liver supernatant and MAO	RR		(2)	5.7	55.8	14.7	16.4	3.8	3.7	29.1
Liver supernatant and MAO	RR	Phenobarbital	(2)	5.8	44.9	12.2	30.9	2.8	3.4	27.6
MAO			(2)	16.4	51.2	6.9	8.9	6.5	10.8	29.6
Liver supernatant and MAO	rr		(3)	15.8 ± 0.8	56.0 ± 1.6	4.7 ± 2.5	18.0 ± 4.0	2.6 ± 0.8	3.0 ± 0.8	31.8 ± 1.9
Liver supernatant and MAO	rr	TCDD	(3)	17.1 ± 1.3	57.9 ± 2.3	5.6 ± 2.6	13.7 ± 1.2	2.2 ± 0.6	3.5 ± 1.6	27.1 ± 4.1

The isolated liver supernatant fractions were incubated with 10 mM $MgCl_2$, 10 mM sodium phosphate, pH 7.4, [^{14}C]dopamine (2 μmoles, 2 μCi), MAO preparation partially purified from beef liver mitochondria and the following concentrations of pyridine nucleotide coenzymes: 0.5 mM NAD, 0.5 mM NADPH, 1 μM NADH, and 5 μM NADP. The incubations each contained 3.0 mg of cytosolic protein except for lines 1 and 4 where no cytosol was added to the MAO incubation. Line 1 was then concentrated for RR and line 4 for rr. The results are expressed as the mean or mean ± SEM; the number of rats is shown in parentheses.

ation in DOPAL oxidation brought about by the action of ethanol (line 1 vs line 5).

Incubations of subcellular liver fractions with dopamine. To determine whether the ϕ-isoenzyme induced by phenobarbital was capable of oxidizing DOPAL, experiments were performed with the cytosolic fraction of the rat liver supplemented with MAO partially purified from beef liver mitochondria [13]. The results of control incubations containing dopamine and MAO are presented in line 1 of Table 3. The levels of DOPAC and DOPET in these incubations were taken as blank values.

Unlike incubations performed with rat liver slices in which DOPAC was the major product in liver cytosol, the productions of DOPAC and DOPET were about equal. DOPAL was the major metabolite found (lines 2 and 5, Table 3).There was a 2-fold increase in DOPAC and a decrease in DOPAL in the cytosol isolated from phenobarbital-treated RR rats (line 3), indicating that the induction of the ϕ-enzyme enhanced DOPAL oxidation in the cytosol fraction.

Similar experiments were performed using liver cytosol isolated from rr rats treated with TCDD. A different preparation of MAO was used for these experiments. The results of the control incubations of dopamine and MAO are presented in line 4 of Table 3. The induction of the τ-isoenzyme by TCDD did not increase the production of DOPAC in the cytosol of livers isolated from TCDD-treated rats (line 5 vs line 6, Table 3), suggesting that the τ-isoenzyme did not oxidize DOPAL to any measurable degree under the conditions of the incubation.

Incubations of liver slices with norepinephrine. The metabolism of norepinephrine was essentially the same in liver slices obtained from both RR and rr rats (lines 1 and 2, Table 4). The effect of phenobarbital itself on norepinephrine metabolism was shown by the results obtained from slice incubations of phenobarbital-treated rr rats (line 3). There was a 25% decrease in DHPG levels which was probably due to inhibition of ALR by phenobarbital. This inhibition resulted in a slight accumulation of NORAL. Such an inhibition was not observed in dopamine metabolism, probably because DOPAL is a poor substrate for ALR. No changes in dihydroxymandelic acid (DHMA) levels were found. Incubations using phenobarbital-induced liver slices from RR rats also exhibited a 25% inhibition of DHPG formation, but in these incubations there was an increased production of DHMA and no increase in NORAL (line 4). The metabolism of norepinephrine in liver slices from TCDD-treated rr rats was identical to that observed in slices from untreated rr rats (line 1 vs line 5).

Incubations of liver slices with norepinephrine and ethanol. Ethanol did not change dramatically the metabolism of norepinephrine in liver slices from either rr or RR rats (lines 1 and 2, Table 5). There was again a decrease in DHPG formation, and a concomitant build-up of NORAL levels in the incubation of liver slices obtained from phenobarbital-treated rr rats when incubated in the presence of ethanol; however, no increase in DHMA production was seen. In slices from RR rats treated with phenobarbital, DHPG formation was again decreased, but the 10-fold increase in the enzyme activity promoted to a small extent the oxidation of NORAL to DHMA (line 4). Analogous to dopamine metabolism in liver slices from TCDD-treated animals, no increase in DHMA levels were observed in tissue slices from TCDD-treated rr rats.

DISCUSSION

It is established using Wistar rats that both acetaldehyde and DOPAL are oxidized primarily to acetate and DOPAC, respectively, in liver mitochondria [2, 4]. Thus, even though cytosol and microsomes possess ALDH activity *in vivo*, the mitochondrial enzyme appears to be responsible for the oxidation of these aldehydes.

Investigators often invoke a K_m-argument to attempt to explain specificity found *in vivo*. In many cases K_m values determined for a substrate with isolated enzyme will not predict the subcellular site of oxidation of the substrate unless the total metabolic capacity is known ([Enzyme] $\cdot$ k_{cat}). That is, even if the K_m is high, metabolism (in the case of these aldehydes, oxidation) could occur. This is due to the fact that, barring any enzyme regulation, the overall

Table 4. Percentages of the deaminated products formed from the metabolism of norepinephrine in incubations of liver slices from drug-treated and control rats

Rat genotype	Drug treatment	N	Percent of deaminated products			
			NORAL	DHPG	DHMA	Percent deaminated
rr		(6)	41.7 ± 0.7*	39.4 ± 0.7†	18.8 ± 1.7	71.9 ± 4.5‡
RR		(8)	34.2 ± 2.5	41.0 ± 2.6	24.8 ± 12.7	47.3 ± 1.6
rr	Phenobarbital	(5)	49.8 ± 2.2*	30.4 ± 1.9†	19.8 ± 2.2	51.5 ± 5.2‡
RR	Phenobarbital	(2)	34.8	30.0	35.3	47.4
rr	TCDD	(4)	40.7 ± 3.1	44.6 ± 3.2	14.7 ± 0.7	73.6 ± 2.7

Liver slices were obtained from RR or rr rats treated with either phenobarbital or TCDD. Control slices were obtained from untreated rats. The radioactivity isolated from the incubations after the paper electrophoresis was found at the R_f values for 3,4-dihydroxyphenylglycoaldehyde (NORAL), 3,4-dihydroxyphenylglycol (DHPG) and 3,4-dihydroxymandelic acid (DHMA). The results are expressed as the mean ± SEM; the number of rats is shown in parentheses.

*–‡ Statistical significance was determined by Student's *t*-test; values that the significantly different from each other have the same superscript: *$P < 0.05$, †$P < 0.01$, and ‡$P < 0.01$.

Table 5. Effect of 40 mM ethanol in incubations of liver slices from drug-treated and control rats on percentages of the deaminated products formed from the metabolism of norepinephrine

Rat genotype	Drug treatment	N	Percent of deaminated products NORAL	DHPG	DHMA	Percent deaminated
rr		(6)	36.2 ± 4.1*	46.0 ± 5.9†	17.8 ± 2.7‡	71.9 ± 4.5§
RR		(2)	36.8	47.4	15.8	45.1
rr	Phenobarbital	(5)	47.9 ± 7.0*	34.1 ± 4.8†	17.9 ± 5.7	55.4 ± 5.7§
RR	Phenobarbital	(2)	33.3	35.7	31.0	44.2
rr	TCDD	(4)	35.1 ± 4.3	53.1 ± 5.3	11.7 ± 1.2‡	69.0 ± 8.9

Liver slices were obtained from RR or rr rats treated with either phenobarbital or TCDD. Control slices were obtained from untreated rats. All the incubations contained 40 mM ethanol added 5 min prior to the addition of dopamine. The radioactivity isolated from the incubations after the paper electrophoresis was found at the R_f values for NORAL, DHPG and DHMA. The results are expressed as the mean ± SEM; the number of rats is shown in parentheses.

*–§ Statistical significance was determined by Student's t-test; values that are significantly different from each other have the same superscript: *$P < 0.02$, †$P < 0.01$, ‡$P < 0.01$, and §$P < 0.01$.

velocity of metabolism is related to the sum of the individual Michaelis–Menten terms.

$$v = \sum_i \frac{[\text{Enzyme}]_i \cdot k_{\text{cat}_i} \cdot [S]}{K_{m_i} + [S]}$$

There are many ways in which one can investigate the role of different organelles in a metabolic process. One is to study the metabolism in the isolated organelle in question. This was the approach used in our previous study with dopamine metabolism [22]. The second approach is to selectively inhibit an enzyme (or pathway) in just one organelle, as was utilized in our investigation of acetaldehyde metabolism in rat liver [4]. In this study, a third approach was employed—that is, the selective induction of particular isozymes. Since the specific induction of cytosolic ALDH by drugs has been shown to occur in rodent models, we were able to increase the total ALDH activity in the cytosol. If that organelle, or more precisely, the isozyme induced, is involved in the metabolic process of interest, it should be possible to increase the involvement of that organelle or isozyme in the overall metabolic process. This approach was employed previously to study acetaldehyde oxidation [15].

Drugs or chemical used to alter a cellular function are usually not totally specific for only the pathway or enzyme in question. Some of the potential problems of non-specific alterations were eliminated in this study by using animals that responded to the chemicals (RR) and those that did not (rr). Phenobarbital and TCDD both induce ALDH activity in the rat liver cytosol; however, phenobarbital may also act as an inhibitor of ALR activity [13, 14]. Both actions of phenobarbital must be considered when interpreting its effect on biogenic amine metabolism. Some effect of phenobarbital inhibition of ALR activity on dopamine and norepinephrine metabolism was observed in the rr rats. Assuming that the same level of inhibition of reductase occurs in both RR and rr rats, the effect of phenobarbital on biogenic amine metabolism due to ALDH induction can be calculated from the data obtained with the two genotypes.

As reported elsewhere [2, 25], the major metabolite of dopamine in rat liver slices was DOPAC; however, significant quantities of DOPET, DOPAL and THP, the condensation product of dopamine and DOPAL, were also produced. When the rr rats were treated with phenobarbital, an unexpected result occurred; DOPAC production decreased, while DOPET production increased. Phenobarbital inhibition of ALR would be expected to produce the opposite effect. We do not have an explanation for this phenomenon. When RR rats are treated with phenobarbital, a 10-fold induction of ϕ-ALDH occurs in the rat liver [6], but as presented here there was no change in dopamine metabolism in slices obtained from these livers when compared to slices obtained from livers of RR rats not treated with phenobarbital. However, if phenobarbital has the same intrinsic effect on dopamine metabolism in liver slices from RR rats as seen in slices from rr rats, then the enzyme induced in the RR rats increased the production of DOPAC by about 50% (line 3 vs line 4, Table 1). In either case, DOPAL accumulation remained high and DOPAC levels did not increase dramatically. These results suggest that the phenobarbital-induced enzyme may be capable of oxidizing DOPAL to some extent but is not normally the principal enzyme involved in this oxidation. If the enzyme principally responsible for DOPAL oxidation were induced 10-fold, a greater accumulation of DOPAC and a lesser level of DOPAL would be expected.

Induction of the τ-isoenzyme by TCDD did not produce an increase in DOPAC formation in liver slices. In fact, a small decrease in DOPAC formation was observed. This result suggests that the τ-isoenzyme plays no role in DOPAL oxidation.

The ingestion of ethanol causes marked changes in the products of the biogenic amines excreted in the urine [26]. In man, the amines are normally excreted as their free or conjugated acid derivatives. However, after ethanol consumption, the reduced products predominate. This shift in the metabolism of the biogenic amines from oxidative to reductive can also be observed in liver slices [22, 24]. As shown from the data in Table 2, the formation of DOPAC

was totally depressed when liver slices were incubated in the presence of 40 mM ethanol. DOPET and DOPAL levels increased dramatically. Though increases in DOPAC formation were observed in both rr and RR rats treated with phenobarbital, the data clearly show that neither the 10-fold increase in the ϕ-isoenzyme nor the induction of τ-isoenzyme significantly reversed the effect of ethanol.

To test further the ability of cytosolic ALDHs to oxidize DOPAL, experiments were performed with homogenates free of mitochondria. Thus, any oxidation of DOPAL to DOPAC would be catalyzed by cytosolic ALDHs. Very little of the MAO-generated aldehyde was metabolized by liver supernatant fractions during the course of the incubations. In supernatant fractions obtained from RR rats treated with phenobarbital, the 10-fold induction of the enzyme produced only a 2-fold increase in DOPAC formation compared to controls. This result confirms the data from the slice incubations, which showed that the ϕ-isoenzyme is capable of oxidizing DOPAL but that this enzyme form must not be very important in the overall metabolism of dopamine. No increase in DOPAL oxidation was observed in supernatant fractions obtained from TCDD-treated rats. These data support the results from slices, which indicate that the τ-isoenzyme played no role in DOPAL oxidation. These results agree with earlier findings which show in Wistar rats the cytosol ALDHs are not very important in DOPAL oxidation [2].

In incubation using liver slices obtained from either RR and rr rats, norepinephrine was metabolized primarily to its alcohol product, DHPG. Large amounts of the aldehyde accumulated and small quantities of DHMA were formed. A significant decrease of *ca.* 25% in DHPG formation was observed in slices obtained from rr rats treated with phenobarbital. This decrease was due most probably to the inhibitory effect of phenobarbital on ALR activity in the liver and provides evidence that phenobarbital acts as an *in vivo* inhibitor of the reductase involved in DHPG formation. However, the decrease in the alcohol product was not accompanied by a corresponding increase in the acid product; instead NORAL accumulated as would be expected.

Inducing the ϕ-isoenzyme in the RR animals by phenobarbital treatment did not lead to a major alteration in the oxidation of NORAL to DHMA in the presence or absence of added ethanol. Similarly, induction of the τ-isozyme had no effect on noradrenaline metabolism in slices isolated from TCDD-treated animals. These results suggest that the ϕ-isoenzyme is capable of oxidizing NORAL and may play a small role in its normal oxidation. However, the results indicate that this ALDH isoenzyme does not compete favorably with the reductive enzymes. The τ-isoenzyme apparently plays no role in the oxidation of this aldehyde.

We can conclude that, even though the phenobarbital-induced ALDH isozyme appears to be capable of oxidizing biogenic aldehydes, the isoenzyme at its normal cellular concentration is not important in dopamine or norepinephrine metabolism. The TCDD-induced ALDH appeared to play no role in biogenic aldehyde oxidation at the 0.3 to 0.5 mM substrate concentrations formed during the incubations. This was an unexpected finding as these inducible enzymes appear to have specificity towards aromatic aldehydes [11, 27]. Thus, the physiological role of these cytosolic ALDHs is still unknown.

The findings reported in this study show that total ALDH activity is not as important as the levels of specific isozymes. Other examples of this are known, especially in detoxication systems, such as those catalyzed by the microsomal P_{450}s [28].

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