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INDUCTION OF ALDEHYDE DEHYDROGENASES*

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Abstract—Rat liver contains two inducible aldehyde dehydrogenases in the cytosol. Previously it was found that the ϕ enzyme is maximally induced 30-fold by phenobarbital but only in genetically selected rats, while the τ enzyme is maximally induced 100-fold by tetrachlorodibenzo-*p*-dioxin (TCDD) in all rats tested. A number of other compounds have now been tested for their ability to induce one or the other of these enzymes *in vivo*. The decision as to which enzyme is induced by these compounds is made by a combination of genetic, kinetic, physical and immunological techniques. The major conclusions are that phenobarbital, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl-ethane) (DDT), and dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuto[cd]pentalene (Mirex) induce only the ϕ enzyme significantly, TCDD induces only the τ enzyme, while 3-methylcholanthrene and polychlorinated biphenyl induce both enzymes. Neither the normal function of the enzymes nor the advantage of their induction is known yet.

Rat liver contains a number of aldehyde dehydrogenase enzymes (EC 1.2.1.3) which are distributed in the mitochondrial[1–4], microsomal[1, 2] and supernatant[1, 2] fractions of rat liver homogenates. Two of these enzymes, located in the supernatant fraction, are inducible[5]. One of the supernatant enzymes, the phi enzyme (ϕ enzyme), is induced by prior treatment of rats with phenobarbital. This induction is controlled genetically by a single, incompletely dominant, autosomal gene[6–8]. Two lines of rats, RR (inducible) and rr (noninducible), are maintained by this laboratory. A second supernatant enzyme, the tau enzyme (τ enzyme), is inducible by treatment of the rats with tetrachlorodibenzo-*p*-dioxin (TCDD)[5]. In contrast to induction of the ϕ enzyme, induction of the τ enzyme is observed in all rats so far tested[5]. The ϕ and τ enzymes have been purified, and antisera to them prepared in rabbits[5, 9].

It is not known what function these enzymes may serve or what significance can be attached to their induction. They are capable of oxidation of acetaldehyde and may function in the fine control of blood acetaldehyde levels[10, 11]. However, their substrate specificity is so broad that numerous other possibilities exist. It is known that there is no apparent relationship between induction of these aldehyde dehydrogenases and induction of microsomal drug-metabolizing enzymes[5]. Rat liver tumors, induced by feeding acetylamino-fluorene (AAF), contain an enzyme apparently identical to the τ enzyme[12].

In the present study, we report the use of physical, kinetic, genetic and immunological techniques to investigate the induction by a variety of compounds of these two supernatant aldehyde dehydrogenases as a step toward understanding both the mechanism of induction and the function of the enzymes *in vivo*.

METHODS

Supernatant and mitochondrial fractions of rat liver homogenates were obtained by the usual differential centrifugation techniques. Twenty per cent homogenates of rat liver were prepared in 0.25 M sucrose. The homogenates were centrifuged at 600 *g* for 10 min. The pellet was homogenized in 0.25 M sucrose, centrifuged again at 600 *g* for 10 min and the supernatant fractions were combined. Mitochondria were sedimented by centrifugation at 9000 *g* for 10 min. The mitochondria, after being washed three times in 0.25 M sucrose, were suspended in a volume of 0.25 M sucrose containing 20 mM phosphate (pH 7.4) and 2 mM β-mercaptoethanol equal to half the wet weight of the liver. This suspension was treated by sonic irridation for a total of 6 min in 10-sec bursts, followed by 10 sec to allow for cooling, and centrifuged at 218,000 *g* for 1 hr. The post-mitochondrial supernatant fraction was also centrifuged at 90,000 *g* for 1 hr to yield the final supernatant for assay of cytosolic aldehyde dehydrogenase.

Mitochondrial aldehyde dehydrogenase activity was determined in a solution containing 0.03 M pyrophosphate, pH 9.6, 3.3 mM propionaldehyde or 0.33 mM phenylacetaldehyde, 10 mM NAD, and enzyme. The assay system for aldehyde dehy-

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drogenase activity in the supernatant fraction was identical except that 1 mM pyrazole was added.

D-Glucuronolactone dehydrogenase was assayed as described by Marselos and Hänninen[7]. Esterase activity was determined using *p*-nitrophenylacetate as substrate as described by Feldman and Weiner[13].

Water insoluble compounds were dissolved or suspended in corn oil and administered intraperitoneally (i.p.) to adult rats. TCDD was administered i.p. in dioxane (100 μg/ml) in a single dose of 10 μg/kg. The livers were taken on day 12 after the injection. Dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuto[cd]pentalene (Mirex), 20 mg/ml; 1,1,1-trichloro-2,2-bis-(*p*-chlorophenylethane) (DDT), 20 mg/ml; polychlorinated biphenyl (PCB), 6 mg/ml; acetylaminofluorene (AAF), 20 mg/ml; and 3-methylcholanthrene (3-MC), 20 mg/ml, were injected at a dose of 80 mg/kg i.p. using corn oil as the carrier. The animals were treated for 3 consecutive days and the livers taken on day 12. β-Napthoflavone (8 mg/ml of corn oil) gave a maximal response if the animals received 80 mg/kg/day for each of 3 days and the livers were taken on day 4. Phenobarbital (10 mg/ml of water), at a dose of 50 mg/kg/day, was given i.p. for 3 days and livers were excised on day 4.

Ouchterlony gel double diffusion was carried out with the supernatant fraction of liver homogenates and the antisera prepared against the ϕ or τ aldehyde dehydrogenase protein. Several days were allowed for diffusion, after which the plates were washed and examined for precipitin lines. Protein was stained with 0.5% Amido Schwartz dye in 7% acetic acid. The plates were then washed with 7% acetic acid. Aldehyde dehydrogenase activity was detected by incubating the Ouchterlony plates in a solution containing 14.3 mM Na pyrophosphate, pH 9.3; 1 mM NAD, 3.2 mM propionaldehyde, 0.78 mM *p*-iodonitrotetrazolium violet, and 0.5 mM phenazinemethosulfate for 15–20 min in the dark. The plates were then washed, dried and photographed. In some cases propionaldehyde was omitted to check for non-specific staining.

Previous results[5] showed that the majority of the ϕ enzyme precipitates between 50 and 60% saturation of $(NH_4)_2SO_4$ and most of the τ enzyme between 60 and 70% saturation. The ϕ enzyme will not bind to DEAE-cellulose columns equilibrated with 8 mM phosphate buffer, pH 7.4, containing 1 mM dithiothreitol. This characteristic was used to further characterize the type of aldehyde dehydrogenase induction afforded by the chemicals used in this study.

RESULTS

It had been shown previously that the ϕ and τ enzymes are induced by phenobarbital and TCDD respectively. Induction of the ϕ enzyme is indicated by an increase in phenylacetaldehyde dehydrogenase activity as well as propionaldehyde dehydrogenase activity using the RR line. Phenobarbital has no effect on aldehyde dehydrogenase activity of the rr line (Table 1). TCDD treatment at 10 μg/kg induced propionaldehyde dehydrogenase enzyme activity about 20-fold in both lines of rats, but only a 2-fold induction of phenylacetaldehyde dehydrogenase activity was observed after TCDD treatment. Thus, a compound inducing only the ϕ enzyme will induce both propionaldehyde and phenylacetaldehyde dehydrogenase in RR rats but is ineffective in rr rats, whereas compounds inducing only the τ enzyme will induce propionaldehyde activity (about 8-fold) in both lines but have essentially no effect on the phenylacetaldehyde activity (<2-fold). Using this criterion we may then classify Mirex and DDT as inducers of the ϕ type enzyme activity. PCB and 3-MC must be regarded as inducing both the ϕ and τ enzymes. AAF appeared to elicit only a marginal induction of either enzyme under these conditions. β-Naphthoflavone (β-NF) induces the ϕ enzyme marginally. A relatively large increase in τ enzyme activity is seen in rr rats treated with β-NF but no statistically significant difference can be detected due to the large standard error. Several notes of caution are necessary. While several doses and times were tried, no systematic study of dose–response or time dependency curves has been made other than for TCDD, phenobarbital and β-naphthoflavone by this laboratory[5] and for DDT by Marselos and Hänninen[7]. Thus, the doses and time employed may not be optimal, and a compound which appears inactive by these tests might prove to be an inducing agent under other conditions. A positive response, however, is consistent with induction by the compounds.

To further show that the response noted in Table 1 was indeed enzyme induction, antibodies to the ϕ and τ enzymes were used to test for increased ϕ and τ antigens in the supernatant fraction of liver homogenates from animals treated with the various inducing compounds. The results of these studies are shown in Fig. 1. In all cases ϕ locates the well which contains antibody directed against phenobarbital-induced rat liver supernatant aldehyde dehydrogenase and τ is the location of the well containing antibody directed against the τ aldehyde dehydrogenase. The stain is for aldehyde dehydrogenase activity with propionaldehyde as substrate. The ϕ antibody gives only one precipitin line which reacts as aldehyde dehydrogenase but a second precipitin line of protein. The τ antibody shows only one coincident line for both enzyme and protein.

Well 1 contains rat liver supernatant from a phenobarbital-treated animal. Well 2 contains rat liver supernatant from an untreated animal. It is apparent that the ϕ antibody recognizes the phenobarbital-induced aldehyde dehydrogenase, but not the τ (well 5) or control aldehyde dehydrogenase. However, higher titer ϕ antibody will react with an aldehyde dehydrogenase in the liver supernatant from untreated animals indicating the presence of some ϕ enzyme in untreated animals. From the patterns we can conclude that 3-MC (well 3), PCB (well 6) and β-NF (well 7) induce both the ϕ and τ enzymes, while DDT (well 4), Mirex (well 8) and phenobarbital (well 1) induce only the ϕ enzyme. TCDD (well 5) induces only the τ enzyme. These results are consistent with those of Table 1 and indicate that β-NF does, in

Table 1. Effect of administration of foreign compounds on rat liver aldehyde dehydrogenase*

		NADH [nmoles/min/mg protein ± S.E.M.; (*n*)]		
		Cytosol		*Mitochondria*
Line	*Compound*	*Propionaldehyde*† (3.3 *mM*)	*Phenylacetaldehyde*† (0.33 *mM*)	*Propionaldehyde*† (3.3 *mM*)
RR	None	9.7 ± 1.4 (8)	11.0 ± 1.6 (14)	48.4 ± 2.5 (14)
RR	TCDD‡	180.6 ± 9.2§ (3)	26.6 ± 2.7 (3)	
RR	Phenobarbital	160.3 ± 15.6§ (4)	166.7 ± 27.2§ (4)	
RR	Mirex‡	250.9 ± 43.8§ (5)	169.0 ± 25.2§ (5)	65.9 ± 19.9 (5)
RR	DDT‡	241.5 ± 31.8§ (8)	158.7 ± 15.2§ (8)	122.7 ± 8.1§ (8)
RR	3-MC‡	194.4 ± 44.9§ (6)	23.7 ± 1.56§ (6)	47.6 ± 4.5 (6)
RR	PCB‡	287.5 ± 54.0§ (8)	95.8 ± 10.7§ (7)	63.3 ± 3.0‖ (8)
RR	AAF‡	34.1 ± 1.9§ (6)	27.5 ± 3.7‖ (6)	46.2 ± 2.0 (6)
RR	β-NF‡	66 ± 9.4§ (4)	29.6 ± 0.4§ (4)	93.9 ± 0.4§ (4)
rr	None	6.9 ± 0.68 (7)	8.6 ± 0.50 (7)	64.5 ± 4.90 (7)
rr	TCDD	222 ± 29.4§	19 ± 0.8 (3)	
rr	Mirex	7.7 ± 0.92 (4)	11.4 ± 1.77 (4)	69.7 ± 5.56 (4)
rr	DDT	10.1 ± 0.68‖ (8)	11.7 ± 0.38 (8)	131.8 ± 5.74§ (8)
rr	3-MC	67.1 ± 12§ (6)	12.5 ± 2.83 (6)	
rr	PCB	294.0 ± 37.4§ (5)	9.4 ± 0.8 (7)	55.7 ± 3.02 (7)
rr	β-NF	30 ± 10.5 (4)	6.7 ± 0.3 (4)	

*Adult rats of both sexes were injected intraperitoneally with either the compound or vehicle in the dosage schedule outlined in Methods. Livers were taken at the peak of the response and mitochondrial and cytosolic fractions prepared and analyzed for aldehyde dehydrogenase activity.

†Substrate.

‡Abbreviations are: TCDD: tetrachlorodibenzo-*p*-diopin; Mirex: dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuto[cd]pentalene (recrystallized Mirex was a gift from Dr. Casida of the University of California, Berkeley, CA.); DDT: 1,1,1-trichloro-2,2-bis-(*p*-chlorophenylethane) (purchased from Eastman); PCB1254 is a mixture of polychlorinated biphenyl molecules containing 54% chlorine by weight (this was a gift from Dr. N. Young, Colorado State University, Fort Collins, CO); 3-MC: 3-methylcholanthrene; AAF: acetylaminofluorene; and β-NF: β-naphthoflavone.

§$P < 0.001$.

‖$P < 0.01$.

Table 2. Partial purification of aldehyde dehydrogenase from Mirex-treated RR animals*

Fraction	Total activity (nmoles/min)	% Recovery	Specific activity (nmoles/min/mg protein)	Fold purification
Supernatant	4.5×10^5	100	99	
$(NH_4)_2SO_4$ 30–70% saturated	4.25×10^5	94	330	3.3
DEAE-cellulose	3.0×10^5	67	890	9.0

*After $(NH_4)_2SO_4$ fractionation, the enzyme which precipitated in the 30–70% saturated fraction was dissolved in and dialyzed against 8 mM Na phosphate and 1 mM dithiothreitol, pH 7.4, overnight. The column was equilibrated with the same buffer. The enzyme was applied to the column and eluted without binding.

fact, induce the τ enzyme in spite of the statistically insignificant figure in Table 1 (rr supernatant fraction with propionaldehyde as substrate).

Since both the φ and τ enzymes have been purified and their behavior during purification is known, a similar purification scheme was applied to aldehyde dehydrogenase from livers of rats treated with Mirex. The prediction was that the enzyme would behave as the φ enzyme on the basis of data in Table 1 and Fig. 1. The results are given in Table 2. A broad cut of ammonium sulfate saturation was used to assure obtaining both the φ and τ enzymes. The material obtained by ammonium sulfate precipitation was applied to a DEAE column. The enzyme eluted without binding and with nearly the same fold purification, 9.0 as obtained previously, 8.7, for the φ enzyme[5]. Taken alone these data would provide little information as to the nature of the enzyme induced by Mirex, but it does provide corroboration of the assertion that Mirex induces the φ enzyme in rat liver.

Statistically significant induction of mitochondrial aldehyde dehydrogenase activity was seen with DDT in both RR and rr rats. Induction of mitochondrial aldehyde dehydrogenase by PCB

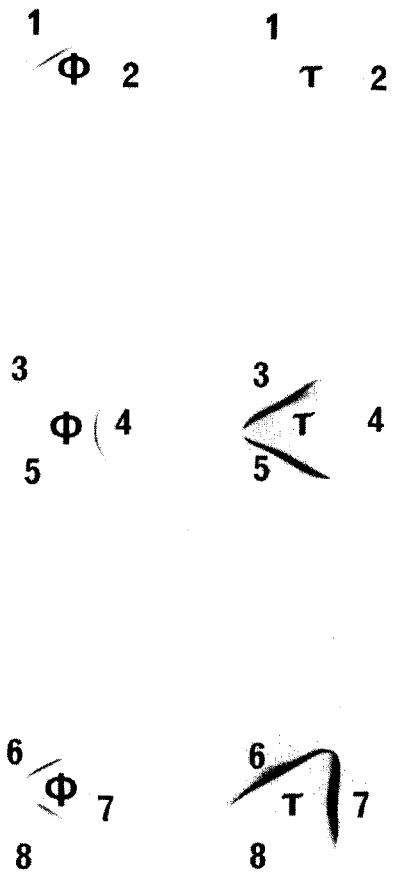

Fig. 1. Ouchterlony double diffusion with antibody prepared against ϕ and τ enzymes and aldehyde dehydrogenase antigens from liver supernatant fractions of treated rats. ϕ and τ locate the antibody-containing wells. The other wells contained 90,000 g supernatant fractions from rats treated with (1) phenobarbital, (2) untreated (corn oil or dioxane-injected animals gave similar response), (3) 3-MC, (4) DDT, (5) TCDD, (6) PCB, (7) β-NF and (8) Mirex. The plates were incubated at 4° for several days, washed thoroughly and stained for aldehyde dehydrogenase with propionaldehyde as substrate.

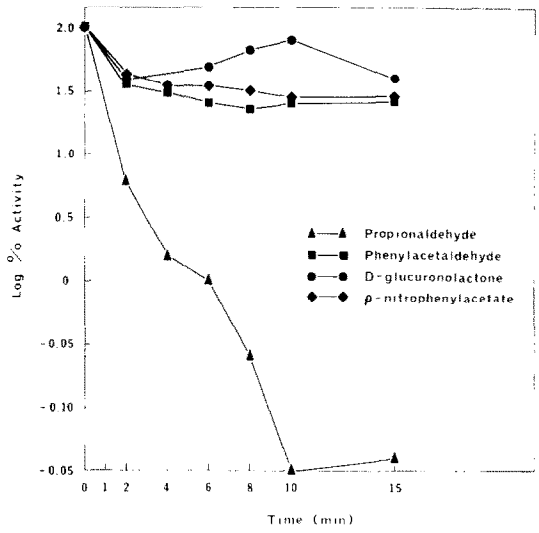

Fig. 2. Heat denaturation curves for rat liver enzymes from TCDD-treated animals. Enzyme was prepared by ammonium sulfate fractionation and DEAE column chromatography. A solution of 0.1 M phosphate, pH 7.4, was heated to 55° and enzyme added at zero time. Aliquots were removed at the time indicated, chilled, centrifuged and analysis for the various enzyme activation was carried out as outlined in the text. The results are given as log % of initial activity and are from three independent incubations.

and β-NF was only observed in RR rats. The substrate concentration was sufficiently high to measure both the matrix and intermembrane space enzymes[2, 3]. Previously we have found no significant induction of the mitochondrial enzyme by either TCDD[5] or phenobarbital[6]. This finding may complicate *in vivo* studies of the effect of DDT on aldehyde metabolism.

Aldehyde dehydrogenase enzymes have been reported to have other enzymatic activities. There is evidence that D-glucuronolactone dehydrogenase is induced by phenobarbital and is associated with the ϕ enzyme[7]. The homogenous horse liver aldehyde dehydrogenase has esterase activity[13]. Figure 2 provides evidence that a preparation of the τ propionaldehyde dehydrogenase can be separated from D-glucuronolactone dehydrogenase and phenylacetaldehyde dehydrogenase as well as from *p*-nitrophenylacetate esterase by heat denaturation. This is consistent with previous unpublished results which show that, as the τ enzyme is purified, phenylacetaldehyde dehydrogenase activity is lost. We have not yet tested either the ϕ or τ antiserum for ability to precipitate the other enzyme activities (esterase, glucuronolactone or phenylacetaldehyde dehydrogenase).

DISCUSSION

The work reported here shows that a large number of agents are capable of bringing about induction of one or the other rat liver supernatant aldehyde dehydrogenase enzymes. This could be demonstrated by use of the genetic susceptibility to induction of the ϕ enzyme, the kinetic properties of the ϕ and τ enzymes with phenylacetaldehyde as substrate, the use of antiserum prepared against the ϕ or τ enzyme, and finally physical characteristics of the ϕ enzyme as evidenced by behavior on an ion exchange column.

It is difficult to visualize a common parameter which the compounds have that brings about induction of the ϕ and τ enzymes since their structures are markedly different. The induction of both ϕ and τ enzymes by PCB may be due to different isomers contained in the mixture[14], but the induction by 3-methylcholanthrene and β-naphthoflavone would indicate interaction of these compounds with both systems. What advantage

there is to the rat in being able to induce these enzymes is unknown. Perhaps further work will lead to discovery of the "physiological substrate" for these enzymes.

A recent report by Törrönen *et al.*[15] indicates that phenobarbital induces a low K_m aldehyde dehydrogenase besides the high K_m ϕ enzyme in the cytosol of rat liver and that the induction is not controlled genetically since rr animals also show the increase. The increase is relatively small (79 per cent for 0.05 mM propionaldehyde and 56 per cent for 50 mM D-glucuronolactone). Verification of these inductions must await purification and physical separation of this low K_m enzyme, however. It is apparent that there is no relationship between this enzyme and the τ enzyme, whatever the relationship to the ϕ enzyme may prove to be.

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