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EFFECT OF PROPRANOLOL ON ETHANOL METABOLISM—EVIDENCE FOR THE ROLE OF MITOCHONDRIAL NADH OXIDATION*

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Abstract—Ethanol metabolism in the rat as measured *in vivo* by $^{14}CO_2$ production or *in vitro* by the removal of ethanol by liver slices was inhibited approximately 30 per cent by propranolol. There was no inhibitory effect of propranolol on rat liver alcohol dehydrogenase, catalase, NADPH-dependent microsomal ethanol oxidation or formate oxidation to $^{14}CO_2$. Propranolol inhibited fatty acid oxidation to $^{14}CO_2$ *in vivo* as well as by liver slices and isolated hepatic mitochondria. NADH oxidation by hepatic mitochondria was also reduced by propranolol. 2,4-Dinitrophenol treatment or chronic ethanol feeding of rats stimulated alcohol metabolism as well as hepatic mitochondrial NADH oxidation. These increases were abolished by propranolol. The effect of propranolol in blocking the increase in ethanol oxidation after chronic alcohol feeding appears to be related to its action on the mitochondrial re-oxidation of NADH to NAD. Propranolol inhibits mitochondrial NADH oxidation, while 2,4-dinitrophenol or chronic ethanol feeding stimulates this process. The present studies support the concept that the rate of hepatic ethanol metabolism is limited, at least in part, by the mitochondrial oxidation of NADH.

Propranolol is used in a variety of clinical disorders [1] and it has been suggested that this drug may also be useful in the treatment of alcoholism because of its apparent ability to modify some of the psychological or behavioral effects of alcohol [2–4]. We have examined some of the effects of propranolol on ethanol metabolism in the rat and found this agent to inhibit ethanol oxidation. The present studies suggest that this effect may be mediated by the action of propranolol on mitochondrial functions, especially by its inhibition of mitochondrial NADH oxidation. The fact that the action of 2,4-dinitrophenol, a drug known to stimulate mitochondrial NADH oxidation, is prevented by propranolol, lends further support to the concept that the effect of propranolol on ethanol metabolism is at the level of the mitochondria. The studies also support the hypothesis [5, 6] that the stimulation of ethanol metabolism by chronic alcohol feeding may involve enhanced mitochondrial oxidation of NADH.

METHODS

Propranolol (propranolol HCl, Inderal) was a generous gift from Dr. Henry L. LeMein, Ayerst Laboratories, New York.

The livers of female Sprague–Dawley rats (175–200 g) were excised and homogenized in 9 vol. of 0·25 M sucrose. The resulting homogenate was spun at 700 *g* for 10 min. The supernatant was then separated and centrifuged at 8700 *g* for 10 min. The resulting pellet was resuspended in 0·25 M sucrose and spun once more at 8700 *g* for 10 min, after which the pellet was resuspended in 0·25 M sucrose. The original 8700 *g* supernatant was centrifuged at 105,000 *g* for 1 hr to obtain a soluble and a microsomal pellet fraction.

The oxidation of ethanol *in vivo* and by liver slices, as well as the determination of alcohol dehydrogenase (ADH), catalase and NADPH-dependent microsomal ethanol oxidation (MEOS) were performed as previously described [7]. In the case of ^{14}C-ethanol oxidation to $^{14}CO_2$, the initial 2-hr point was used to monitor $^{14}CO_2$ production from ethanol, since previous studies indicated that under these conditions 2,4-dinitrophenol (DNP) produced the maximum increase in total ethanol oxidation to $^{14}CO_2$ *in vivo* [7]. Fatty acid oxidation *in vivo* was measured according to Reboucas and Isselbacher [8], while isolated mitochondrial fatty acid oxidation was measured by the method of Gordon [9]. Mitochondrial NADH oxidation was determined as described by Mackler [10] with the cofactor content determined fluorometrically [11]. In preliminary experiments it was shown that the disappearance of NADH and the appearance of NAD were linear over a 45-min period. In the absence of mitochondria, NADH was not spontaneously converted to NAD. NADH-dependent ethanol oxidation in the presence of mitochondria was assayed by a modification of the system of Rawat and Kuriyama [6] with the ethanol content determined by gas–liquid chromatography [12]. In preliminary experiments it was found that ethanol removal from the incubation medium was linear over a 90-min period. Mitochondrial NADH oxidation and NADH-dependent ethanol oxidation were carried out within 2 hr after sacrifice of the animal. For both the NADH oxidation and the NADH-dependent ethanol removal assays, the mitochondrial preparations were gassed with 95% O_2–5% CO_2 for 30 sec (10 liters/min) before use. Protein was determined according to Lowry *et al.* [13].

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Table 1. Effect of propranolol and 2,4-dinitrophenol on ^{14}C-ethanol oxidation to $^{14}CO_2$ *in vivo*

Treatment	Ethanol oxidation* (m-moles $^{14}CO_2$ produced/kg body wt/2 hr)	% Change from control
Control	8·7 ± 0·4	0
Propranolol, 2·5 mg/kg	8·1 ± 0·4	−7
Propranolol, 25 mg/kg	6·7 ± 0·2	−23†
Propranolol, 50 mg/kg	5·4 ± 1·2	+38†
2,4-Dinitrophenol	14·2 ± 1·1	+63†
2,4-Dinitrophenol plus propranolol, 50 mg/kg	5·7 ± 3·0	−34†

* Animals were injected intraperitoneally with 1-^{14}C-ethanol (1 μCi/m-mole), 1 g/kg, 12% w/v, in 0·154 M NaCl. Respiratory $^{14}CO_2$ was collected as described previously [7]. Animals were injected intraperitoneally with either 100 mM sodium phosphate buffer, pH 7·4 (control), sodium 2,4-dinitrophenolate (34 mg/kg, 3·2 mg/ml in 154 mM NaCl given 30 min before alcohol) or propranolol at the various doses listed (given as a solution of 25 mg/ml in 100 mM sodium phosphate, pH 7·4 just before the ethanol administration). There were three or four animals in each group.

† Statistically significant compared to the control, $P < 0{\cdot}01$.

Chronic ethanol administration was achieved by feeding rats for 10 days a liquid diet according to Lieber *et al.* [14], which was modified by using casein hydrolysate to replace part of the amino acid mixture with 36 per cent of the calories present as ethanol. Controls received the same diet with ethanol replaced by glucose. Chronic ethanol-fed animals and controls were fasted overnight before use while normal animals were not. Propranolol was dissolved in 100 mM sodium phosphate buffer, pH 7·4, and was injected intraperitoneally, at the various doses indicated, just prior to alcohol administration. DNP was injected intraperitoneally (34 mg/kg, 3·2 mg/ml in saline) 30 min prior to alcohol administration. Controls were injected with phosphate buffer or saline respectively.

RESULTS

Intraperitoneal injection of propranolol at a dose of 2·5 mg/kg produced no effect on ethanol oxidation to $^{14}CO_2$ *in vivo* (Table 1). However, when this dose was increased to 25 mg/kg, there was a 23 per cent inhibition and at 50 mg/kg a 38 per cent inhibition of ethanol oxidation. In contrast to the actions of propranolol, DNP (34 mg/kg) stimulated alcohol oxidation *in vivo* (Table 1), in confirmation of the previous observations by Israel *et al.*[15]. When propranolol and DNP were used together, there was a reduction in alcohol oxidation (34 per cent) which was comparable to that observed with propranolol alone. Since DNP stimulates ethanol oxidation by uncoupling oxidative phosphorylation and speeding reoxidation of NADH to NAD [15] and since propranolol blocked this effect, the data suggested that propranolol might be interfering with mitochondrial NADH oxidation, thereby inhibiting ethanol oxidation. It should be noted that Sakurada *et al.* [16] previously reported that oxidative phosphorylation and oxidation of NAD-linked substrates by heart mitochondria were depressed by propranolol (1·44 mM).

To determine if propranolol injection could affect mitochondrial function, we measured the effect of this drug on fatty acid oxidation. Propranolol was found to inhibit markedly the oxidation of 1-^{14}C-palmitate to $^{14}CO_2$ *in vivo* (Fig. 1). At a dose of 50 mg/kg, there was a 70 per cent reduction at 2 hr. However, under the same conditions, there was no effect of propranolol on ^{14}C-formate oxidation to $^{14}CO_2$ (Fig. 1). This suggested that the inhibition of $^{14}CO_2$ production from 1-^{14}C-ethanol and 1-^{14}C-palmitate produced by propranolol injection was not due to an inadequate distribution of $^{14}CO_2$ into the body pools of CO_2 and HCO_3^-. However, to clarify this aspect,

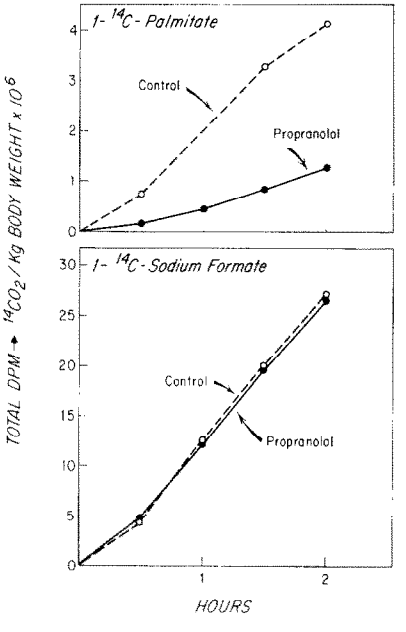

Fig. 1. Effect of propranolol on 1-^{14}C-palmitate and 1-^{14}C-formate oxidation to $^{14}CO_2$ by rats *in vivo*. Normal rats were injected intraperitoneally with 1-^{14}C-palmitate complexed to albumin (0·4 μmole/kg, 25 μCi/m-mole), sodium 1-^{14}C-formate (1 g/kg, 147 μCi/m-mole; 12%, w/v, in saline), propranolol (50 mg/kg, 25 mg/ml in sodium phosphate buffer, pH 7·4) or sodium phosphate buffer alone. Respiratory $^{14}CO_2$ was collected as described previously [7].

Table 2. Effect of propranolol on the activity of certain hepatic enzymes and on the oxidation of ethanol and 1-^{14}C-palmitate by liver slices

Propranolol (addition or treatment)		Liver slice oxidation*				Enzyme activity†					
		Ethanol		1-^{14}C-palmitate		Alcohol dehydrogenase		Catalase		MEOS	
In vivo‡	*In vitro*§	Rate	% Control	Rate	% Control	Rate	% Control	Rate	% Control	Rate	% Control
0	0	745	100	38,481	100	13·5	100	298	100	5·7	100
0	+	526	71‖	12,373	32‖	13·2	98	266	89	7·3	128
+	0	694	93	35,766	93	17·1	127	295	99	6·3	111
+	+	518	70‖	12,720	33‖	14·4	107	317	106	5·9	104

* Assay mixture of 3 ml consisted of 100 mM sodium phosphate, pH 7·4, 50 mM ethanol or 1 μM 1-^{14}C-palmitate (25 μCi/m-mole), 500 mg of liver slices and 24 mM sodium bicarbonate when fatty acid oxidation was measured. Rates of ethanol oxidation were expressed as μg ethanol removed/100 mg of liver slices/3 hr, while the rates of fatty acid oxidation were expressed as dis./min $\rightarrow$ $^{14}CO_2$/100 mg of liver slices/3 hr. The results are the means of three animals in each control and experimental group; duplicate samples of liver slices were taken from each rat. Ethanol content was determined by gas–liquid chromatography [12].

† Assay conditions were as follows: for alcohol dehydrogenase, the assay mixture of 3·3 ml consisted of 100 mM sodium phosphate, pH 7·4, 1·5 mM NAD, 50 mM ethanol and 0·1 ml of 105,000 *g* liver supernatant (1 mg protein). Rates were expressed as nmoles NADH formed/mg protein/min. For catalase, the assay mixture of 10 ml consisted of 1·5% sodium perborate, 150 mM sodium phosphate, pH 6·8, and 1·5 ml of whole liver homogenate (0·01 mg protein). Rates were expressed as μmoles perborate oxidized/mg protein/min. For microsomal ethanol oxidation (MEOS), the assay mixture of 3·0 ml consisted of 100 mM sodium phosphate, pH 7·4, 1·5 mM NADPH, 50 mM ethanol and washed microsomes (3·0 mg protein). Rates were expressed as nmoles acetaldehyde formed/mg protein/min.

‡ Propranolol was injected intraperitoneally (50 mg/kg, 25 mg/ml in sodium phosphate, pH 7·4) 3 hr prior to sacrifice. Controls were injected with phosphate buffer.

§ 1·7 mM propranolol in the incubation medium.

‖ Statistically significant compared to the control injected with phosphate buffer and assayed in the absence of propranolol *in vitro* ($P < 0·01$).

we measured the effect of propranolol on ethanol and palmitate oxidation by liver slices.

We observed that when 1·7 mM propranolol was added to normal liver slices, ethanol removal was inhibited by 29 per cent and oxidation of 1-^{14}C-palmitate to $^{14}CO_2$ was inhibited by 68 per cent (Table 2). However, as seen in Table 2, propranolol *in vitro* had no effect on ADH activity in the hepatic 105,000 *g* supernatant fraction, on catalase activity of crude homogenates, or on MEOS activity in washed microsomes. Our results with rat liver ADH differ from those of Duncan [17], who found that propranolol inhibited purified horse liver and yeast ADH *in vitro*, a finding we confirmed using our assay conditions at pH 7·4. In all likelihood, the failure of propranolol to inhibit rat liver 105,000 *g* supernatant ADH was related to the large amount of nonspecific protein in this fraction, leading to binding of the drug and thus reducing the free propranolol concentration.

Duncan [17] found that propranolol inhibited aldehyde dehydrogenase to a greater degree than purified horse liver or yeast ADH. Although we did not examine the effect of propranolol on the various aldehyde dehydrogenases of the rat liver [18], we did attempt to determine if propranolol pretreatment altered the activity of the enzymes believed responsible for the primary oxidation of ethanol, namely ADH, catalase and MEOS. It should be noted that when propranolol (50 mg/kg) was injected 3 hr prior to sacrifice there was no significant effect on either ethanol removal or palmitate oxidation (Table 2). Under the same conditions, 3-amino-1,2,4-triazole injected 3 hr prior to sacrifice inhibited catalase and MEOS activity [7]. This lack of effect of propranolol may have been due to a low effective drug concentration in the tissues at 3 hr or possibly to rapid conversion of propranolol to metabolites which were not inhibitory [19]. Propranolol (50 mg/kg) injected 0·5 or 1 hr prior to sacrifice also had no effect on ethanol removal or palmitate oxidation in liver slices (unpublished observations). However, as expected, when propranolol was added *in vitro* to liver slices from animals pretreated 3 hr earlier with the same drug, ethanol and palmitate oxidation were inhibited 29 and 69 per cent respectively. In contrast, ADH, catalase and MEOS activities were unaffected by propranolol either *in vitro* or *in vivo* (Table 2).

In view of these observations, it seemed possible that propranolol might be interfering with mitochondrial function. We therefore examined the effect of propranolol on isolated mitochondria and found that mitochondrial NADH oxidation was inhibited by about 30 per cent (Table 3). On the other hand, pretreatment of animals with DNP (34 mg/kg) stimulated mitochondrial NADH oxidation, and this effect was blocked by propranolol addition. The addition of DNP *in vitro* (0·1 mM) also enhanced mitochondrial NADH oxidation and the addition of propranolol also reduced this increase. When DNP was given *in vivo* as well as being added *in vitro*, mitochondrial NADH oxidation increased to a greater extent than could be demonstrated with either treatment alone. Again, the addition of propranolol to mitochondria inhibited this stimulation. Thus, propranolol inhibited mitochondrial NADH oxidation and blocked the stimulatory effect of DNP on this reaction.

To gain some insight on the mechanism of the inhibition of NADH oxidation, isolated mitochondria were examined in a fluorometer having an excitation wavelength of 340 nm and an emission wavelength of 450 nm. As seen in Fig. 2, when mitochondria were added to the cuvette containing only buffer, there was a decrease in fluorescence, suggesting oxidation of NADH. The addition of propranolol to the assay medium slowed this decrease but did not cause an increase in fluorescence. By contrast, the addition of succinate, which is a substrate for mitochondrial enzymes and leads to the production of NADH,

Table 3. Effect of propranolol and 2,4-dinitrophenol on NADH oxidation by washed mitochondria

Addition or treatment			Mitochondrial NADH oxidation*	
Propranolol (1·7 mM)	2,4-Dinitrophenol†		(nmoles oxidized/mg protein/min)	% Change from control
	In vitro	*In vivo*		
0	0	0	10·6 ± 0·8	0
+	0	0	7·4 ± 0·4	−30
0	0	+	14·1 ± 1·0	+33
+	0	+	9·4 ± 0·6	−11
0	+	0	12·9 ± 1·1	+21
+	+	0	7·5 ± 0·7	−29
0	+	+	15·1 ± 0·8	+42
+	+	+	9·7 ± 0·3	−8

* Assay mixture of 3 ml consisted of 100 mM sucrose, 100 mM sodium phosphate, pH 7·4, 80 mM potassium chloride, 5 mM $MgCl_2$, 25 mM sodium bicarbonate, 1·5 mM NADH and washed mitochondria (10 mg protein). The results are the mean of three experiments (± S.D.).

† Sodium 2,4-dinitrophenol was injected intraperitoneally (34 mg/kg, 3·2 mg/ml in 154 mM NaCl) 0·5 hr before sacrifice or was added at a final concentration of 0·1 mM. Controls received an equal volume of 154 mM NaCl. There were three animals in each group.

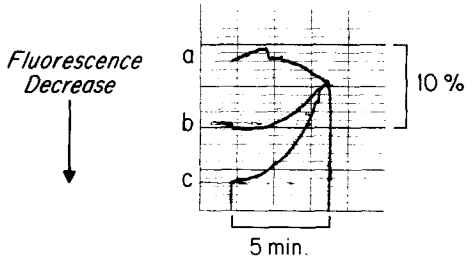

Fig. 2. Effect of propranolol and succinate of fluorescence by isolated mitochondria. The assay conditions were as described in Table 3, except that NADH was omitted. The mitochondria were examined in the fluorometer set at 340 nm excitation and 450 nm emission. Curve a represents the addition of succinate (10 mM); curve b results with the addition of propranolol (1·7 mM); and curve c is the control. The tracings are read from right to left. The 10% refers to change from the baseline fluorescence.

resulted in an increase in fluorescence. Thus, it appeared that propranolol did not inhibit mitochondrial NADH oxidation by acting as a substrate like succinate.

We also examined the effect of propranolol and DNP on ethanol oxidation in a system containing mitochondria, NADH and horse liver ADH. As seen in Table 4, in the absence of either mitochondria or horse liver ADH, no significant ethanol oxidation was detected; however, a small amount of ethanol was oxidized in the absence of NADH. In the complete system, the addition of propranolol inhibited ethanol oxidation, while the addition of DNP stimulated this process. The addition of propranolol completely blocked the stimulatory effect of DNP.

In other experiments (not shown), mitochondria prepared from animals pretreated with DNP led to enhanced ethanol oxidation in the presence of NADH

Table 4. Effect of propranolol and 2,4-dinitrophenol on ethanol oxidation by washed mitochondria

Incubation system*	Ethanol oxidation (μg ethanol removed/mg mitochondrial protein/1 hr)	% Change from control
Complete system	18·5 ± 3·6	0
− Mitochondria	0·7 + 0·1	−90
− Horse liver ADH	0·8 ± 0·1	−95
− NADH	3·7 ± 0·4	−80
+ Propranolol†	5·2 ± 1·3	−71
+ 2,4-Dinitrophenol†	33·3 ± 2·1	+80
+ 2,4-Dinitrophenol plus propranolol	8·8 ± 1·5	−52
− NADH + NAD†	38·8 ± 1·7	+109
− NADH + NAD plus 2,4-dinitrophenol	39·0 ± 1·9	+110

* Assay conditions as in Table 3, except that horse liver ADH (0·5 mg protein) and 6 mM ethanol were included. The complete system included NADH, 1·5 mM.

† Where indicated, 2,4-dinitrophenol was present at a concentration of 0·1 mM, propranolol, 1·7 mM, and NAD, 1·5 mM.

Table 5. Effect of chronic ethanol feeding and propranolol on ethanol oxidation *in vivo* and *in vitro* and on various hepatic mitochondrial functions

Assay	Propranolol (addition or treatment)	Activity: Control (n=6)	Activity: Chronic alcohol-fed group* (n=6)	P
1 1-^{14}C-ethanol oxidation *in vivo*†	0	6·0 ± 0·7	8·9 ± 1·0	< 0·01
	+	5·4 ± 0·9	5·4 ± 0·8	NS
2 Ethanol oxidation *in vitro*‡	0	600 ± 30	760 ± 40	< 0·01
	+	460 ± 40	475 ± 45	NS
3 Mitochondrial NADH oxidation§	0	13·0 ± 0·7	17·8 ± 0·7	< 0·01
	+	12·9 ± 0·2	12·6 ± 0·3	NS
4 NADH-dependent mitochondrial ethanol oxidation‖	0	6·9 ± 0·7	8·4 ± 0·8	< 0·01
	+	3·0 ± 1·0	3·1 ± 1·1	NS
5 Mitochondrial fatty acid oxidation¶	0	523 ± 14	370 ± 25	< 0·05
	+	0	0	NS

* Animals were fed for 10 days a liquid diet containing 36% of the calories as ethanol. The control was fed the same diet with ethanol replaced by glucose.

† Assay conditions, see Table 1. Rates were expressed as m-moles $^{14}CO_2$ produced/kg body wt/2 hr. (mean + S.D.). Propranolol was injected just prior to ethanol at a dose of 50 mg/kg.

‡ Assay mixture of 3·0 ml consisted of 100 mM sodium phosphate, pH 7·4, 50 mM ethanol and 500 mg of liver slices. Rates were expressed as μg ethanol removed/100 mg wet wt liver slice/3 hr (mean ± S.D.). In this and in experiments 3–5, propranolol, when added, was present at 1·7 mM.

§ For assay conditions, see Table 3. Rates were expressed as nmoles NADH oxidized/mg protein/min (mean ± S.D.).

‖ For assay conditions, see Table 4. Rates were expressed as nmoles ethanol removed/mg protein/min (mean ± S.D.).

¶ Assay conditions as in Table 3, except that the substrate was 1 μM 1-^{14}C-palmitate (25 μCi/m-mole). Rates were expressed as dis./min → $^{14}CO_2$/mg protein/1 hr (mean ± S.D.). Flasks with no mitochondria yielded, on the average, 170 dis./min.

and horse liver ADH; this effect could also be blocked by the addition of propranolol. As indicated in Table 4, when ADH was present in the medium and NAD was substituted for NADH, there was a significantly enhanced rate of alcohol oxidation which was not affected by DNP.

We also studied the effect of chronic ethanol feeding and propranolol on ethanol oxidation and on a number of mitochondrial functions (Table 5). Chronic ethanol feeding, as observed previously [20], increased ethanol oxidation *in vivo* and *in vitro*, and this increase was abolished by propranolol. Chronic ethanol feeding also stimulated mitochondrial NADH oxidation, and alcohol oxidation by mitochondria in the presence of NADH and ADH; these increases were also blocked by propranolol. In confirmation of the findings of Gordon [9], fatty acid oxidation by isolated mitochondria was decreased by chronic ethanol feeding. The addition of propranolol to mitochondria completely blocked all fatty acid oxidation.

DISCUSSION

Recent studies indicate that factors other than the amount or activity of hepatic ADH are responsible for the regulation of alcohol metabolism [20]. Of the other possible mechanisms, it would appear that the mitochondrial re-oxidation of NADH, formed during the ADH reaction, may be a rate-limiting factor [5,21–24]. Consistent with this concept have been the observations from several laboratories (15, 25, 26) showing that 2,4-dinitrophenol (DNP), which uncouples mitochondrial oxidative phosphorylation and thereby accelerates NADH re-oxidation, increases ethanol metabolism *in vivo* and *in vitro*.

The present studies with propranolol as well as DNP emphasize the important role of mitochondrial re-oxidation of NADH in ethanol metabolism. Thus, we observed that propranolol inhibited ethanol metabolism and interfered with mitochondrial function, especially fatty acid oxidation to CO_2. Propranolol did not inhibit ADH, catalase, NADPH-dependent microsomal ethanol oxidation (MEOS) or formate oxidation to CO_2. Propranolol also blocked the stimulatory effects of DNP on mitochondrial NADH oxidation and ethanol metabolism. The mechanism for the effect of propranolol in inhibiting mitochondrial NADH oxidation is not clear. While this action could be due to β-adrenergic receptor blockade, the high concentrations needed for the NADH oxidation effect suggest that the action may have been due to nonspecific stabilization of the mitochondrial membranes.

It has also been shown by many observers that chronic alcohol administration results in increased ethanol metabolism [20], but the mechanism for this effect remains debatable. There are conflicting data on the possible roles of ADH, catalase and MEOS as explanations for this phenomenon [20]. One theory is that chronic ethanol administration leads to altered mitochondrial function and increasing mitochondrial NADH oxidation [5, 6]. In the present report, mitochondrial NADH oxidation was increased in chronic ethanol-fed rats and was blocked by propranolol. However, Cederbaum *et al.* [27] observed that in their experiments NAD-dependent

oxidation of ethanol in the presence of mitochondria was not stimulated with chronic ethanol feeding.

The discrepancy between the data of Cederbaum *et al.* [27] and those of the present report and the results of Rawat and Kuriyama [6] may be explained in part by the methods used to measure mitochondrial ethanol oxidation. Thus Cederbaum *et al.* [27] added NAD plus ADH to mitochondria, while NADH was added in the present study and in that of Rawat and Kuriyama [6].

In the present experiments, when NADH and ethanol were added to mitochondria, in all likelihood the NADH was first oxidized to NAD, which then functioned together with ADH to convert ethanol to acetaldehyde. The latter was then oxidized to acetate by means of the mitochondrial acetaldehyde dehydrogenase [18]. Under these conditions, the rate limiting step appeared to be the conversion of NADH to NAD. Support for this concept was the fact that when NADH re-oxidation to NAD by mitochondria was stimulated by DNP *in vivo* or *in vitro*, ethanol oxidation also increased (Tables 3 and 4). Furthermore, as shown in Table 4, ethanol oxidation also was greater when NAD rather than NADH was added to mitochondria and, under these conditions, the addition of DNP provided no further increase in ethanol metabolism (in contrast to its action when NADH was the added cofactor). Therefore, it would appear that when NAD is added to the system, as in the studies of Cederbaum *et al.* [27], NADH re-oxidation is no longer rate limiting, but the more likely limiting step is the oxidation of acetaldehyde to acetate. In all probability, the failure of Cederbaum *et al.* [27] to have observed changes in ethanol metabolism with mitochondria from chronic ethanol-fed rats may have been due to the fact that these investigators added ADH plus NAD rather than NADH to isolated mitochondria.

We conclude that the effects of propranolol and DNP on ethanol metabolism indicate that mitochondrial NADH oxidation must play an important role in the overall metabolism of ethanol. The present studies also support the concept of Videla and Israel [5] and of Rawat and Kuriyama [6] that the increased ethanol metabolism associated with chronic ethanol feeding may be due, at least in part, to increased mitochondrial NADH oxidation.

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