Effects of acute ethanol administration on rat liver 5-aminolaevulinate synthase activity

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1. Liver 5-aminolaevulinate (ALA) synthase activity of 24 h-starved rats is maximally increased at 4 h after intraperitoneal administration of a 1.6 g/kg body wt. dose of ethanol. Larger doses cause a dose-dependent decrease in the extent of this stimulation, exhibiting a reciprocal relationship with an elevation of hepatic haem concentration, as suggested by the simultaneous increase in the haem saturation of tryptophan pyrrolase. 2. ALA synthase induction by ethanol is abolished if the above increase in pyrrolase saturation with haem is enhanced by theophylline, but is potentiated when the increase in the haem saturation is inhibited by anti-lipolytic agents. 3. ALA synthase induction by ethanol is also inhibited by inhibitors of alcohol dehydrogenase and aldehyde dehydrogenase. Acetaldehyde and acetate are, however, not responsible; they both decrease ALA synthase activity and increase the haem saturation of tryptophan pyrrolase. These latter effects of acetaldehyde are not mediated by acetate. 4. ALA synthase activity is also stimulated by succinate, which, however, also increases the haem saturation of tryptophan pyrrolase. 5. Ethanol does not influence the rate of ALA synthase degradation. 6. It is suggested that ethanol increases rat liver ALA synthase activity as a result of its own metabolism by the alcohol dehydrogenase-dependent pathway by a mechanism not involving decreased degradation of the former enzyme or the participation of the metabolites acetaldehyde and acetate.

INTRODUCTION

Of the various effects of ethanol on haem metabolism (for reviews, see Elder, 1976; Ostroff, 1981), the enhancement of rat liver ALA synthase (5-aminolaevulinate synthase, EC 2.3.1.37) activity by acute administration of the drug (Shanley *et al.*, 1968; Rubin *et al.*, 1970; Beattie *et al.*, 1973) is of particular interest from at least two viewpoints. First, very little is known about its mechanism(s), other than the suggestion that it involves ethanol metabolism by NAD⁺-dependent alcohol dehydrogenase (EC 1.1.1.1) and/or the influence of such metabolism on availability of haem precursors. The second point concerns the relationship between the activity of ALA synthase (whose synthesis is subject to feedback control by haem) and the haem saturation of tryptophan pyrrolase (tryptophan 2,3-dioxygenase, EC 1.13.11.11). This saturation is used as an indirect means of assessing changes in intracellular haem availability and is generally inversely related to ALA synthase activity (Welch & Badawy, 1980; Badawy *et al.*, 1981; Badawy & Morgan, 1982). The effects of the amino acid tryptophan on these two parameters are of particular importance in this context. Thus tryptophan, which decreases ALA synthase activity in rat liver (Badawy *et al.*, 1981; Badawy & Morgan, 1982), activates tryptophan pyrrolase by the so-called substrate-type mechanism, which involves both increased haem saturation and stabilization of the enzyme (Schimke *et al.*, 1965; Badawy & Evans, 1975b; Badawy *et al.*, 1981). Because acute ethanol administration increases rat liver tryptophan pyrrolase activity by a substrate-type mechanism

involving a non-esterified-fatty-acid-mediated displacement of serum-protein-bound tryptophan and a consequent increase in its availability to the liver (Badawy & Evans, 1975a, 1976b), the drug-induced increase in pyrrolase saturation with haem is at variance with its ability to induce ALA synthase activity. The experiments whose results are described and discussed in the present paper were therefore designed to address these points. A brief summary of this work has appeared in abstract form (see Badawy *et al.*, 1986).

MATERIALS AND METHODS

Chemicals

The aldehyde dehydrogenase inhibitor disulfiram (tetraethylthiuram disulphide) and the β -adrenoceptor blocker propranolol [1-(isopropylamino)-3-(naphthyl-oxy)propan-2-ol] were gifts from Berk Pharmaceuticals (Eastbourne, East Sussex, U.K.) and Imperial Chemical Industries (Alderley Park, Macclesfield, Cheshire, U.K.) respectively. The alcohol dehydrogenase inhibitor pyrazole was purchased from Koch–Light (Colnbrook, Bucks., U.K.), and the hydrochloride of the opiate-receptor antagonist naloxone (17-allyl-4,5 α -epoxy-3,14-dihydroxynormorphinan-6-one), which was in ampoules, each containing 0.4 mg/ml, was obtained from Winthrop Laboratories (Surrey, Surrey, U.K.). All other chemicals (of the purest commercially available grades) were purchased from BDH Chemicals or Sigma Chemical Co. (both of Poole, Dorset, U.K.).

Abbreviation used: ALA, 5-aminolaevulinate.

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Animals and treatment

Locally bred male Wistar rats (150–170 g) were maintained on cube diet 41B (Oxoid) and water, and were starved for 24 h before being killed (between 13:00 and 15:00 h) either by decapitation (for the determination of concentrations of serum non-esterified fatty acids and serum and liver tryptophan) or by stunning and cervical dislocation (for all other determinations).

All chemicals were dissolved in 0.9% (w/v) NaCl (saline) and were administered intraperitoneally in the following doses: acetaldehyde (156 mg/kg), cycloheximide (10 mg/kg), disulfiram (100 mg/kg), ethanol (0.4–4 g/kg), naloxone hydrochloride (5 mg/kg), propranolol (10 mg/kg), pyrazole (272 mg/kg) and sodium acetate, sodium succinate and theophylline (100 mg/kg each). Except for the naloxone ampoules (12.5 ml/kg) and the ethanol solutions (20 ml/kg each), solutions of all other administered compounds were given in a volume of 2 ml/kg body wt. each. Control rats received an appropriate volume of saline.

Chemical, enzymic and other determinations

Tryptophan pyrrolase activity was determined in fresh-liver homogenates either in the absence (holoenzyme activity) or in the presence (total enzyme activity) of added ($2\ \mu\text{M}$) haematin (Badawy & Evans, 1975b; Badawy, 1981). The saturation of tryptophan pyrrolase with its cofactor haem was expressed as the percentage haem saturation ($100 \times \text{holoenzyme activity} / \text{total enzyme activity}$). ALA synthase activity was determined in fresh-liver homogenates as described by Badawy & Morgan (1980). The concentration of serum non-esterified fatty acids was determined by the method of Mikac-Dević *et al.* (1973), whereas those of free (ultrafiltrable) serum, total (acid-soluble) serum and liver tryptophan were determined fluorimetrically as described by Badawy & Evans (1976a); the latter was determined in pieces of liver isolated by a freeze-clamp. The results were analysed statistically by Student's *t* test.

RESULTS AND DISCUSSION

Effects of administration of various doses of ethanol on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

These effects, examined at 4 h in 24 h-starved rats, are illustrated in Fig. 1. As shown, ALA synthase activity was not significantly altered by a 0.4 g/kg, but was increased by 71% ($P < 0.005$) by a 0.8 g/kg, and by 388% ($P < 0.001$) by a 1.6 g/kg, body wt. dose of ethanol. The effects of larger doses of ethanol were unexpected in that there was a dose-dependent decrease in the extent of the above maximum induction. The results in Fig. 1 also show that the saturation of tryptophan pyrrolase with haem (an index of haem availability, expressed as the percentage haem saturation of the enzyme) was not significantly altered by the 0.4 g/kg dose of ethanol, but was increased in a dose-dependent fashion by doses of the drug of 0.8–4 g/kg body wt., by 30–178% ($P = 0.025$ – 0.001).

The time course of induction of ALA synthase activity by the 1.6 g/kg dose of ethanol is shown in Fig. 2. ALA synthase activity was not significantly altered at 0.5 h, but was increased by 55% ($P < 0.005$) at 1 h and by a maximum of 403% ($P < 0.001$) at 4 h before returning

Fig. 1. Effects of acute administration of various doses of ethanol on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

Rats were starved for 24 h and received, 4 h before death, an intraperitoneal injection of various doses of ethanol as shown or an equal volume (20 ml/kg body wt.) of 0.9% (w/v) NaCl (saline). ALA synthase activity (O) and the percentage haem saturation of tryptophan pyrrolase (●) were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. (bars) for each group of four rats. The ethanol-induced increase in pyrrolase saturation with haem was due to significant increases only in the holoenzyme, but not the total enzyme, activity.

to basal values at 8 h after ethanol administration. This latter increase is not dissimilar to that (388%) given in Fig. 1. The results in Fig. 2 also show that the haem saturation of tryptophan pyrrolase was significantly increased (though moderately) only at 3, 4 and 5 h after administration of the 1.6 g/kg dose of ethanol by 23%, 51% and 25% respectively ($P = 0.01$ – 0.001). The increase at 4 h was not much different from that (43%) shown in Fig. 1.

The results described so far have demonstrated that liver ALA synthase activity of starved rats is induced by acute administration of doses of ethanol of up to 1.6 g/kg body wt., and that larger doses are less effective (i.e. they cause a decrease in the extent of this induction). The next two sections deal respectively with possible mechanisms of this decrease and the greater induction caused by smaller doses of ethanol.

Mechanism of the weaker induction of rat liver 5-aminolaevulinate synthase activity caused by administration of large doses of ethanol

This weaker induction (Fig. 1) can best be explained by the larger doses of ethanol (> 1.6 g/kg) exerting a haem-mediated repression of synthesis of ALA synthase induced fully by the smaller doses. That larger doses of ethanol increase intracellular hepatic haem availability is suggested by the ability of such doses to increase the haem saturation of tryptophan pyrrolase, by the tryptophan-dependent mechanism described in the In-

production. Further support for this interpretation is provided by the data in Tables 1 and 2 from experiments with a 4 g/kg body wt. dose of ethanol, which show that prevention by the β -adrenoceptor-blocking agent propranolol of the ethanol-induced increase in the haem saturation of tryptophan pyrrolase is associated with a

Fig. 2. Time course of the effects of acute administration of a 1.6 g/kg body wt. dose of ethanol on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

Rats were starved for 24 h before being killed and received an intraperitoneal injection of ethanol (1.6 g/kg) at zero time. ALA synthase activity (○) and the percentage haem saturation of tryptophan pyrrolase (●) were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. (bars) for each group of four rats.

concomitant potentiation of ALA synthase induction (Table 1), whereas potentiation by the phosphodiesterase inhibitor theophylline of the ethanol-induced increase in pyrrolase saturation with haem totally abolishes the ALA synthase induction (Table 2). In experiments not detailed here, we found that the opiate antagonist naloxone modified the ethanol effects on ALA synthase activity and pyrrolase saturation with haem in a manner similar to propranolol. We have previously shown (Badawy & Morgan, 1982) that the reciprocal effects on these two parameters of haem metabolism exerted by the lipolytic agents endotoxin, morphine and theophylline can be prevented by the above two anti-lipolytic agents propranolol and naloxone, as in the present work with ethanol. Also, as shown previously with lipolytic agents (Badawy & Morgan, 1982), we found in the present work that ethanol (4 g/kg) increased tryptophan availability to the liver by increasing circulating non-esterified fatty acid concentration (presumably by enhancing lipolysis, lipogenesis or both) and that both the former effects are prevented by pretreatment of rats with propranolol or naloxone (results not shown). It may therefore be concluded that the weaker induction of ALA synthase activity by doses of ethanol above 1.6 g/kg body wt. is caused by a tryptophan-dependent haem-mediated repression of the synthesis of the enzyme.

Mechanism of induction of rat liver 5-aminolaevulinate synthase activity by acute ethanol administration

That such an induction (Figs. 1 and 2) does not involve simple activation of the enzyme by ethanol is suggested by the results of previous experiments *in vitro* (Rubin *et al.*, 1970). The possibility proposed by Beattie *et al.* (1973) that ethanol may act by inducing a conformational change in mitochondrial membranes has not, however, been examined. That ALA synthase induction by acute ethanol administration involves increased synthesis *de novo* of the enzyme, rather than a decrease in the rate of its degradation, is suggested by the ability of the metabolic inhibitors actinomycin D and puromycin to block this induction (Rubin *et al.*, 1970) and by the failure of

Table 1. Modification by propranolol of the effects of acute ethanol administration on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

Rats were starved for 24 h and received, 4 h before death, an intraperitoneal injection of either ethanol (4 g/kg body wt.) or an equal volume (20 ml/kg) of 0.9% (w/v) NaCl (saline). The animals also received, at 0.5 h before these treatments, a similar injection of either propranolol (10 mg/kg) or an equal volume (2 ml/kg) of saline. ALA synthase and tryptophan pyrrolase activities and the haem saturation of the latter enzyme were determined as described in the Materials and methods section. Synthase activity is expressed in nmol of ALA formed/min per g wet wt. of liver, whereas pyrrolase activities are in μ mol of kynurenine formed/h per g wet wt. Pyrrolase saturation with haem is expressed as the percentage haem saturation ($100 \times$ holoenzyme activity/total enzyme activity). Values are means $\pm$ S.E.M. for each group of four rats. The values in columns (2) and (3) are compared with those in column (1), whereas those in column (4) are compared with those in column (3), and the significance of the differences is indicated as follows: * $P < 0.01$; *** $P < 0.001$.

Determination	Pretreatment ...	Saline		Propranolol	
	Treatment ...	Saline (1)	Ethanol (2)	Saline (3)	Ethanol (4)
Synthase activity		0.42 $\pm$ 0.04	1.58 $\pm$ 0.10***	0.44 $\pm$ 0.05	2.82 $\pm$ 0.34***
Pyrrolase activity:					
Holoenzyme		2.1 $\pm$ 0.15	7.5 $\pm$ 0.38***	2.2 $\pm$ 0.06	3.6 $\pm$ 0.36*
Total enzyme		7.0 $\pm$ 0.10	13.2 $\pm$ 0.91***	7.1 $\pm$ 0.30	12.3 $\pm$ 0.34***
Pyrrolase haem saturation (%)		30 $\pm$ 2	57 $\pm$ 3***	31 $\pm$ 1	29 $\pm$ 3

Table 2. Modification by theophylline of the effects of acute ethanol administration on rat liver 5-aminolaevulinate synthase activity and the haem saturation of tryptophan pyrrolase

Experimental details, design, expression of results and their comparisons are as described in Table 1, except that the rats were pretreated, 0.5 h before administration of ethanol or saline, with an injection of either theophylline (100 mg/kg) or an equal volume (2 ml/kg) of saline. The significance of the differences is indicated as follows: $\dagger\dagger P < 0.02$; $*P < 0.01$; $**P < 0.005$; $***P < 0.001$.

Determination	Pretreatment ...	Saline		Theophylline	
	Treatment ...	Saline (1)	Ethanol (2)	Saline (3)	Ethanol (4)
Synthase activity		0.50 ± 0.03	$1.59 \pm 0.12^{***}$	$0.31 \pm 0.03^{**}$	$0.54 \pm 0.06^{\dagger\dagger}$
Pyrrolase activity:					
Holoenzyme		2.8 ± 0.27	$6.7 \pm 0.35^{***}$	$6.0 \pm 0.13^{***}$	$11.6 \pm 0.45^{***}$
Total enzyme		7.4 ± 0.21	$10.3 \pm 0.70^*$	$8.9 \pm 0.36^{\dagger\dagger}$	$13.4 \pm 0.81^{**}$
Pyrrolase haem saturation (%)		38 ± 2	$65 \pm 2^{***}$	$67 \pm 3^{***}$	$87 \pm 4^*$

ethanol in the present work to influence the rate of ALA synthase degradation. The half-life [as determined in 24 h-starved rats treated 4 h earlier with either ethanol (1.6 g/kg) or saline and examined for ALA synthase activity both then and at 1 or 2 h after treatment with cycloheximide (10 mg/kg) to block further synthesis of the enzyme protein] was found to be 1.60 h in control (saline-treated) and 1.50 h in ethanol-treated rats. Although Tischler (1980) suggested that an increase in the $[NADH]/[NAD^+]$ ratio, which is one of the major effects of ethanol oxidation by NAD^+ -dependent alcohol dehydrogenase (see Krebs, 1968), decreases proteolysis, the above results clearly suggest that this is not so in the case of ALA synthase induction by ethanol. It may therefore be concluded that acute ethanol administration increases rat liver ALA synthase activity by stimulating the synthesis of the enzyme.

That ethanol metabolism by alcohol dehydrogenase could be involved in the drug-induced increase in ALA synthase activity is suggested by the finding by Beattie *et al.* (1973) that pyrazole [an inhibitor of the former enzyme (Lester *et al.*, 1968)] causes a strong (75%) inhibition of this increase. A similar inhibition by pyrazole (76%) is also seen in the present work (Table 3), which also shows that an even stronger (90%) inhibition is caused by pretreatment of rats with the aldehyde dehydrogenase inhibitor disulfiram. Neither agent exerted any significant effects on ALA synthase activity in control (saline-treated) rats. In the experiments described in Table 3, a large dose of ethanol (4 g/kg) was used to examine the possibility that the marked increase in the pyrrolase saturation with haem caused by large doses of ethanol could be secondary to ALA synthase induction and a consequent increase in hepatic haem formation, i.e. whether ethanol activates tryptophan pyrrolase by a cofactor-type mechanism, as suggested by Sardesai & Provido (1972) and initially by us (Badawy & Evans, 1973), rather than by the well-documented substrate (tryptophan)-type mechanism (for references see the Introduction). The results in Table 4 demonstrate that the ethanol-induced increase in pyrrolase activity and saturation with haem are not influenced by pyrazole or disulfiram, despite the ability of these two agents to block the ALA synthase induction (Table 3), thus excluding the above (cofactor-type) possibility and also

Table 3. Inhibition by pyrazole and disulfiram of the enhancement of rat liver 5-aminolaevulinate synthase activity by acute ethanol administration

Rats were starved for 24 h and received, 4 h before death, an intraperitoneal injection of either ethanol (4 g/kg body wt.) or an equal volume (20 ml/kg) of saline. The animals also received, at 0.5 h before the above treatments, a similar injection of pyrazole (272 mg/kg), disulfiram (100 mg/kg) or an equal volume (2 ml/kg) of saline. ALA synthase activity was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. for each group of four rats. The effects of ethanol alone are compared with values observed in the saline-treated controls in the corresponding column, and the significance of the differences is indicated as follows: $***P < 0.001$.

Pretreatment	Treatment ...	ALA synthase activity (nmol of ALA formed/min per g wet wt. of liver)	
		Saline	Ethanol
Saline		0.42 ± 0.04	$2.38 \pm 0.18^{***}$
Pyrazole		0.44 ± 0.03	$1.05 \pm 0.05^{***}$
Disulfiram		0.43 ± 0.02	$0.62 \pm 0.04^{***}$

suggesting that the pyrrolase activation is unrelated to ethanol metabolism.

The results in Table 3 suggest that ethanol metabolism by alcohol dehydrogenase is involved in the drug-induced induction of ALA synthase activity and that the products of ethanol oxidation (acetaldehyde, acetate or both) may play a role. The failure of administered acetaldehyde or acetate to increase ALA synthase activity (Table 5), however, excludes these two metabolites as direct mediators of the ethanol effect. Both agents actually decreased ALA synthase activity by 19% and 29% respectively at 4 h after administration and caused simultaneous increases in the haem saturation of tryptophan pyrrolase, of 169% and 97% respectively (Table 5). The ability of acetaldehyde to exert this latter effect has previously been demonstrated in fed rats (Badawy & Evans, 1976b), whereas the similar effect exerted by acetate in 24 h-starved animals is reported here for the first time and

Table 4. Failure of pyrazole and disulfiram to inhibit the increase in the haem saturation of rat liver tryptophan pyrrolase caused by acute ethanol administration

Experimental details, design and comparison of results are as described in Table 3, except that tryptophan pyrrolase activity was determined as described in the Materials and methods section and is expressed in μmol of kynurenine formed/h per g wet wt. of liver. The haem saturation of the pyrrolase is expressed as the percentage haem saturation ($100 \times \text{holoenzyme activity}/\text{total enzyme activity}$). Values are means $\pm$ S.E.M. for each group of four rats. The significance of the differences is indicated as follows: $\dagger P < 0.05$; $*P < 0.01$; $**P < 0.005$; $***P < 0.001$.

Pretreatment	Saline			Ethanol		
	Pyrrolase activity		Pyrrolase saturation with haem (%)	Pyrrolase activity		Pyrrolase saturation with haem (%)
	Holoenzyme	Total enzyme		Holoenzyme	Total enzyme	
Saline	2.0 ± 0.13	7.6 ± 0.56	26 ± 2	$5.2 \pm 0.11^{***}$	$11.2 \pm 0.53^{**}$	$46 \pm 1^{***}$
Pyrazole	2.2 ± 0.02	7.6 ± 0.36	29 ± 1	$5.8 \pm 0.55^{***}$	$11.2 \pm 0.82^*$	$52 \pm 2^{***}$
Disulfiram	2.2 ± 0.14	7.3 ± 0.64	30 ± 1	$5.4 \pm 0.46^{***}$	$9.8 \pm 0.82\dagger$	$55 \pm 4^{***}$

Table 5. Effects of administration of acetaldehyde, acetate and succinate on rat liver 5-aminolaevulinate synthase and tryptophan pyrrolase activities

Rats were starved for 24 h and received, 4 h before death, an intraperitoneal injection of acetaldehyde (156 mg/kg body wt.), sodium acetate (100 mg/kg), sodium succinate (100 mg/kg) or an equal volume (2 ml/kg) of saline. ALA synthase and tryptophan pyrrolase activities and the haem saturation of this latter enzyme were determined as described in the Materials and methods section and are expressed as in Table 1. Values are means $\pm$ S.E.M. for each group of four rats. The significance of the differences between experimental groups and the control (saline) group is indicated as follows: $\dagger\dagger P < 0.02$; $**P < 0.005$; $***P < 0.001$.

Treatment	Pyrrolase activity		Pyrrolase saturation with haem (%)	ALA synthase activity
	Holoenzyme	Total enzyme		
Saline	2.7 ± 0.23	8.4 ± 0.03	32 ± 2	0.52 ± 0.02
Acetaldehyde	$13.4 \pm 0.78^{***}$	$17.5 \pm 0.29^{***}$	$77 \pm 3^{***}$	$0.42 \pm 0.01^{**}$
Acetate	$7.0 \pm 0.18^{***}$	$11.2 \pm 0.45^{***}$	$63 \pm 1^{***}$	$0.37 \pm 0.04\dagger\dagger$
Succinate	$4.1 \pm 0.35\dagger\dagger$	8.5 ± 0.09	$48 \pm 4\dagger\dagger$	$0.77 \pm 0.07\dagger\dagger$

should be contrasted with the decrease in haem saturation known (Badawy, 1977) to occur at a later time interval (24 h) after administration of a much larger dose (1.9 g/kg).

In experiments not shown here, we found that the increase in the haem saturation of tryptophan pyrrolase (56%) and the associated decrease in ALA synthase activity (41%) observed at 4 h after administration to 24 h-starved rats of acetaldehyde (156 mg/kg) were not influenced by pretreatment of rats with the aldehyde dehydrogenase inhibitor disulfiram (100 mg/kg). These findings therefore suggest that the above effects of acetaldehyde are not mediated by acetate. This latter suggestion is also compatible with the idea that acetaldehyde could exert its reciprocal effects on ALA synthase activity and pyrrolase saturation with haem by enhancing lipolysis [as do other lipolytic agents such as endotoxin, morphine and theophylline (Badawy & Morgan, 1982) and possibly also large doses of ethanol (Fig. 1 of the present work)], possibly by releasing catecholamines (see, e.g., Schneider, 1974; O'Neill & Rahwan, 1975). In support of this latter suggestion is the finding that non-esterified fatty acid concentration in 24 h-starved rats (means $\pm$ S.E.M. for each group of 4–5

rats) was increased at 1 h after acetaldehyde administration (156 mg/kg) to 0.77 ± 0.04 mM, compared with a control value (observed at 1 h after saline) of 0.53 ± 0.02 mM. The reciprocal effects on ALA synthase activity and pyrrolase saturation with haem exerted by administered acetate (Table 5) could also be mediated by non-esterified fatty acids, whose concentration was also increased at 1 h after a 100 mg/kg dose to 1.48 ± 0.08 mM. The mechanism of this latter increase may involve stimulation by acetate of lipogenesis (see, e.g., Topping *et al.*, 1984), but not lipolysis, because this latter process is known to be inhibited by acetate (Crouse *et al.*, 1968; Nilsson & Belfrage, 1978; Lieber, 1984).

If acetaldehyde and acetate are not the mediators of the ethanol-induced induction of ALA synthase activity, then other aspects of ethanol metabolism must be responsible. One such aspect, the increase in the $[\text{NADH}]/[\text{NAD}^+]$ ratio, has been suggested to promote protein synthesis (Hedden & Buse, 1982), and the data in Table 3 of the present work with the alcohol dehydrogenase inhibitor pyrazole, which prevents the ethanol-induced rise in the above ratio (Bustos *et al.*, 1970), are not inconsistent with this suggestion, though clearly much more work is required to examine this point.

Another possibility, proposed by Shanley *et al.* (1968) and Beattie *et al.* (1973), is that of the influence of ethanol metabolism on intermediary metabolic processes leading to increased availability to ALA synthase of its succinate substrate (as succinyl-CoA), as originally proposed by Labbe (1967). It is not, however, known whether ALA synthase could be induced *in vivo* by its succinate substrate simply through an increase in its availability, as in the case of pyrrolase activation by tryptophan. A regulatory link between ALA synthase and succinyl-CoA has, however, been proposed by Jenkins & Weitzman (1988) on the basis of their observation of a specific increase in activity of the GTP-specific succinate thio-kinase in experimental hepatic porphyria. Also, although succinate was tested in the present work and found (Table 5) to increase both ALA synthase activity by 48% and the haem saturation of tryptophan pyrrolase by 50% at 4 h after intraperitoneal administration of a 100 mg/kg dose, it is not clear if such non-reciprocal effects are direct ones, because we have not ascertained whether the administered succinate has entered the liver. Further work is therefore required to examine these and other possible effects of succinate.

General conclusions and comments

The present results have dissociated two dose-dependent opposing actions of acute ethanol administration on rat liver ALA synthase activity: an overall induction of the enzyme related to the metabolism of the drug, and a haem-mediated repression evident with large doses and most likely mediated by tryptophan. The major ethanol metabolites acetaldehyde and acetate are unlikely to be involved in the above induction, but the possible roles of the disturbed [NADH]/[NAD⁺] ratio (as a stimulus for protein synthesis) and of intermediary metabolites, such as succinate and/or related precursors and also related enzymes require further assessment utilizing experimental systems and animal strains and/or species differing in their metabolic responses to ethanol and in their rates of, and enzyme systems involved in, its metabolism. The present discussion has been limited to the acute effects of ethanol on ALA synthase activity in a mammalian liver. The drug is also known to increase the activity of the enzyme in rat liver after chronic (Rubin *et al.*, 1970; Teschke *et al.*, 1987), and in human leukocytes after acute (McColl *et al.*, 1980), administration. In isolated cell systems, however, the effects of the drug are variable, with inhibition in isolated rabbit reticulocytes (Ibrahim *et al.*, 1979) and cultured chick-embryo hepatocytes (Sinclair *et al.*, 1986) and induction in this latter system (Geiger & Meyer, 1980) and in cultured rat hepatocytes (Lane & Stewart, 1983). These controversial findings also clearly require further experimental scrutiny.

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