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Prevention by Pyrazole of the Effects of Chronic Ethanol Administration on the Redox States of the Hepatic Nicotinamide–Adenine Dinucleotide (Phosphate) Couples and on Liver and Brain Tryptophan Metabolism in the Rat

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Chronic administration of pyrazole in the diet of rats does not cause toxicity and prevents the chronic effects of ethanol on: (1) the redox states of the hepatic NAD(P) couples; (2) liver tryptophan pyrrolase activity; (3) brain tryptophan and 5-hydroxytryptamine metabolism.

We have previously shown that chronic ethanol administration to rats decreases liver tryptophan pyrrolase (EC 1.13.11.11) activity (Badawy & Evans, 1973) and enhances brain 5-hydroxytryptamine synthesis (Badawy *et al.*, 1978). The former effect is associated with increased hepatic concentrations of the allosteric inhibitors NAD(P)H, and we suggested that these cause the decrease in pyrrolase activity because it is reversed by addition *in vitro* of NAD(P)⁺ or by administration of regenerators of these oxidized dinucleotides (Badawy & Evans, 1975*b*). The enhancement of brain 5-hydroxytryptamine synthesis is associated with an increased availability of circulating tryptophan to the brain secondary to the decreased liver pyrrolase activity, because reversal of this decrease by NAD(P)⁺ regenerators prevents these changes (Badawy *et al.*, 1979). These results therefore suggest that liver NADH plays a central role in the chronic ethanol-induced changes in liver and brain tryptophan metabolism. If this is so, then these changes should also be reversed by preventing the ethanol-induced increase in [NADH] (and possibly also [NADPH]) by pyrazole, an alcohol dehydrogenase inhibitor (Lester *et al.*, 1968). Although pyrazole has been shown (Bustos *et al.*, 1970) to prevent the acute effect of ethanol on the [NAD⁺]/[NADH] ratio, it is surprising that no similar studies have been reported, as far as we could ascertain, in relation to the chronic ethanol effects on the redox states of the NAD(P) couples. Moreover, chronic administration of pyrazole in single daily doses causes toxicity (as assessed by morphological and histological examination of liver and by lethality), particularly in the presence of joint ethanol administration (Leibach, 1969; Kalant *et al.*, 1972), and, perhaps, a safer method would be to give pyrazole in the diet. Another point of interest in the present context is whether pyrazole is capable of preventing

the chronic ethanol-induced increase in liver [NADPH] as well as [NADH], if the former is a consequence of the latter through the transdehydrogenase system. In the present paper, we report that incorporation into the diet is a safe method of administration of pyrazole and that such administration prevents the chronic effects of ethanol on the redox states of the hepatic NAD(P) couples and on liver and brain tryptophan metabolism.

Materials and Methods

Locally bred male Wistar rats were housed three per cage and were maintained on cube diet 41B (Oxoid, Basingstoke, Hants., U.K.) and water. Ethanol and pyrazole were freely administered for 2 weeks before death. Ethanol was given in drinking water in concentrations (v/v) of 5% for 2 days, 7.5% for 2 more days and finally 10% for the remaining 10 days. Pyrazole was given in the diet. Each kg of diet was powdered, suspended in 0.5 litre of tap water containing 0.5 g of pyrazole, reconstituted into small lumps and dried at about 30°C for 1–2 days. Control diet was similarly treated, except that pyrazole was not included in the suspending water. Dietary intake was recorded every 4–5 days, whereas fluid intake and body weight were measured daily.

The sources of various chemicals were described by Badawy & Evans (1975*a,b*, 1976). Concentrations of liver nicotinamide–adenine dinucleotides (phosphates) were determined in whole-liver samples, obtained by a locally manufactured freeze-clamp, by the method of Slater *et al.* (1964) with two minor modifications involving the use of a 2 mg/ml solution of phenazine methosulphate (T. F. Slater, personal communication) and performing the reaction cycles at 20 ± 0.5°C. Tryptophan pyrrolase activity was determined in liver homogenates (Badawy & Evans, 1975*a*)

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in either the absence (holoenzyme activity) or the presence (total enzyme activity) of added haematin. The apoenzyme activity was obtained by difference. The concentrations of liver, free (ultrafiltrable) serum, total (acid-soluble) serum and brain tryptophan and those of brain 5-hydroxytryptamine and 5-hydroxyindol-3-ylacetic acid were determined by standard procedures (for references see Badawy *et al.*, 1979). Serum glucose concentration was determined fluorimetrically (Slein, 1963). Student's *t* test was used to assess some of the differences between control and test results.

Results and Discussion

When pyrazole was administered in the solid diet in the present work, it was found (Fig. 1*a*) that, although it caused a transient loss of weight during the first 3 days, it did not prevent the rats from

achieving a net weight gain of 19% after 2 weeks. The corresponding gain by control rats was 34%. Ethanol caused about the same (20%) weight gain as pyrazole. The average final body weight of ethanol-treated rats was similar to that of controls because the starting weight of the former animals was deliberately 13% higher than that of the latter. Joint administration of pyrazole and ethanol caused a 16% net gain in weight, with the final average body weight being somewhat lower than that of control or ethanol-treated rats. These changes in body weight mirrored the dietary intake (results not shown). Pyrazole intake was calculated from that of diet, and averaged 37.5–48.6 mg/kg body wt. per day in pyrazole-treated rats and 31.2–48.6 mg/kg body wt. per day in those treated with pyrazole plus ethanol. These doses of pyrazole resemble those used by Lebach (1969) and Kalant *et al.* (1972).

Fluid intake is also shown in Fig. 1. In Fig. 1(*b*), it is clear that pyrazole caused only a small effect on water intake. Ethanol intake (Fig. 1*c*), however, was moderately decreased by pyrazole by 11–26% (except for the 14% increase on day 8). When these values are corrected for body weight, ethanol intake in the absence of pyrazole varied between 7.9 and 8.9 ml/kg body wt. per day, whereas in its presence, it was 7.1–8.0 ml/kg body wt. per day. Ethanol intake was therefore decreased by pyrazole by only 10%. These latter values do not include the first 4 days when the doses of ethanol were less than 10%. Although we could not obtain values for blood ethanol concentrations because of a methodological error, it is clear from the above results that both pyrazole-treated and untreated rats received large doses of ethanol.

None of the animals treated with ethanol, pyrazole or both died, nor did their livers exhibit any abnormality of appearance, colour or texture. Taken together, these results suggest that incorporation into the diet is a safe method of administration of pyrazole and this may be explained by the fact that it has been taken by rats in repeated small doses.

The effects of chronic administration of ethanol, pyrazole or both on concentrations of serum glucose and liver nicotinamide-adenine dinucleotides (phosphates) and on various aspects of tryptophan metabolism are shown in Table 1. Serum glucose concentration was not significantly altered by any of the above treatments and this is consistent with the above dietary results.

Ethanol treatment increased [NADH] and [NADPH]. These effects of ethanol (at 2 weeks) agree with those reported by Kalant *et al.* (1970), except for the increase in [NADPH]. The latter effect has been reported to occur at 6 weeks by Kalant *et al.* (1970) and at 4 weeks by Badawy & Evans (1975*b*).

Pyrazole alone only caused a small increase (7%) in [NADH] and a small decrease (8%) in the [NAD⁺]/[NADH] ratio. When given with ethanol, pyrazole

Fig. 1. Effects of chronic administration to rats of ethanol, pyrazole or both on body weight and fluid intake. Experimental details are given in the Materials and Methods section. Values are means for at least 18 rats per group. Symbols: ○, control; ●, ethanol; △, pyrazole; ▲, ethanol plus pyrazole. (a) Body weight; (b) water intake; (c) intake of ethanol in water.

Table 1. *Effects of chronic administration to rats of ethanol, pyrazole or both on concentrations of serum glucose and liver nicotinamide-adenine dinucleotides (phosphates) and on various aspects of tryptophan metabolism*

Experimental details are given in the Materials and Methods section. Liver tryptophan pyrrolase activity is expressed in μmol of kynurenine formed/h per g wet wt. of liver, whereas serum glucose concentration is given in mg/100 ml. All other values (except ratios and percentages) are in $\mu\text{g/g}$ wet wt. of tissue or in $\mu\text{g/ml}$ of serum. Values are means $\pm$ S.E.M. for each group of four rats (tryptophan pyrrolase activities) or for six rats (all other determinations). The values [NADH] and related expressions in the water-treated control group are means for five animals. The results in columns 2 and 3 are statistically compared with those in column 1, whereas those in column 4 are compared with those in column 3. Differences are signified as follows: * $P < 0.05$; ** $P < 0.02$; *** $P < 0.005$; † $P < 0.001$.

		Glucose, nicotinamide-adenine dinucleotide (phosphate) and tryptophan-metabolism parameters			
Determination	Treatment ...	Water	Ethanol	Pyrazole	Ethanol + pyrazole
[NAD ⁺]		329 ± 9	293 ± 13*	324 ± 4	321 ± 7
[NADH]		176 ± 3	222 ± 8†	189 ± 4*	186 ± 5
[NAD ⁺]+[NADH]		507 ± 14	515 ± 19	513 ± 6	507 ± 11
[NAD ⁺]/[NADH]		1.87 ± 0.03	1.32 ± 0.05†	1.71 ± 0.03***	1.73 ± 0.05
[NADP ⁺]		40 ± 3	41 ± 2	40 ± 2	42 ± 2
[NADPH]		173 ± 6	219 ± 8***	170 ± 4	173 ± 8
[NADP ⁺]+[NADPH]		213 ± 8	259 ± 8***	210 ± 5	215 ± 9
[NADPH]/[NADP ⁺]		4.33 ± 0.24	5.45 ± 0.35*	4.25 ± 0.27	4.12 ± 0.15
Total sum		715 ± 18	781 ± 21*	723 ± 5	722 ± 9
Holo-(tryptophan pyrrolase)		2.1 ± 0.10	1.8 ± 0.07*	2.1 ± 0.05	2.2 ± 0.18
Total tryptophan pyrrolase		5.5 ± 0.23	2.6 ± 0.19†	5.9 ± 0.21	5.7 ± 0.29
Apo-(tryptophan pyrrolase)		3.4 ± 0.14	0.8 ± 0.14†	3.8 ± 0.22	3.5 ± 0.12
Serum glucose		99 ± 5	97 ± 2	92 ± 2	95 ± 4
Free serum tryptophan		1.25 ± 0.07	1.55 ± 0.07**	1.20 ± 0.06	1.20 ± 0.06
Total serum tryptophan		19.68 ± 0.58	23.67 ± 1.46*	18.83 ± 1.00	19.52 ± 0.37
Free serum tryptophan (%)		6.35 ± 0.36	6.55 ± 0.25	6.37 ± 0.31	6.15 ± 0.33
Liver tryptophan		4.00 ± 0.21	5.00 ± 0.15***	4.23 ± 0.14	4.01 ± 0.14
Brain tryptophan		1.78 ± 0.07	2.38 ± 0.05†	1.77 ± 0.05	1.89 ± 0.07
Brain 5-hydroxytryptamine		0.63 ± 0.009	0.77 ± 0.018†	0.61 ± 0.01	0.62 ± 0.007
Brain 5-hydroxyindol-3-ylacetic acid		0.31 ± 0.005	0.38 ± 0.009†	0.29 ± 0.007*	0.28 ± 0.006

prevented all the ethanol effects on dinucleotide concentrations, ratios and sums. These results therefore show that pyrazole prevents the chronic ethanol-induced changes in the redox states of the NAD(P) couples and also suggest that the ethanol-induced increase in [NADPH] is a consequence of that in [NADH], presumably through activation of transdehydrogenase.

Support for the suggestion (Badawy & Evans, 1975b) that the decrease in liver tryptophan pyrrolase activity associated with chronic ethanol administration is caused by increased [NAD(PH)] is provided by the finding (Table 1) that pyrazole, which prevents the increase in NAD(P)H, also prevents the decrease in pyrrolase activity. Pyrazole alone exerted no significant effects on the basal enzyme activity, and this contrasts with its ability to cause a decrease after acute administration (Rouach *et al.*, 1976). Although the possibility that the NADH-mediated decrease in pyrrolase activity is caused by acetaldehyde produced from ethanol has not been excluded in the present work, it is unlikely in view of the finding (Badawy & Evans, 1975b) that NAD⁺ regenerators, which

presumably enhance ethanol metabolism and therefore acetaldehyde production, reverse rather than potentiate the ethanol-induced decrease in pyrrolase activity. Also, direct inhibition by acetaldehyde or acetate is excluded because both agents added *in vitro* (5 mM) exerted no significant effects on the basal enzyme activities (results not shown).

We have previously suggested (Badawy *et al.*, 1979) that chronic ethanol administration enhances brain 5-hydroxytryptamine synthesis by increasing the availability of circulating tryptophan to the brain secondary to the decreased liver tryptophan pyrrolase activity. The ethanol effects (Table 1) on concentrations of tryptophan and/or its indole metabolites in liver, serum and brain support the above suggestion. Pyrazole alone only caused a slight (6%) decrease in brain 5-hydroxyindol-3-ylacetic acid concentration, but when given with ethanol it prevented all the effects of ethanol on the above aspects of tryptophan metabolism. These findings provide further evidence for the involvement of decreased liver pyrrolase activity in the chronic ethanol-induced enhancement of brain tryptophan and 5-hydroxytryptamine

metabolism, and, together with the other results of the present work, support the hypothesis that liver [NADH] plays a central role in the chronic effects of ethanol on liver and brain tryptophan metabolism.

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