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The induction of ethanol dependence and the ethanol withdrawal syndrome: the effects of pyrazole

J. M. LITTLETON, P. J. GRIFFITHS AND A. ORTIZ

Department of Pharmacology, King's College, Strand, London W.C.2., U.K.

Daily administration of an inhibitor of alcohol dehydrogenase (pyrazole, 1 m mol kg^{-1}, i.p.) appeared to prevent the development of metabolic tolerance to ethanol administered chronically to mice by inhalation, but increased the duration and intensity of the behavioural change associated with ethanol withdrawal, despite the absence of any marked difference in blood or brain ethanol and acetaldehyde concentrations during ethanol administration in the two groups. (Pyrazole-treated mice were exposed to lower concentrations of ethanol.) Changes in brain monoamine concentrations which occur in mice during chronic ethanol administration were not prevented by pyrazole, but differed in time course under these conditions. Repeated administration of pyrazole intraperitoneally caused weight loss and hypothermia in mice, whether or not ethanol was also given. It is concluded that the combination of pyrazole and ethanol is probably not capable of separating primary effects of chronic ethanol administration from secondary (metabolic) effects, and that inhibition of alcohol dehydrogenase is unlikely to be the sole reason for the potentiation of the ethanol withdrawal syndrome by pyrazole.

The administration of ethanol to mice by inhalation has been reported to induce dependence (Goldstein & Pal, 1971; Griffiths, Littleton & Ortiz, 1973a) as evidenced by a characteristic behavioural syndrome when the drug is withdrawn. Goldstein & Pal administered ethanol at a steady high concentration for periods of two to five days and combined this with daily injections of pyrazole (an inhibitor of alcohol dehydrogenase), whereas Griffiths, Littleton & Ortiz administered ethanol alone, in increasing concentration, for a period of ten days. Despite the longer time course and the much higher total dose of ethanol in the latter study, the reported withdrawal syndrome was shorter and less marked than that described by Goldstein & Pal. There are many possible reasons for this discrepancy, but the major difference between the experimental methods seems to be the combination of pyrazole with the ethanol treatment in the earlier studies.

Goldstein & Pal (1971) and Goldstein (1972) reported that the administration of pyrazole reduced variation in blood ethanol concentrations in grouped mice exposed to ethanol vapour. This finding is difficult to reconcile with the evidence that rate of ethanol elimination is largely unrelated to differences in alcohol dehydrogenase activities between individuals (Hawkins & Kalant, 1972), but pyrazole probably affects other enzyme systems involved in alcohol metabolism as well as alcohol dehydrogenase (Lieber, Rubin & others, 1970). It has also been shown to have central nervous system depressant properties (Goldberg, Hollstedt & others, 1972). However, these authors have suggested that pyrazole could be used as a tool to separate the primary effects of ethanol on the central nervous system from its second-

ary metabolic effects (Goldberg & others, 1972). It is therefore of interest to evaluate further some of the changes produced by the administration of pyrazole to mice inhaling ethanol over a long period. This approach may help to elucidate some of the biochemical mechanisms involved in ethanol dependence.

In particular, many authors have suggested that acetaldehyde may play some part in the induction of ethanol dependence. Pyrazole would be expected to reduce the formation of acetaldehyde from ethanol, and this has in fact been reported (Lester, Keokosky & Felzenberg, 1968). If pyrazole potentiates the induction of ethanol dependence as suggested by the work of Goldstein & Pal (1971), then the hypothesis of a role for acetaldehyde is clearly difficult to sustain.

After chronic exposure to ethanol inhalation, mice are able to eliminate ethanol more rapidly than after acute exposure by the same route (Griffiths & others 1973a). If this is due to the induction of alcohol dehydrogenase as suggested by the work of Abe (1964) and Raskin & Sokoloff (1970), then pyrazole administration during ethanol inhalation would be expected to prevent this aspect of tolerance to ethanol. Once again current concepts of dependence would suggest that this might reduce the ability of ethanol to induce physical dependence rather than the reverse.

Griffiths, Littleton & Ortiz (1973b) have recently reported that chronic ethanol administration by inhalation causes changes in mouse brain monoamine concentrations. It was suggested that these changes were due to changes in metabolism of the monoamines secondary to changes imposed by the metabolism of ethanol. If the assumption that pyrazole can be used as a tool for separating the primary effects of ethanol from the secondary metabolic effects is correct, then one would expect that pyrazole administration would prevent these changes if they stem from changes produced by ethanol metabolism.

Obviously there are several paradoxes inherent in the seeming ability of pyrazole to potentiate ethanol dependence, and these may relate to the fundamental mechanisms involved. We have sought to answer some of these questions by comparing the administration to mice of ethanol alone with that of ethanol plus pyrazole. Behavioural changes, body weight and rectal temperature, blood and brain ethanol and acetaldehyde concentrations, and brain monoamine concentrations have all been measured in this study.

METHODS

Ethanol administration and withdrawal

Groups of 30 male mice (T.O. strain—Animal Suppliers Ltd.), 18–22 g, were exposed to ethanol vapour for periods of up to ten days, using the apparatus described by Griffiths, Littleton & Ortiz (1973c). Some groups received daily intraperitoneal injections of pyrazole (1 m mol kg^{-1}, i.p.) in saline until the day before withdrawal. The others received only physiological saline (0·25 ml, i.p.). Exposures to ethanol were of two sorts: short exposures, in which ethanol concentrations remained constant for a period of four days before withdrawal, and long exposures, in which ethanol concentrations were gradually increased over a period of ten days before withdrawal. A small number of mice was harvested at random at intervals during ethanol exposure to determine whether blood ethanol concentrations were in the same range in pyrazole-treated and non-pyrazole-treated mice. Pyrazole-treated mice required lower ethanol vapour concentrations to reach the same blood ethanol

concentration. For short exposures pyrazole-treated mice received 5–10 mg litre^{-1} ethanol, whereas non-pyrazole-treated mice received 10–15 mg litre^{-1}. For long exposures the ethanol concentrations were increased from 5 mg litre^{-1} up to about 10 mg litre^{-1} for pyrazole-treated mice, and 10 mg litre^{-1} up to about 20 mg litre^{-1} for non-pyrazole-treated mice. Approximate values only can be given, because ethanol concentrations often needed to be altered to prevent high mortality in the groups of mice. Cage ethanol concentrations were estimated by g.l.c. (see below).

Food and water were freely provided throughout exposure to ethanol and withdrawal. Ambient temperature was maintained at 28–30°. Mice were weighed daily and core temperatures measured by inserting a Grant thermistor probe into the rectum to a depth of 0·5 cm for 15 s.

Ethanol was withdrawn after four or ten days. Ethanol concentrations in inspired air fell to almost undetectable levels within 10 min.

Behavioural signs of ethanol withdrawal were scored in the way described by Goldstein (1972) except that locomotor activity was assessed separately. Locomotor changes were measured by an Animex type S activity meter (sensitivity 40 μA unless otherwise stated) with the output displayed on a Grass type 7B polygraph.

Control treatments included animals injected daily with saline or pyrazole intraperitoneally, and kept under identical environmental conditions, except that ethanol was absent from the inspired air.

Ethanol and acetaldehyde determinations

Concentrations of ethanol and acetaldehyde in air, blood and tissues were estimated in the way described by Griffiths, & others (1973c). This method utilizes g.l.c. on a column containing 20% PEG 20M on Chromosorb W-HP, 80–100 mesh, column temperature 110°, detector (F.I.D.) temperature 200° and carrier flow 50 ml min^{-1}. Peak areas and retention times were determined by a Vidar 6300 integrator. One ml gas samples were injected, either of air, or of the head space above the supernatant from protein-precipitated tissue or blood samples. Under these conditions the recoveries of ethanol and acetaldehyde were linear over the range encountered in this study. Retention times were: acetaldehyde 150 s and ethanol 270 s.

Estimation of brain monoamine concentrations

Mice were killed by total immersion in liquid nitrogen. Brains were dissected in the cold and monoamines extracted by the method of Shore & Olin (1958). The final acid extracts were taken for fluorimetric estimation of noradrenaline and dopamine (Laverty & Taylor, 1968) or 5-hydroxytryptamine (Curzon & Green, 1970). Pooled mouse brains, usually three, were used for these determinations. Ethanol, acetaldehyde, and pyrazole when added to brain homogenates in the concentrations expected *in vivo* did not affect the recoveries of the monoamines using these methods.

Drugs and chemicals

Analytical grade reagents were used whenever these were available. Ethanol (A.R. grade 99·8% v/v) was supplied by James Burroughs Ltd. Acetaldehyde was supplied by BDH (Chemicals) Ltd. and pyrazole (98% pure) was obtained from Ralph Emmanuel Ltd.

RESULTS

Ethanol administration

The concentrations of ethanol vapour to which mice were exposed all initially caused locomotor excitement followed by depression and ataxia. Mice treated with pyrazole and ethanol remained ataxic, whereas mice receiving ethanol alone stayed ataxic only in the groups exposed to increasing concentrations of ethanol. Blood and brain concentrations of ethanol and acetaldehyde were comparable in all the groups exposed to ethanol under the conditions of these experiments (Table 1). Pyrazole treated mice were exposed to very roughly half the concentration of ethanol to which untreated mice were exposed.

Table 1. *Blood and brain ethanol and acetaldehyde concentrations in mice exposed to ethanol vapour alone, in increasing concentrations, or to ethanol vapour plus pyrazole (1 m mol kg^{-1}, i.p.) daily.* These results are from a representative experiment in which 5 mice were killed on each day of ethanol administration for estimation of ethanol and acetaldehyde concentrations. Normally only one or two mice were taken at intervals of two or three days to ensure that ethanol concentrations were of the same order between groups.

	Ethanol					Ethanol + pyrazole				
		Blood		Brain			Blood		Brain	
Day of administration	Cage EtOH concn mg litre^{-1}	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}	Cage EtOH concn mg litre^{-1}	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}
1	8·2	2·30 ±0·1	0·68 ±0·02	2·08 ±0·1	6·05 ±0·2	5·7	2·40 ±0·1	0·74 ±0·02	2·29 ±0·1	7·10 ±0·3
2	9·4	2·40 ±0·2	0·70 ±0·04	2·18 ±0·2	7·50 ±0·4	6·4	2·14 ±0·2	0·76 ±0·03	2·14 ±0·1	6·94 ±0·3
3	10·5	2·15 ±0·2	0·73 ±0·04	1·94 ±0·2	6·51 ±0·3	7·4	2·01 ±0·1	0·71 ±0·03	2·20 ±0·2	7·31 ±0·4
4	11·4	2·22 ±0·2	0·74 ±0·04	2·14 ±0·2	6·80 ±0·3	8·0	2·30 ±0·2	0·78 ±0·02	2·34 ±0·2	7·41 ±0·4
5	13·1	2·48 ±0·1	0·74 ±0·06	2·54 ±0·3	7·40 ±0·4	8·3	2·46 ±0·2	0·79 ±0·04	2·41 ±0·2	7·60 ±0·3

The administration of pyrazole to mice even for short periods caused weight loss and hypothermia. This was true, whether or not ethanol was also administered. Ethanol itself did not cause weight loss, and rarely caused hypothermia. Some mice became comatose during the experiments, and these had very low rectal temperatures; for this reason they were assessed separately. These results are shown in Table 2. Mortality was low (0–5%) in short exposures to ethanol unless pyrazole was also administered when it increased (10–15%). Mortality increased during longer exposures to ethanol (10–15%) and was further increased (25–40%) when pyrazole treatment was combined. Pyrazole itself when given for a period of nine days was associated with a mortality of 10–15%.

Ethanol withdrawal

There were large differences between behavioural changes in ethanol withdrawal after short or long exposure to ethanol, and so these are assessed separately.

Withdrawal after short exposure to ethanol. Mice receiving ethanol alone showed a period of locomotor excitement lasting about 3 h after ethanol withdrawal. With-

Table 2. *Body weight and rectal temperature in mice exposed to ethanol, ethanol and pyrazole, and pyrazole alone.* Each value represents the mean (±s.e.) of at least 20 determinations, except for values for rectal temperature in comatose mice, where a minimum of 4 determinations were made.

Day of administration	Ethanol			Pyrazole			Ethanol + pyrazole		
	Mean body wt (g)	Rectal temp.°		Mean body wt (g)	Rectal temp.°		Mean body wt (g)	Rectal temp.°	
		Conscious	Comatose		Conscious	Comatose		Conscious	Comatose
1	19·43 ±0·42	38·24 ±0·2	—	19·39 ±0·43	38·30 ±0·1	—	19·31 ±0·3	38·42 ±0·2	—
3	20·0 ±0·57	36·43 ±0·1	35·2 ±0·05	19·20 ±0·6	37·06 ●0·2	—	18·48 ±0·3	36·6 ±0·3	33·6 ±0·4
5	19·85 ±0·4	37·16 ±0·1	—	18·65 ±0·2	36·72 ±0·2	—	18·15 ±0·4	36·45 ±0·2	34·4 ±0·2
10	20·45 ±0·47	37·42 ±0·2	34·8 ±0·1	17·20 ±0·5	36·2 ±0·1	—	15·41 ±0·3	35·89 ±0·2	34·6 ±0·1

drawal signs were not marked, and were very variable between individual mice. The withdrawal syndrome lasted for a period of about 8 h. These results are shown in Fig. 1a.

The administration of pyrazole to mice during short exposure to ethanol markedly increased the duration and intensity of the withdrawal syndrome. There was no phase of locomotor excitement in these mice. The withdrawal signs reached a peak about 8 h after withdrawal, and persisted up to 24 h in some instances. These results are shown in Fig. 1b.

Blood and brain ethanol and acetaldehyde concentrations were similar between the two groups before withdrawal. During withdrawal, blood and brain ethanol concentrations fell much more slowly in the pyrazole-treated animals. The ratio of ethanol to acetaldehyde in blood, or in brain, remained similar between the groups. It is of

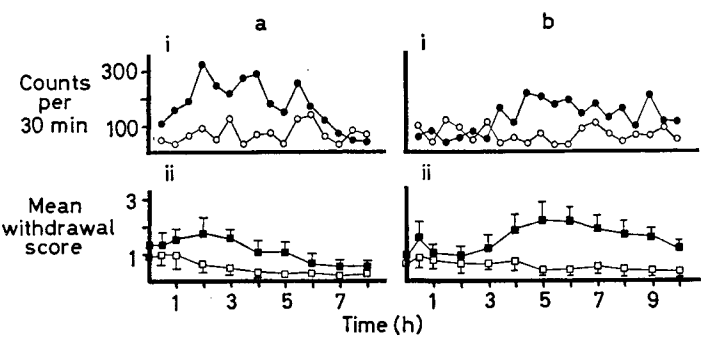

FIG. 1. Withdrawal syndrome after administration of ethanol, or ethanol and pyrazole for 4 days. (a) Locomotor activity and withdrawal score in mice given ethanol by inhalation for 4 days. Locomotor activity—ethanol withdrawal (●——●); controls (○——○). Groups of 15 mice. Withdrawal score—ethanol withdrawal (■——■); controls (□——□). Each value represents the mean score from at least 15 mice. Vertical bars represent s.e.

(b) Locomotor activity and withdrawal score in mice given pyrazole (1 m mol kg^{-1} i.p.) for 3 days and ethanol by inhalation for 4 days. Locomotor activity—ethanol + pyrazole withdrawal (●——●); controls (pyrazole only) (○——○). Groups of 15 mice. Withdrawal score—ethanol + pyrazole withdrawal (■——■); controls (pyrazole only) (□——□). Each value represents the mean score from at least 15 mice. Vertical bars represent s.e.

Table 3. *Ethanol and acetaldehyde concentrations in blood and brain during ethanol, or ethanol and pyrazole withdrawal after 4 days' exposure.* Each value represents the mean (±s.e.) of at least 5 determinations. Horizontal bars indicate a concentration of ethanol or acetaldehyde not significantly higher than that obtained in untreated control mice.

	Ethanol				Ethanol + pyrazole			
	Blood		Brain		Blood		Brain	
Hours after withdrawal	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}
0	2·49 ±0·1	0·83 ±0·1	2·54 ±0·2	8·74 ±0·8	2·71 ±0·2	0·89 ±0·1	2·64 ±0·2	9·41 ±0·8
1	1·84 ±0·2	0·71 ±0·1	1·78 ±0·3	6·13 ±0·6	2·01 ±0·1	0·84 ±0·1	1·94 ±0·2	7·85 ±0·7
2	1·41 ±0·1	0·64 ±0·1	1·30 ±0·2	5·30 ±0·6	1·90 ±0·1	0·76 ±0·1	1·80 ±0·1	7·43 ±0·6
3	0·89 ±0·1	0·38 ±0·1	0·91 ±0·1	4·13 ±0·6	1·74 ±0·1	0·71 ±0·1	1·64 ±0·1	6·68 ±0·5
4	0·41 ±0·1	0·29 ±0·1	0·43 ±0·1	3·01 ±0·3	1·41 ±0·1	0·69 ±0·1	1·31 ±0·1	5·35 ±0·3
6	—	—	—	—	0·41 ±0·1	0·44 ±0·1	0·33 ±0·1	2·69 ±0·2

interest that brain acetaldehyde concentrations were found to be considerably higher than those in blood by this method. These results can be seen in Table 3.

Withdrawal after long exposure to ethanol. Mice receiving ethanol alone showed a period of locomotor excitement lasting about 6 h when ethanol was withdrawn. Withdrawal signs were exhibited by all animals and reached a peak after about 3 h. The withdrawal syndrome lasted for about 12–15 h (Fig. 2a). Pyrazole administration during long exposure to ethanol increased withdrawal signs during ethanol withdrawal, so that these became very marked indeed. Mice initially showed locomotor depression and ataxia during withdrawal. Withdrawal signs reached a peak about 6 h after withdrawal, and were still evident after 24 h (Fig. 2b).

Blood and brain ethanol and acetaldehyde concentrations were similar between the two groups before withdrawal. Concentrations of ethanol and acetaldehyde fell much more slowly during withdrawal in mice which had received pyrazole (Table 4).

Rate of elimination of ethanol

Mice exposed to a high concentration of ethanol acutely until they developed locomotor ataxia metabolized ethanol at a rate of 500 mg kg^{-1} h^{-1}, whereas mice exposed to ethanol vapour (10–15 mg litre^{-1}) for four days and then made ataxic by increasing the ethanol concentration eliminated ethanol at a rate of 620 mg kg^{-1} h^{-1}. These figures represent an increase in the rate of ethanol elimination of 120 mg kg^{-1} h^{-1} in mice exposed to ethanol for four days (Fig. 3a).

Mice given daily injections of pyrazole (1 m mol kg^{-1}, i.p.) for three days and then exposed to ethanol vapour until ataxic on the fourth day eliminated ethanol at a rate

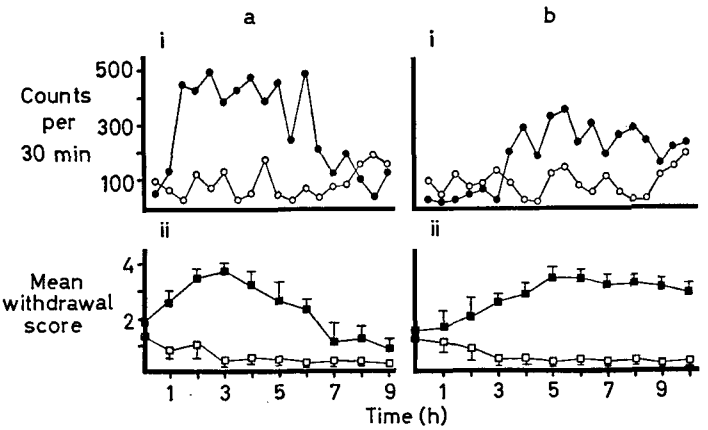

FIG. 2. Withdrawal syndrome after administration of ethanol, or ethanol and pyrazole for 10 days. (a) Locomotor activity and withdrawal score in mice given ethanol by inhalation for 10 days. Locomotor activity—ethanol withdrawal (●——●); controls (○——○). Groups of 15 mice. Withdrawal score—ethanol withdrawal (■——■); controls (□——□). Each value represents the mean score from at least 15 mice. Vertical bars represent s.e.

(b) Locomotor activity and withdrawal score in mice given pyrazole (1 m mol kg^{-1}, i.p.) for 9 days and ethanol by inhalation for 10 days. Locomotor activity—ethanol + pyrazole withdrawal (●——●); controls (pyrazole only) (○——○). Groups of 15 mice. Withdrawal score—ethanol + pyrazole withdrawal (■——■); controls (pyrazole only) (□——□). Each value represents the mean score from at least 15 mice. Vertical bars represent s.e.

Table 4. *Ethanol and acetaldehyde concentrations in blood and brain during ethanol, or ethanol and pyrazole withdrawal after 10 days of exposure.* Each value represents the mean (±s.e.) of at least 5 determinations. Horizontal bars indicate a concentration of ethanol or acetaldehyde not significantly higher than that obtained in untreated control mice.

	Ethanol				Ethanol + pyrazole			
	Blood		Brain		Blood		Brain	
Hours after withdrawal	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}	EtOH mg ml^{-1}	Acet. μg ml^{-1}	EtOH mg g^{-1}	Acet. μg g^{-1}
0	2·40 ±0·1	0·86 ±0·1	2·14 ±0·1	7·74 ±0·2	3·60 ±0·1	1·02 ±0·1	3·42 ±0·1	10·87 ±0·6
1	1·06 ±0·1	0·55 ±0·1	0·92 ±0·1	5·74 ±0·5	3·41 ±0·1	0·92 ±0·1	3·25 ±0·1	8·61 ±0·6
2	0·46 ±0·1	0·33 ±0·1	0·41 ±0·1	3·46 ±0·5	2·62 ±0·1	0·88 ±0·1	2·82 ±0·1	7·14 ±0·5
3	0·06 ±0·01	0·14 ±0·01	0·09 ±0·05	0·74 ±0·1	2·41 ±0·1	0·71 ±0·1	2·35 ±0·1	5·24 ±0·5
4	—	—	—	—	2·00 ±0·1	0·61 ±0·1	2·24 ±0·1	4·81 ±0·3
6	—	—	—	—	1·12 ±0·1	0·51 ±0·1	1·28 ±0·1	3·58 ±0·4

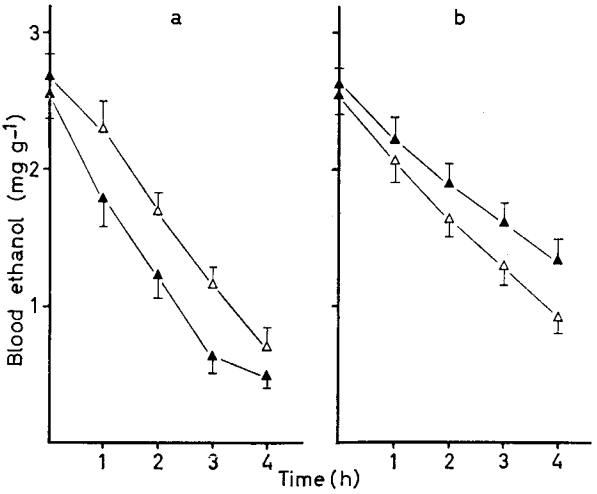

FIG. 3. Ethanol elimination after acute and chronic administration of ethanol; the effect of pyrazole. (a) Ethanol elimination after acute administration of ethanol (△——△), and after chronic administration of ethanol (▲——▲). Each point represents the mean ± s.e. of at least 4 determinations of blood ethanol concentration.

(b) Ethanol elimination after acute administration of ethanol and after chronic administration of pyrazole (△——△), and after chronic administration of ethanol and chronic administration of pyrazole (▲——▲). Each point represents the mean ± s.e. of at least 4 determinations of blood ethanol concentration.

of 380 mg kg^{-1} h^{-1}. Mice exposed to ethanol vapour (about 10 mg $litre^{-1}$) for four days and given pyrazole (1 m mol kg^{-1}, i.p.) on the first three days eliminated ethanol at a rate of 320 mg kg^{-1} h^{-1} when made ataxic by increasing the ethanol concentration on the fourth day. This represents a decrease of 60 mg kg^{-1} h^{-1} in the rate of ethanol elimination in mice exposed to ethanol for four days (Fig. 3b).

These results suggest that pyrazole administration prevents the increase in rate of ethanol elimination which occurs on chronic administration of ethanol.

Brain monoamine concentrations

Mouse brain noradrenaline, dopamine and 5-HT concentrations were increased during long exposure of mice to ethanol, and during short exposure if this was combined with pyrazole. Short exposure of mice to ethanol alone raised monoamine

Table 5. *Mouse brain monoamine concentrations after chronic administration of ethanol, or ethanol plus pyrazole.* Values represent concentrations expressed as a percentage of untreated control. Means (±s.e.) of at least 4 determinations are given. Absolute control concentrations were: noradrenaline 0·76 ± 0·05 μg g^{-1}; dopamine 1·32 ± 0·04 μg g^{-1}; 5-hydroxytryptamine 0·93 ± 0·03 μg g^{-1} (expressed per wet weight of brain tissue).

Monoamine	Ethanol 4 days	Ethanol 4 days + pyrazole 3 days	Ethanol 10 days
Noradrenaline	122·4 ± 5·3%	173·7 ± 22·4%	172·4 ± 19·7%
Dopamine	106·1 ± 5·3%	181·6 ± 20·5%	137·1 ± 12·1%
5-Hydroxytryptamine	118·4 ± 6·5%	149·5 ± 6·5%	134·4 ± 9·7%

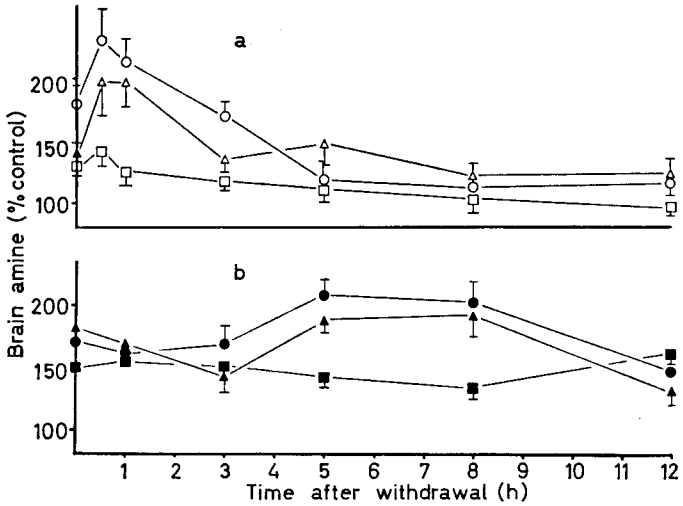

FIG. 4. Brain monoamine concentrations in mice during withdrawal from ethanol (10 days) or ethanol + pyrazole (4 days). (a) Brain monoamines during ethanol withdrawal: noradrenaline (△——△); dopamine (○——○); 5-hydroxytryptamine (□——□). Each value represents the mean of at least 6 determinations expressed as % untreated control. Vertical bars represent s.e.

(b) Brain monoamines during withdrawal from ethanol + pyrazole: noradrenaline (▲——▲); dopamine (●——●); 5-hydroxytryptamine (■——■). Each value represents the mean of at least 6 determinations expressed as % untreated control. Vertical bars represent s.e.

concentrations, but this increase was not significant ($P > 0{\cdot}05$). Too few of the animals exposed to ethanol for ten days and also given pyrazole survived to allow estimation of brain monoamines. The results referred to above are shown in Table 5.

During ethanol withdrawal after long exposure, brain catecholamines showed a marked transient rise; brain 5-HT showed a smaller rise. Ethanol withdrawal after four days exposure combined with pyrazole treatment was associated with similar changes in brain noradrenaline and dopamine, but with a considerably protracted time course; brain 5-HT concentrations changed little from their pre-withdrawal concentrations. Ethanol withdrawal after four days exposure to ethanol alone had no significant effect on brain amine concentrations. These results are shown in Fig. 4.

Chronic pyrazole administration produced no significant alteration in brain amines at any time interval.

DISCUSSION

Administration of pyrazole by intraperitoneal injection during ethanol administration by inhalation increases the intensity and duration of the behavioural changes observed in mice when ethanol is withdrawn. This effect occurs in the absence of any marked effect of pyrazole on ethanol and acetaldehyde concentrations in the treated mice (pyrazole-treated mice inhaled lower concentrations of ethanol) but ethanol and acetaldehyde concentrations remained high for a longer period in pyrazole-treated, ethanol-withdrawn mice.

An accumulation of brain monoamines was observed in mice given ethanol chronically by inhalation, whether or not pyrazole was also given. Pyrazole appeared to accelerate this accumulation, although pyrazole itself was without effect on brain

monoamines. During withdrawal from ethanol there was a further transient rise in brain monoamine concentrations. This change was also seen during withdrawal when pyrazole was administered with ethanol, but with a much longer time course.

All mice given daily intraperitoneal injections of pyrazole became hypothermic (despite the high ambient temperature) and lost weight, whether or not they were also receiving ethanol. Mice receiving ethanol alone did not lose weight and most maintained normal body temperature. In both groups some mice became comatose, and these were observed to have very low body temperatures. These mice usually died.

It is difficult to reconcile these effects of pyrazole with inhibition of alcohol dehydrogenase alone. Lieber & others (1970) have reported that pyrazole has hepatotoxic effects, whereas Kalant, Khanna & Bustos (1972) suggest that it enhances the hepatotoxic effects of ethanol. It may be that these actions are related to the toxic effects seen in our experiments. In addition, Goldberg & others (1972) have shown that pyrazole appears to have central nervous system depressant effects. We believe that the increase in ethanol dependence caused by pyrazole may be a consequence of these actions rather than of alcohol dehydrogenase inhibition. Thus, it is possible that summation of the central nervous system depressant effects of pyrazole and ethanol could lead to the more rapid development of dependence on the combined treatment.

In our studies, pyrazole was observed not to lower the acetaldehyde concentration for any given blood or tissue level of ethanol. These findings are at variance with the observations of Lester & others (1968). No reason can be given for this discrepancy, but differences in methodology for measuring acetaldehyde and ethanol, dose of pyrazole and route of administration of ethanol may all contribute. If our findings are correct, they indicate either that brain and blood concentrations of acetaldehyde *in vivo* are relatively independent of the rate of formation, or that pyrazole inhibits acetaldehyde elimination.

If acetaldehyde is still formed from ethanol in the presence of pyrazole at the concentrations obtained here, then this regime cannot be used to separate primary and secondary effects of ethanol as suggested by Goldberg & others (1972). The marked toxic effects seen in our experiments make it unlikely that a higher dose would be tolerated by mice for repeated intraperitoneal injection. This approach is, therefore, probably unable to resolve whether changes in brain monoamine concentrations are a direct effect of ethanol, or whether they are consequent on ethanol metabolism. It is interesting that during ethanol withdrawal, brain monoamines changed much more slowly in pyrazole-treated mice than in mice withdrawn from ethanol alone. The different time course appears to reflect the much slower rate of fall of ethanol concentration in the pyrazole-treated animals. These results are thought to suggest a direct relation between these changes in brain amines, the fall in blood ethanol concentration and the withdrawal signs, since these also began much later in withdrawal in pyrazole-treated animals.

Mention has been made of the slow rate of fall of blood ethanol concentrations in withdrawn, pyrazole-treated mice. Other workers (Lieber & DeCarli, 1970) have reported that pyrazole does not prevent the increase in ethanol elimination resulting from chronic administration, and this has been adduced as evidence that the increased rate of elimination is unlikely to be due to induction of alcohol dehydrogenase. Our results do not agree since chronic ethanol administration decreased the rate of elimination of ethanol in pyrazole-treated mice. This suggests that induction of alcohol

dehydrogenase may contribute to metabolic tolerance to ethanol, but there are other explanations. For example, Rydberg, Buijten & Neri (1972) have reported that ethanol increases the half-life of pyrazole administered to rats. We therefore find it difficult to come to any conclusion with respect to the effect of pyrazole on metabolic tolerance to ethanol.

In conclusion, pyrazole increased the physical dependence of mice on ethanol as shown by its potentiation of the withdrawal syndrome. This did not appear to be caused by any marked change in ethanol or acetaldehyde concentrations in pyrazole-treated mice. The main observed differences between mice treated with pyrazole plus ethanol, and ethanol alone, were due to the toxic and apparently central nervous system depressant properties of pyrazole. In the absence of other effects, it is tentatively suggested that these actions, rather than inhibition of alcohol dehydrogenase, are responsible for the potentiation of ethanol dependence by pyrazole.

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