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A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: evidence for a role of acetaldehyde in ethanol dependence

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After chronic exposure to ethanol or acetaldehyde vapour in concentrations which depress locomotor activity, mice show similar behavioural changes during withdrawal, and there is some degree of cross dependence. Mice exposed to acetaldehyde vapour had blood acetaldehyde concentrations similar to those of ethanol-treated mice, but brain acetaldehyde concentrations were apparently lower. There was no accumulation of acetaldehyde in blood or brain in either group during chronic administration. Chronic ethanol or acetaldehyde administration to mice is associated with an increase in the concentrations of the brain monoamines noradrenaline, dopamine and 5-HT. Withdrawal of ethanol or acetaldehyde is associated with a further, rapid, transient rise in the brain catecholamines, noradrenaline and dopamine. These results suggest that acetaldehyde may play a role in some of the biochemical and behavioural changes associated with ethanol dependence.

Acetaldehyde is the primary metabolite of ethanol, and in many respects is more potent than the parent compound. Several workers have implicated acetaldehyde in some of the acute effects of ethanol (Stoltz, Westerfeld & Berg, 1944; MacLeod, 1950; Duritz & Truitt, 1966). In addition some recent theories of ethanol dependence ascribe an important role to acetaldehyde (Walsh, Davis & Yamanaka, 1970; Truitt & Walsh, 1971).

One simple way of testing the hypothesis that acetaldehyde is involved in ethanol dependence would be to administer acetaldehyde chronically and observe the behavioural effects associated with its withdrawal. If acetaldehyde is responsible for ethanol dependence, then it should produce similar withdrawal signs to those resulting from chronic ethanol administration. Similarly, cross dependence between ethanol and acetaldehyde should be exhibited. For experiments of this sort to be practicable, a simple model for ethanol dependence is required. However, it is difficult to administer either ethanol or acetaldehyde in such a way that high blood concentrations are maintained, since these compounds are metabolized very rapidly in most laboratory animals. Goldstein & Pal (1971) administered ethanol to mice by inhalation for a period of days and combined the treatment with daily intraperitoneal injection of pyrazole, an inhibitor of alcohol dehydrogenase. Withdrawal of ethanol from these animals produced a characteristic syndrome of behaviour. Goldstein (1972) and Griffiths, Littleton & Ortiz (1973a) reported that ethanol alone, when administered by inhalation, could produce a similar withdrawal syndrome. The effect of pyrazole on induction of ethanol dependence is discussed elsewhere (Littleton, Griffiths & Ortiz, 1974).

The administration of ethanol and acetaldehyde by inhalation could, therefore, allow a comparison of the ability of these two compounds to produce dependence of the same type. In addition, Griffiths, Littleton & Ortiz (1973b) have shown that changes in brain monoamine concentrations may be related to ethanol dependence in mice. It was suggested that these changes might be secondary to an inhibitory effect on brain amine metabolism produced by acetaldehyde derived from ethanol. If this is true, then it would be expected that acetaldehyde itself could initiate similar changes. It is therefore of interest to evaluate possible changes in brain monoamine metabolism occurring during chronic acetaldehyde administration.

Truitt & Walsh (1971) have reported that brain acetaldehyde concentrations are higher than those in blood when ethanol is administered to animals. These authors have speculated that accumulation of acetaldehyde in brain during chronic administration of ethanol may provide the conditions necessary for the induction of ethanol dependence. A study of the type projected here obviously provides an opportunity to discover whether acetaldehyde accumulation does take place in brain, and also whether it is necessary to achieve a particular acetaldehyde concentration (by chronic administration of acetaldehyde or of ethanol) before dependence occurs.

This paper compares chronic administration of ethanol with acetaldehyde with respect to the behavioural changes which occur, and to the changes in monoamine concentrations in the brains of treated mice. The concentrations of ethanol and acetaldehyde in the blood and brains of mice receiving each treatment have also been measured.

METHODS

Ethanol and acetaldehyde administration

Groups of 30 male mice (T/O strain, 18–22 g) were exposed to ethanol vapour for periods of up to 10 days, using the apparatus described by Griffiths & others (1973a). The apparatus for administration of acetaldehyde was identical, except that acetaldehyde was injected continuously into the air feed pipe by an S.R.I. slow infusion apparatus. Environmental temperature was maintained at 28–30° throughout, and food and water were freely provided. Behavioural changes on withdrawal were evaluated at 15 or 30 min intervals early in withdrawal and later hourly by the scoring method of Goldstein (1972) except that locomotor activity was assessed separately.

Concentrations of ethanol in inspired air were increased from 8–12 mg litre^{-1} on the first day to 20–25 mg litre^{-1} on the tenth day of ethanol administration. Mice receiving acetaldehyde obtained about 750 μg litre^{-1} on the first day to about 4 mg litre^{-1} on the tenth day. Concentrations can only be given approximately because they are very close to toxic levels, and often had to be adjusted to prevent large numbers of deaths in some groups. The ethanol and acetaldehyde concentrations were estimated by the method described below.

Estimation of ethanol and acetaldehyde

Air. 1 ml samples of air from the cage were injected onto the g.l.c. column described below. Peak areas obtained were compared with those produced by injection of 1 μl of ethanol and 1 μl of 1% (w/w) acetaldehyde solution (diluted with distilled water).

Mouse blood and brain. Mice were killed by decapitation so that the heads fell into liquid nitrogen, and 0·5 ml of blood was taken into a heparinized tube on ice.

Blood. To 0·5 ml of blood was added 1 ml of 0·4N perchloric acid. The tube was shaken and the mixture centrifuged. The supernatant was placed in a tightly stoppered tube and heated in a water bath at 55° for 30 min. 1 ml of the head space air above the sample was then injected onto the g.l.c. column described below. Standards were made by the addition of ethanol or acetaldehyde to blood from untreated mice before the addition of perchloric acid.

Brain. Individual brains were dissected in the cold and homogenized in 2 ml of 0·4N perchloric acid. Thereafter, these samples were treated in the same way as blood samples. Standards were prepared by the addition of ethanol or acetaldehyde to the brain homogenate of untreated mice. There was no apparent formation of acetaldehyde from added ethanol when standards were prepared in this way.

G.l.c. (Pye series 104) conditions: the column was a 9 foot glass column containing 20% polyethylene glycol 20M on chromosorb W-HP 80-100 mesh. Injection port temperature was 130°, column temperature 110°, detector temperature 200° and carrier flow 50 ml min^{-1}. Peak areas and retention times were determined by a Vidar 6300 digital integrator. Recovery ($\pm$ s.e.) of acetaldehyde from brain was 81·2 $\pm$ 4·7% and from blood 86·2 $\pm$ 5·3% (n = 10). Recovery of ethanol from brain was 99·3 $\pm$ 2·5% and from blood 99·6 $\pm$ 2·8% (n = 10). The addition of thiourea to brain extracts as reported by Eriksson (1973) for liver homogenates did not affect these recoveries significantly. Under the conditions described here acetaldehyde gave a single peak at 150 s and ethanol a single peak at 270 s.

Estimation of brain monoamine concentrations

Mice were killed by total immersion in liquid nitrogen. Brains were dissected in the cold and, after solvent extraction (Shore & Olin, 1958), noradrenaline and dopamine were estimated fluorimetrically according to Laverty & Taylor (1968) and 5-hydroxytryptamine according to Curzon & Green (1970). Pooled mouse brains, usually three, were used for these determinations. Neither ethanol nor acetaldehyde in the concentrations found in these experiments produced any significant change in the recoveries of monoamines when added to mouse brain homogenates.

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 (99·5%) was supplied by British Drug Houses (Chemicals) Ltd. Acetaldehyde from this source gave only one peak on g.l.c. examination.

RESULTS

The results have been divided into sections, based on the time courses of the behavioural and biochemical changes observed, in order to facilitate comparison of results obtained with ethanol and acetaldehyde. In all cases these compounds were administered as in the regime described under Methods. Mice were killed at various stages during administration and withdrawal (times given in parentheses after headings) to obtain the results shown.

Acute administration (0 to 3 h)

Ethanol. Inhalation of ethanol during this period increased blood ethanol and acetaldehyde concentrations. Brain ethanol concentrations were similar to those in

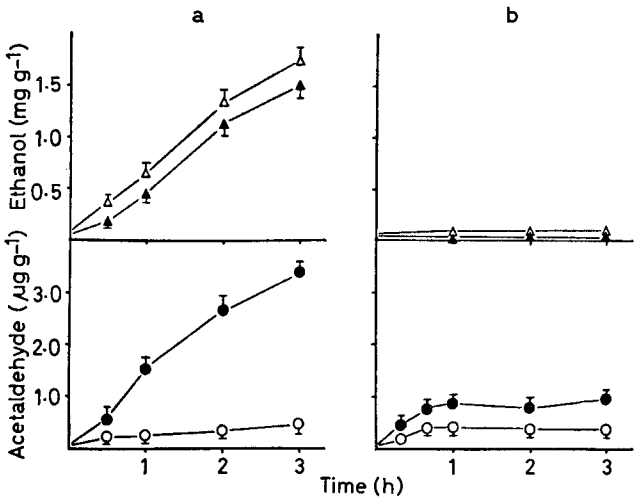

FIG. 1. (a) Accumulation of ethanol and acetaldehyde in mouse blood and brain during administration of ethanol (10 mg litre^{-1}) by inhalation. (b) Accumulation of ethanol and acetaldehyde in mouse blood and brain during administration of acetaldehyde (1 mg litre^{-1}) by inhalation. △, blood alcohol; ▲, brain alcohol concentrations. ○, Blood acetaldehyde; ●, brain acetaldehyde concentrations. Concentrations in blood are expressed in mg ml^{-1} (ethanol) or μg ml^{-1} (acetaldehyde). Each point represents the mean ±s.e. of at least 5 determinations.

blood throughout this period, but brain acetaldehyde concentrations were much higher than those in blood (Fig. 1). Mouse brain monoamine concentrations fell during this period of ethanol administration, but this fall was short-lived (Fig. 2).

Acute ethanol administration increased locomotor activity in grouped mice. This effect reached a maximum after 2 h; later, locomotor depression and ataxia gradually supervened.

Acetaldehyde. Inhalation of acetaldehyde produced a rapid increase in blood and brain acetaldehyde concentrations. These were comparable throughout this period. Very little ethanol was produced from acetaldehyde as shown by the low concentrations in blood and brain (Fig. 1). Mouse brain monoamine concentrations showed a rapid rise during acute acetaldehyde administration (Fig. 2).

Acetaldehyde-treated mice became very excited at first. Locomotor stimulation reached a peak at about 30 min, and after this time locomotor depression and ataxia supervened.

Subacute administration (3 to 24 h)

Ethanol. Blood ethanol concentrations stabilized at about 2·5 mg ml^{-1} during the early stages of chronic administration. Blood acetaldehyde concentrations were low. Brain ethanol concentrations were similar to those in blood while brain acetaldehyde concentrations, although much lower than brain ethanol, were about 10 times blood acetaldehyde concentrations (Table 1).

Ethanol-treated mice showed locomotor depression and ataxia during this period. Brain monoamine concentrations did not differ significantly from those of untreated controls.

Acetaldehyde. Blood acetaldehyde concentrations stabilized at about 0·5 μg ml^{-1} during the early stages of chronic administration. Brain acetaldehyde concentrations

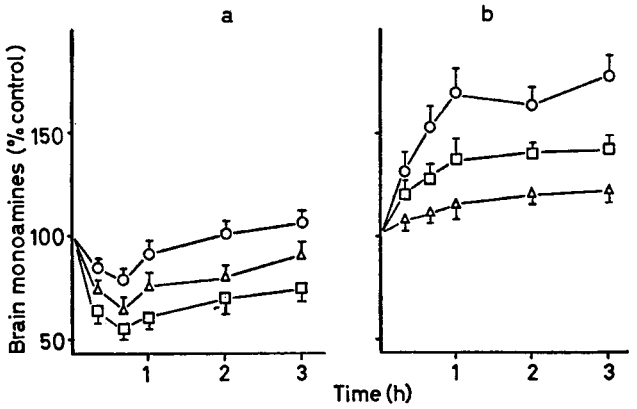

FIG. 2. Monoamine concentrations in mouse brain during administration of (a) ethanol (10mg litre^{-1}) by inhalation; (b) acetaldehyde (1 mg litre^{-1}) by inhalation. ○, noradrenaline; △, dopamine; □, 5-hydroxytryptamine. Concentrations are expressed as percentages of those found in untreated control animals. Each point represents the mean ±s.e. of at least 4 determinations.

Table 1. *Ethanol and acetaldehyde concentrations in blood and brain of mice during chronic ethanol administration. Each value represents the mean ± s.e. of at least 5 determinations.*

Day	Cage concn mg litre^{-1}	Blood		Brain	
		Ethanol mg ml^{-1}	Acetaldehyde μg ml^{-1}	Ethanol mg g^{-1}	Acetaldehyde μg g^{-1}
1	8·2	2·3 ± 0·14	0·69 ± 0·12	2·08 ± 0·12	6·05 ± 0·21
2	9·4	2·4 ± 0·20	0·70 ± 0·04	2·09 ± 0·18	7·50 ± 0·37
3	10·5	2·15 ± 0·16	0·73 ± 0·18	1·94 ± 0·17	6·50 ± 0·31
4	11·4	2·48 ± 0·30	0·74 ± 0·20	2·14 ± 0·20	6·80 ± 0·30
5	13·1	2·20 ± 0·55	0·74 ± 0·16	2·54 ± 0·31	7·42 ± 0·42
10	22·4	2·14 ± 0·07	0·86 ± 0·03	2·40 ± 0·07	7·74 ± 0·46

Table 2. *Ethanol and acetaldehyde concentrations in blood and brain of mice during chronic acetaldehyde administration. Each value represents the mean ±s.e. of at least 5 determinations.*

Day	Cage concn μg litre^{-1}	Blood		Brain	
		Ethanol μg ml^{-1}	Acetaldehyde μg ml^{-1}	Ethanol μg g^{-1}	Acetaldehyde μg g^{-1}
1	750	2·30 ± 0·13	0·34 ± 0·05	1·82 ± 0·08	0·74 ± 0·04
2	900	2·45 ± 0·12	0·46 ± 0·04	1·91 ± 0·08	1·28 ± 0·08
3	1640	2·47 ± 0·13	1·08 ± 0·13	1·98 ± 0·10	1·87 ± 0·07
4	1940	2·61 ± 0·11	2·01 ± 0·08	2·16 ± 0·12	2·03 ± 0·15
5	3000	2·71 ± 0·12	2·56 ± 0·14	2·23 ± 0·14	2·48 ± 0·12
10	4320	3·00 ± 0·14	3·62 ± 0·16	2·48 ± 0·16	2·64 ± 0·13

were higher. Blood and brain ethanol concentrations were low (Table 2).

Acetaldehyde-treated mice exhibited locomotor depression and ataxia, and brain noradrenaline and 5-hydroxytryptamine concentrations were significantly higher than control values.

Chronic administration (1–10 days)

Ethanol. Ethanol concentrations in blood were about 2·5 mg ml^{-1} at withdrawal of ethanol on the tenth day. Blood acetaldehyde concentrations were still very low. Brain ethanol concentrations were similar to those in blood. Brain acetaldehyde concentrations, although higher than those in blood, did not show any marked increase when compared to the brain acetaldehyde concentrations obtained after subacute ethanol administration (Table 1). Brain monoamine concentrations increased during chronic ethanol administration by inhalation (Table 3).

Table 3. *Mouse brain monoamine concentrations during chronic ethanol or acetaldehyde administration.* Values are expressed as percentages of untreated control concentrations. Each value represents means ± s.e. of at least 4 determinations. Absolute concentrations obtained were noradrenaline 0·68 ± 0·05 $\mu g\ g^{-1}$; dopamine 1·32 ± 0·04 $\mu g\ g^{-1}$; 5-hydroxytryptamine 0·82 ± 0·03 $\mu g\ g^{-1}$ (mean of 10 determinations).

	Ethanol administration			Acetaldehyde administration		
Day	Noradrenaline	Dopamine	5-Hydroxy-tryptamine	Noradrenaline	Dopamine	5-Hydroxy-tryptamine
1	75·00 ± 10·52	69·69 ± 6·06	92·47 ± 5·37	165·35 ± 19·73	128·03 ± 19·35	161·17 ± 19·35
3	100·00 ± 11·84	76·21 ± 12·21	106·45 ± 13·97	186·84 28·94	113·63 ± 6·06	162·24 ± 31·05
5	108·83 ± 13·24	98·68 ± 10·24	116·27 ± 10·32	153·94 ± 3·86	149·24 ± 9·84	154·83 ± 3·22
8	138·04 ± 10·29	118·97 ± 12·36	121·25 ± 13·53	169·73 ± 12·94	133·33 ± 6·81	170·96 ± 21·85
10	172·36 ± 19·73	137·12 ± 12·12	134·40 ± 9·67	186·84 ± 31·51	129·54 ± 15·15	152·68 ± 5·37

The increases in ethanol concentration in inspired air during this period were necessary to maintain the ataxia and locomotor depression seen in subacute administration. Treated mice ate and drank less than controls, but maintained body weight compared to controls. Mice did not become hypothermic unless they became comatose during ethanol administration. Comatose mice (5–10% of the total) usually died. These results are shown in Table 4.

Acetaldehyde. Acetaldehyde was toxic when administered chronically by inhalation (see below); for this reason some results refer to animals exposed to acetaldehyde for less than 10 days—in this case the time course of exposure is given in the text.

Blood acetaldehyde concentrations were about 3·5 $\mu g\ ml^{-1}$ after 10 days. Brain acetaldehyde concentrations were similar. Blood and brain ethanol concentrations were very low (Table 2).

Table 4. *Bodyweight and food and water intake of mice receiving ethanol or acetaldehyde by inhalation compared to untreated controls.* Each bodyweight value refers to the mean ± s.e. of 15 estimations (— indicates value not determined). The rectal temperatures of mice after ten days' administration were, ethanol 36·7 ± 0·4°; acetaldehyde 30·5 ± 1·1°; control 37·4 ± 0·2°. Temperatures were measured with a Grant thermistor probe inserted 1·5 cm into the rectum. Each value is the mean ±s.e. of 10 determinations.

	Control			Ethanol administration			Acetaldehyde administration		
Day	g	Water (ml kg^{-1}) in 24 h	Food (g kg^{-1}) in 24 h	g	Water (ml kg^{-1}) in 24 h	Food (g kg^{-1}) in 24 h	g	Water (ml kg^{-1}) in 24 h	Food (g kg^{-1}) in 24 h
1	17·4 ±0·2	—	—	18·2 ±0·4	—	—	18·2 ±0·3	—	—
2	18·3 ±0·4	264·1	—	19·3 ±0·3	259·1	—	17·8 ±0·6	168·2	—
4	19·4 ±0·4	250·9	247·9	18·6 ±0·5	246·9	211·5	15·4 ±0·8	103·8	164·4
6	18·5 ±0·5	300·3	—	19·4 ±0·5	265·2	—	14·4 ±0·5	102·5	—
8	18·6 ±0·5	270·4	—	19·8 ±0·6	209·5	—	13·2 ±0·7	89·7	—
10	18·9 ±0·6	220·5	195·5	20·8 ±0·6	240·5	178·9	12·5 ±0·7	63·9	103·8

Brain monoamine concentrations increased during acetaldehyde administration, monoamine concentrations after up to 10 days of acetaldehyde administration are shown in Table 3.

Locomotor depression and ataxia were shown by mice receiving acetaldehyde during this period. Treated mice ate and drank less than controls and showed a significant loss of weight. Mice receiving acetaldehyde were hypothermic compared to controls, comatose mice were even more hypothermic (Table 4). The death rate in treated mice was 5–10% when treatment did not extend beyond 5 days; it increased to 20% or more when acetaldehyde was given for 10 days.

Withdrawal of ethanol and acetaldehyde

Ethanol. Ethanol was withdrawn after administration by inhalation for 10 days. Blood ethanol concentrations fell from 2·5 mg ml^{-1} to almost undetectable levels in about 3 h. Brain ethanol concentrations fell at a similar rate. Blood and brain acetaldehyde concentrations fell with a similar time course to that shown by ethanol concentrations (Fig. 3). The rate of elimination of ethanol was increased in these mice when compared to mice given ethanol acutely (Fig. 4).

Brain monoamine concentrations rose after ethanol withdrawal to reach a peak after about 1 h. After this, they fell to reach control levels over the next 10 h (Table 5).

Tremor, piloerection, tail lift and convulsions on handling were seen during ethanol withdrawal. These signs reached a maximum after about 3 h and persisted for some 12–15 h (Fig. 5).

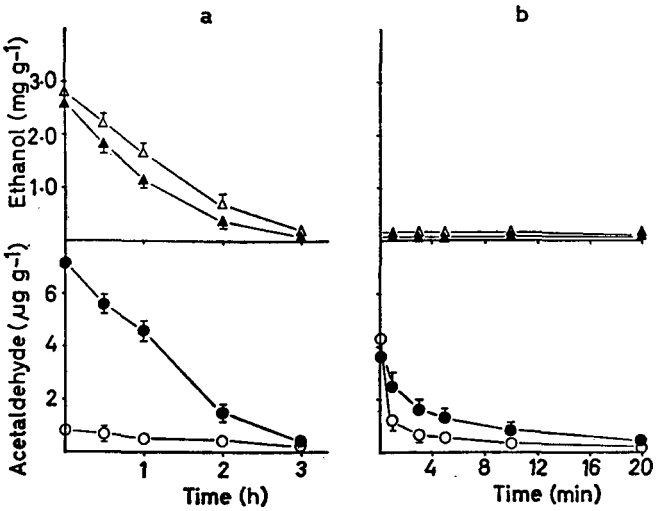

FIG. 3. Ethanol and acetaldehyde concentrations in mouse blood and brain (a) during withdrawal from ethanol; (b) during withdrawal from acetaldehyde. △, blood alcohol; ▲, brain alcohol concentrations. ○, blood acetaldehyde; ●, brain acetaldehyde concentrations. Concentrations in blood are expressed in mg ml^{-1} (ethanol) or μg ml^{-1} (acetaldehyde). Each point represents the mean ±s.e. of at least 5 determinations.

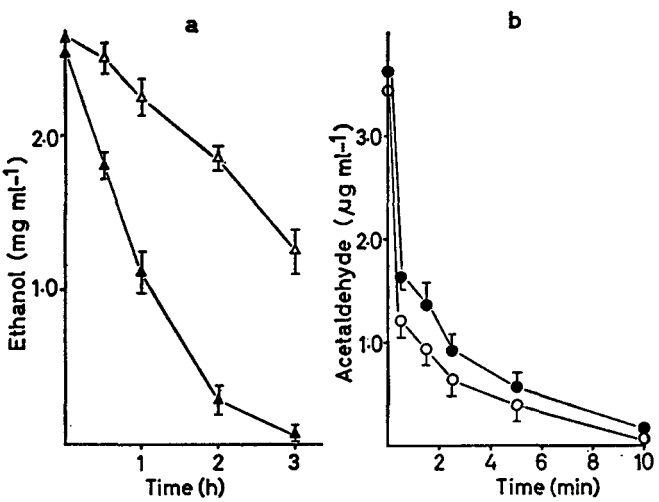

FIG. 4. (a) Elimination of ethanol from blood of mice given ethanol acutely or chronically by inhalation. (b) Elimination of acetaldehyde from blood of mice given acetaldehyde acutely or chronically by inhalation. △, blood alcohol concentrations from mice given ethanol by inhalation for 2 h and then withdrawn at time 0. ▲, blood alcohol concentrations from mice given ethanol for 10 days and then withdrawn at time 0. ○, blood acetaldehyde concentrations from mice given acetaldehyde by inhalation for 2 h and then withdrawn. ●, blood acetaldehyde from mice given acetaldehyde for 10 days. Each point represents mean ±s.e. of 5 determinations.

Acetaldehyde. The results given here refer only to experiments in which acetaldehyde was withdrawn after 8–10 days. Blood acetaldehyde concentrations fell rapidly to become almost undetectable after 20 min. Brain acetaldehyde concentrations fell with a similar time course (Fig. 3). There was no change in the rate of acetaldehyde elimination in these mice compared to mice given acetaldehyde acutely (Fig. 4).

Table 5. *Mouse brain monoamine concentrations during withdrawal of ethanol or acetaldehyde.* Values are expressed as percentages of untreated control concentrations. Each value represents the mean ±s.e. of at least 6 determinations. The symbol — means not determined at that time interval.

Time	Ethanol withdrawal			Acetaldehyde withdrawal		
(min)	Noradrenaline	Dopamine	5-Hydroxy-tryptamine	Noradrenaline	Dopamine	5-Hydroxy-tryptamine
0	172·36 ± 19·73	137·12 ± 12·12	134·40 ± 9·67	185·59 ± 27·63	137·12 ± 13·63	154·83 ± 14·30
10	—	—	—	255·85 ± 31·28	224·24 ± 16·06	158·06 ± 8·60
30	230·26 ± 31·57	207·57 ± 14·39	150·53 ± 8·60	207·89 ± 32·89	156·06 ± 14·39	164·51 ± 5·37
60	214·47 ± 23·68	200·75 ± 7·57	139·78 ± 7·52	136·84 ± 14·47	116·66 ± 6·81	139·78 ± 6·45
120	167·10 ± 13·15	141·66 ± 17·47	129·03 ± 9·58	—	—	—
240	117·10 ± 30·26	158·33 ± 18·93	139·78 ± 24·73	—	—	—
360	114·21 ± 13·15	127·50 ± 16·85	118·27 ± 6·45	102·63 ± 18·43	106·81 ± 7·54	135·48 ± 7·52
480	108·42 ± 16·15	117·42 9·09	112·05 ± 8·59	—	—	—

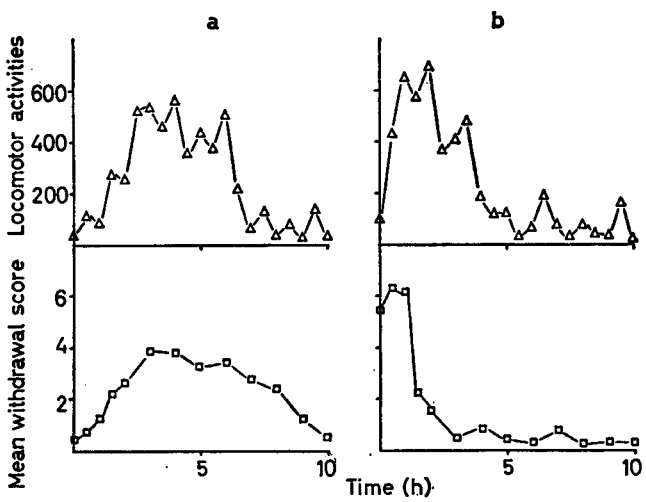

FIG. 5. Effect of (a) ethanol withdrawal in mice and (b) acetaldehyde withdrawal in mice on locomotor activity, mean withdrawal score. △, the activity counts, assessed every 30 min, on an Aminex Activity Meter type S, sensitity 40 μA for a group of 15 mice. □, the mean withdrawal score (determined as described under Methods) for another group of 15 mice. Values represent the mean of 3 separate experiments.

There was a short, transient increase in brain catecholamines after acetaldehyde withdrawal. Brain monoamine concentrations then returned to near control levels after about 6 h (Table 5).

Mice withdrawn from acetaldehyde were initially excited although ataxic, and showed tremor, piloerection, tail lift and convulsions on handling. Piloerection and convulsions on handling were particularly marked and were often evident before withdrawal. In most animals these signs reached a maximum very soon after withdrawal and persisted for only about 2 h (Fig. 5).

Cross dependence between ethanol and acetaldehyde

The administration of ethanol by inhalation during acetaldehyde withdrawal was capable of preventing the withdrawal syndrome for acetaldehyde. Similarly, the administration of acetaldehyde by inhalation during ethanol withdrawal could inhibit the behavioural change associated with ethanol withdrawal. In both cases complete suppression of withdrawal signs could only be obtained with sedative doses of ethanol or acetaldehyde.

DISCUSSION

When mice are exposed to acetaldehyde vapour for periods of up to ten days they appear to develop dependence on acetaldehyde. This dependence shares many characteristics with ethanol dependence produced in a similar manner, and there is some degree of cross dependence. Blood acetaldehyde concentrations were similar in ethanol- and acetaldehyde-treated mice. Brain acetaldehyde concentrations appeared higher in ethanol-treated mice, but in neither group was there marked accumulation of acetaldehyde in brain during chronic administration. Also, chronic acetaldehyde administration and withdrawal are associated with similar changes in brain monoamine concentrations to those produced by chronic ethanol administration and withdrawal. These results suggest that acetaldehyde derived from ethanol may be involved both in the induction of ethanol dependence, and also in the changes in brain monoamine concentrations associated with ethanol dependence.

The toxicity of acetaldehyde was greater than that of ethanol when both were administered by inhalation. This is interesting in view of the observation that brain acetaldehyde concentrations were higher in ethanol-treated mice. However, we feel that this may be explained if toxicity is related to changes induced in respiratory mucosa when acetaldehyde is given by inhalation. In this case the concentration of acetaldehyde in inspired air would be the factor determining toxicity rather than blood or brain acetaldehyde concentrations.

Behavioural changes similar to those of ethanol withdrawal could be elicited from mice actually receiving acetaldehyde, so it is difficult to equate this behaviour with a true *withdrawal* syndrome. However, because acetaldehyde elimination is so rapid, marked fluctuations in blood acetaldehyde concentration probably take place even while mice are receiving acetaldehyde, and therefore the animals which showed this behaviour may have been to some extent "withdrawn" at the time of testing. The rapidity of acetaldehyde elimination may also explain the high withdrawal scores obtained during acetaldehyde withdrawal, since most mice in the group would reach the peak of withdrawal within the same test period. During ethanol withdrawal, ethanol and acetaldehyde concentrations fall more slowly, so that the withdrawal syndrome is less precipitate and a group of mice includes individuals at different stages of withdrawal. It should be noticed that the total withdrawal score is greater in the ethanol withdrawn group.

It seems unlikely that acetaldehyde can be directly responsible for the behavioural changes observed during ethanol withdrawal, since its concentration in blood or brain

is very low, or undetectable during much of the withdrawal syndrome in mice. Therefore, if acetaldehyde is involved in ethanol withdrawal, it is probably because it has initiated some neurochemical change which persists for several hours. The alteration of brain monoamine concentrations described here may be related to such a change, and could indeed be secondary to formation of acetaldehyde from ethanol. It is, however, unlikely that these changes in brain monoamine concentrations can be *directly* related to induction of ethanol dependence and behavioural withdrawal (Griffiths, Littleton & Ortiz, 1974).

Ethanol is known to change monoamine metabolism towards a more reductive pathway, so that an increase occurs in the relative importance of (for example) glycol metabolites, at the expense of acidic metabolites (Smith & Gitlow, 1967). Over a long period such an effect might lead to an increase in the concentration of brain monoamines as a result of reduction in efficiency of monoamine oxidation. This alteration in monoamine metabolism has been suggested to be due to an increase in the NADH/NAD ratio as a result of the metabolism of ethanol to acetaldehyde (Feldstein & Wong, 1965), or to competition between acetaldehyde and aldehyde products of monoamine oxidase for aldehyde dehydrogenase (Walsh, Truitt & Davis, 1970). Our results support the latter concept. Since acetaldehyde, when administered alone, produced an increase in brain monoamine concentrations, then it cannot be the conversion of ethanol to acetaldehyde which is important, and one would not expect the metabolism of the relatively small concentration of acetaldehyde to alter the brain NADH/NAD ratio significantly. Therefore, if the observed change in brain monoamine concentrations is due to reduced oxidation, it is likely to be because acetaldehyde competes with monoamine metabolites for aldehyde dehydrogenase. The further increase in catecholamine concentrations early in withdrawal may be due to increased synthesis and release associated with the excitement phase of withdrawal (Griffiths & others, 1974). This would cause a rapid increase in catecholamine concentrations if it was associated with reduced breakdown as suggested above.

Some hypotheses of ethanol dependence implicate both brain monoamines and acetaldehyde. In particular, Collins & Cohen (1968) and Walsh, Davis & Yamanaka (1970) have shown that acetaldehyde may favour or participate in condensation reactions involving monoamines and their metabolites. It is possible that these compounds may be responsible for the observed increases in brain monoamines, since Yamanaka (1972) and Collins, Cashaw & Davis (1973) have suggested that some of them may have monoamine oxidase inhibitory properties; alternatively, they may interfere with the fluorometric estimation of monoamines in some way. Obviously, some future research must be directed towards identification of these compounds in brains of ethanol- and acetaldehyde-dependent animals.

The changes in brain monoamines demonstrated during chronic administration of ethanol or acetaldehyde may simply reflect the cns depression produced. If this is so, then other cns depressants should have similar effects. Experiments are currently in progress to test this hypothesis, but it should be realized that, even if this is true, changes in brain monoamine metabolism may still be involved in ethanol dependence (and in dependence on other cns depressants) and that acetaldehyde formation from ethanol may still be important in producing ethanol dependence.

Brain acetaldehyde concentrations were higher than those in blood during the early stages of acetaldehyde treatment and throughout chronic ethanol treatment. It is possible that this is an artifact and that oxidation of ethanol in brains after death

is responsible for this finding. However, since the difference between brain and blood acetaldehyde concentrations is apparent in acetaldehyde-treated mice, where brain ethanol concentrations are low, and since ethanol added to brain homogenates did not affect the recovery of acetaldehyde, it seems more likely that the difference in concentration in the two sites is real. We believe that there are two likely explanations for this finding. Firstly, that binding of acetaldehyde takes place in brain and that this reduces the rate of elimination of acetaldehyde from brain. Fig. 3b shows that acetaldehyde elimination after administration of acetaldehyde by inhalation is slower from brain than from blood. It is possible that in brain, acetaldehyde binding may be associated with the formation of acetoin (Stoltz & others, 1944) or tetrahydroisoquinolines (vide supra). Secondly, ethanol may interfere with the metabolism of acetaldehyde in brain perhaps by competition for aldehyde dehydrogenase. A combination of these two factors could explain the observed differences between blood and brain acetaldehyde concentrations in both groups and between brain acetaldehyde concentrations in ethanol-treated and acetaldehyde-treated mice. The possibility exists that acetaldehyde is formed in brain from ethanol during chronic ethanol administration. Our findings suggest that this may be so, but we are unable to speculate further.

The simple experiments reported here suggest a role for both acetaldehyde and brain monoamines in ethanol dependence. It seems probable that these roles are interconnected, but the mechanism remains obscure.

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