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CHANGES IN MONOAMINE CONCENTRATIONS IN MOUSE BRAIN ASSOCIATED WITH ETHANOL DEPENDENCE AND WITHDRAWAL

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1 Chronic administration of ethanol to mice by inhalation induced tolerance to ethanol and produced an increase in the concentration of brain monoamines.

2 Withdrawal of ethanol from dependent mice caused behavioural changes associated with a further transient rise in brain monoamine concentrations which then declined to control levels.

3 Inhibition of the withdrawal syndrome by the administration of ethanol postponed the changes in monoamines associated with withdrawal.

4 Administration of inhibitors of catecholamine synthesis before withdrawal of ethanol modified the withdrawal syndrome.

Introduction

It is difficult to define dependence satisfactorily. The main criterion of physical dependence should be the presence of a recognizable withdrawal syndrome when the drug in question is withdrawn. Most drugs which induce physical dependence require some tolerance to the pharmacological actions of the drug to have developed before withdrawal will produce the characteristic syndrome.

Dependence on ethanol can be induced in a variety of experimental animals as evidenced by a withdrawal syndrome on cessation of ethanol, but it is only recently that adequate models for ethanol dependence have been described for mice (Freund, 1969; Goldstein & Pal, 1971). The usual methods of chronic administration of drugs, either in drinking water or by injection, are probably incapable of maintaining high blood ethanol levels for the periods required to induce dependence in these animals. This is because mice have an extremely high rate of ethanol metabolism (Marshall & Owens, 1955) and exhibit a marked diurnal rhythm in drinking activity.

Freund (1969) overcame this problem by placing mice in a cold (4°C) environment and replacing 35% of dietary calories with ethanol. Goldstein & Pal (1971) administered ethanol by inhalation, but found it necessary to combine this treatment with daily intraperitoneal injections of pyrazole, an inhibitor of alcohol dehydrogenase.

Both methods fall short of a satisfactory model for use in experiments designed to investigate biochemical correlates of ethanol dependence. Freund's technique includes severe diet restriction and low environmental temperature, both factors likely to influence many essential biochemical processes, since such animals are subjected to chronic environmental stress. The administration of ethanol by inhalation is probably more satisfactory (though one should not discount the possible irritant effects of ethanol vapour), but the administration of pyrazole is an undesirable complication, since it is not a specific inhibitor of alcohol dehydrogenase (Lieber, Rubin, DeCarli, Misra & Gang, 1970). Also the administration of pyrazole would be expected to obscure the development of metabolic tolerance to ethanol.

Goldstein (1972a) has shown that the administration of ethanol without intraperitoneal injections of pyrazole can produce a similar withdrawal syndrome to that reported earlier (Goldstein & Pal, 1971). The main problem which occurs is that the marked variation in blood ethanol levels increases mortality during the induction of dependence, and also increases the heterogeneity of the withdrawal syndrome.

Griffiths, Littleton & Ortiz (1973) reported that a modification of the technique of Goldstein, in which ethanol was administered for a much longer period and in increasing concentrations, was capable of producing a very similar withdrawal syndrome to that described by Goldstein & Pal (1971). These modifications reduce the mortality and the variation in the withdrawal syndrome to within acceptable limits.

This paper describes the use of this model for

ethanol dependence to investigate the changes in ethanol metabolism which occur when ethanol is administered to mice. In addition we have measured the concentrations of noradrenaline, dopamine, and 5-hydroxytryptamine occurring in the brains of mice during chronic ethanol administration and withdrawal, since these neurotransmitters have often been implicated in these processes (Feldstein, 1971).

We have sought to modify the withdrawal syndrome by the administration of ethanol during withdrawal, and also by the administration of inhibitors of catecholamine synthesis before withdrawal. The effects of these additional treatments on changes in brain monoamine concentrations occurring during ethanol withdrawal have also been studied.

Methods

Induction of ethanol dependence

Groups of 30 male T.O. strain white mice (18-22 g) were housed in cages enclosed by perspex boxes with a volume of about 100 litres. A high ambient temperature (28° C-30° C) was used in these experiments to prevent hypothermia otherwise experienced by the ethanol-treated mice. Food and water were freely provided.

Air was pumped through the perspex boxes at a rate of 2 litres/minute. Part of the air stream was diverted through ethanol at 50° C. By varying the proportion of air flowing through the ethanol, the concentration in the air of the box was continuously variable from 0 to 60 mg ethanol per litre. Groups of mice were exposed to ethanol vapour for 10 days, during which time the ethanol concentration in the inspired air was raised from 10-15 mg/litre on day 1 to 25-35 mg/litre on day 10. After this period the front of the box was opened and the total air stream diverted past the ethanol. The ethanol concentration in the inspired air then fell to almost undetectable levels within 10 minutes.

Behavioural evidence of ethanol withdrawal was sought in two ways. Locomotor excitation and depression were measured with an Animex type S activity meter. The output was displayed on a Grass type 7B polygraph. Subjective scoring of other behavioural changes during ethanol withdrawal (including piloerection, tremor, tail lift and convulsions on handling) was performed in the way described by Goldstein (1972a).

In all cases, comparison was made with controls which had been kept under identical environmental conditions, except for the absence of ethanol in the inspired air.

Estimation of ethanol

Ethanol concentrations in air were measured by taking 1 ml of air from the perspex box into a gas-tight syringe and injecting this directly on to the gas-liquid chromatograph column described below. The peak areas obtained were compared with those obtained after the injection of 1 μl ethanol on to the column.

To estimate ethanol concentrations in brain and blood, mice were killed by decapitation, so that the heads dropped into liquid nitrogen, and were bled into glass vials containing heparin at 0° C.

Brain. Individual brains were dissected in the cold, and then homogenized in 2 ml 0.4 N perchloric acid. The homogenate was then centrifuged and the supernatant neutralized with 2 ml 0.4 N NaOH. The tube containing the sample was tightly stoppered and shaken for 30 min in a water bath at 55° C. Standards were prepared by the addition of known quantities of ethanol to the brain homogenates of untreated mice.

Blood. To 0.5 ml of blood was added 1 ml of 0.4 N perchloric acid. The tube was shaken and then centrifuged. The supernatant was neutralized with 1 ml of 0.4 N NaOH. The tube containing the sample was tightly stoppered and shaken for 30 min in a water bath at 55° C. Standards were prepared by the addition of known quantities of ethanol to blood from untreated mice before the addition of perchloric acid.

Gas liquid chromatograph (Pye series 104) conditions. One ml of head space air from the samples described above was injected on to the column. Peak areas were measured by a Vidar 6300 digital integrator. The column was a 9 foot glass column containing 20% P.E.G. 20 M on Chromosorb W-HP, 80-100 mesh. Column temperature was 100° C, detector temperature 200° C, and carrier gas flow 50 ml/minute. The retention time for ethanol under these conditions was 270 seconds.

Estimation of mouse brain monoamines

Mice were killed by total immersion in liquid nitrogen. Brains were dissected in the cold and were taken for fluorimetric estimation of dopamine, noradrenaline and 5-hydroxytryptamine. Noradrenaline and dopamine were estimated either by the method of Brownlee & Spriggs (1965), or by the method of Laverty & Taylor (1968). 5-Hydroxytryptamine was measured by the method of Curzon & Green (1970). Pooled mouse brains, usually three, were used for these

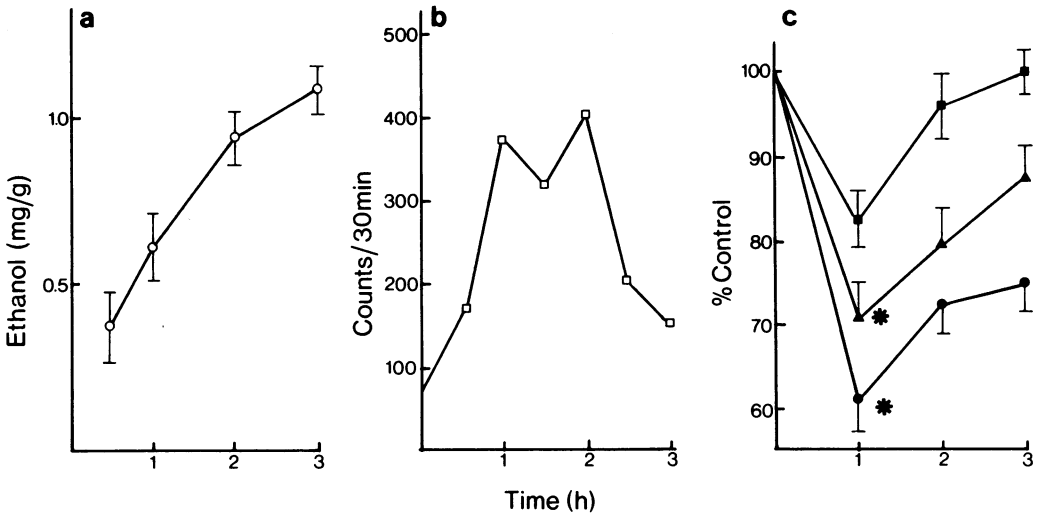

Fig. 1 Effect of ethanol administered acutely by inhalation in mice. Times on the abscissae refer to duration of ethanol administration. (a) The increase in brain ethanol concentration of mice exposed to 15 mg/litre ethanol vapour. (b) Locomotor excitation in mice treated in this way. Values given are the sums of counts obtained on an Animex Activity meter in 30 min periods with an instrumental sensitivity of 40 μA. (c) Mouse brain monoamine concentrations after the acute administration of ethanol expressed as a percentage of untreated control concentrations. Noradrenaline (■), dopamine (▲) and 5-hydroxytryptamine (●). Vertical bars represent s.e. mean of at least five determinations. * Indicates a monoamine concentration significantly ($P < 0.05$) below control.

determinations. Ethanol added to brain homogenates had no significant effect on the recovery of monoamines when they were estimated in this way.

Administration of drugs

Administration by injection was always made intraperitoneally in a volume of 0.25 ml. Solutions were made in distilled water unless otherwise stated.

Drugs and chemicals

Analytical grade reagents were used whenever these were available. Ethanol (99.8% v/v A.R. quality) was supplied by James Burroughs Ltd. α-Methyltyrosine methyl ester was obtained from R.N. Emmanuel and bis-(4-methyl-1-homopiperazinyl thiocabonyl) disulphide (FLA-63) was a gift from Astra Chemicals.

Results

The results are given under separate headings to emphasize the changes observed in relationship to different phases of ethanol administration and withdrawal. The divisions themselves are arbitrary, but are based on the behavioural and biochemical changes observed.

In all cases ethanol administration followed the basic procedure described in the methods section, but experiments were stopped after various time intervals (given in parentheses after each heading, and the mice were killed to obtain the results given below.

Acute ethanol administration (0 to 3 hours)

During the first 3 h of ethanol administration by inhalation, the concentration of ethanol in blood rose to about 1.0 mg/ml. Brain ethanol concentrations were very similar to those obtained in blood.

Mice receiving ethanol at first showed a period of locomotor excitation which lasted about 3 hours. After this time ataxia and locomotor depression gradually supervened.

There was an initial fall in the concentrations of noradrenaline, dopamine and 5-hydroxytryptamine in the brains of treated mice. Noradrenaline concentrations recovered to those in control brains after 2 h, but dopamine and 5-hydroxytryptamine concentrations remained low for 3 hours.

The results obtained for the acute administration of ethanol are shown in Figure 1.

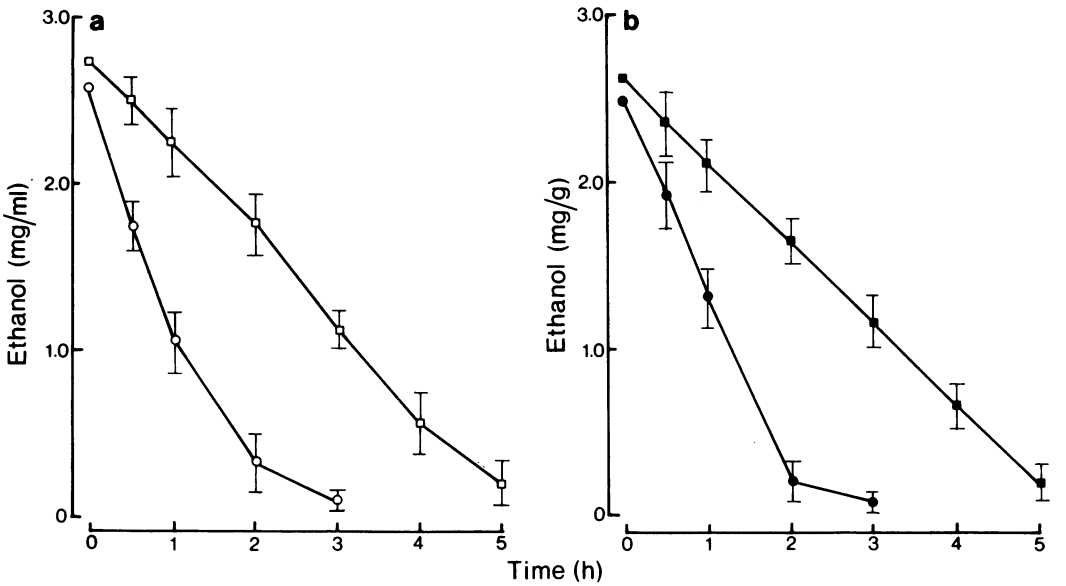

Fig. 2 Rate of elimination of ethanol by mice. Times on the abscissae refer to the time after ethanol withdrawal. Ethanol was administered by inhalation for a period of 3 h (acute) or 10 days (chronic) before withdrawal. (a) Elimination of ethanol from blood, acute (□) and chronic (○). (b) Elimination of ethanol from brain, acute (■) and chronic (●). Points represent means with s.e. mean of at least five determinations.

Subacute ethanol administration (3 to 24 hours)

During this period, blood and brain ethanol concentrations became stable at about 2.25 mg/ml (blood) and 2.0 mg/g (brain).

Locomotor depression and ataxia were evident in mice receiving ethanol. There were no significant changes in brain monoamine concentrations.

Chronic ethanol administration (24 h to 10 days)

Ethanol concentrations in blood and brain increased gradually over this period, because the ethanol concentration in inspired air was increased daily as an integral part of the experiment. This increase in ethanol concentration was necessary to maintain the ataxia and locomotor depression seen during subacute administration. We have found it necessary to maintain these behavioural changes in order to obtain a reproducible withdrawal syndrome.

Blood ethanol concentrations rose to between 2.5-3.5 mg/ml after 10 days' administration. Brain ethanol concentrations were very similar. These concentrations of ethanol in blood and brain were toxic in naive mice.

The rate of ethanol elimination from blood and brain was markedly increased by chronic administration. Naive mice metabolized ethanol at a rate (as determined by the blood curve method) of 540 mg $kg^{-1} h^{-1}$, whereas after 10 days' administration the equivalent value was 1125 mg $kg^{-1} h^{-1}$. Ethanol elimination from the brain showed a similar increase in rate (Figure 2).

Brain noradrenaline and dopamine concentrations were increased during this phase of ethanol administration. The increase was significant after seven days, and concentrations continued to increase throughout the period. After 10 days' administration catecholamine concentrations were 40-50% higher than those in untreated control brains. 5-Hydroxytryptamine concentrations also rose, but less markedly than those of the catecholamines. These results are shown in Table 1.

Mice receiving ethanol in this way ate and drank less than controls, but these differences were not marked, and test mice showed no significant change in weight as compared to controls. During this period of ethanol administration, 5-10% of mice became comatose and these animals usually died. Comatose mice were markedly hypothermic, despite the high ambient temperature. Healthy ethanol-treated mice showed no significant difference in rectal temperature from untreated controls.

During ethanol administration there were no

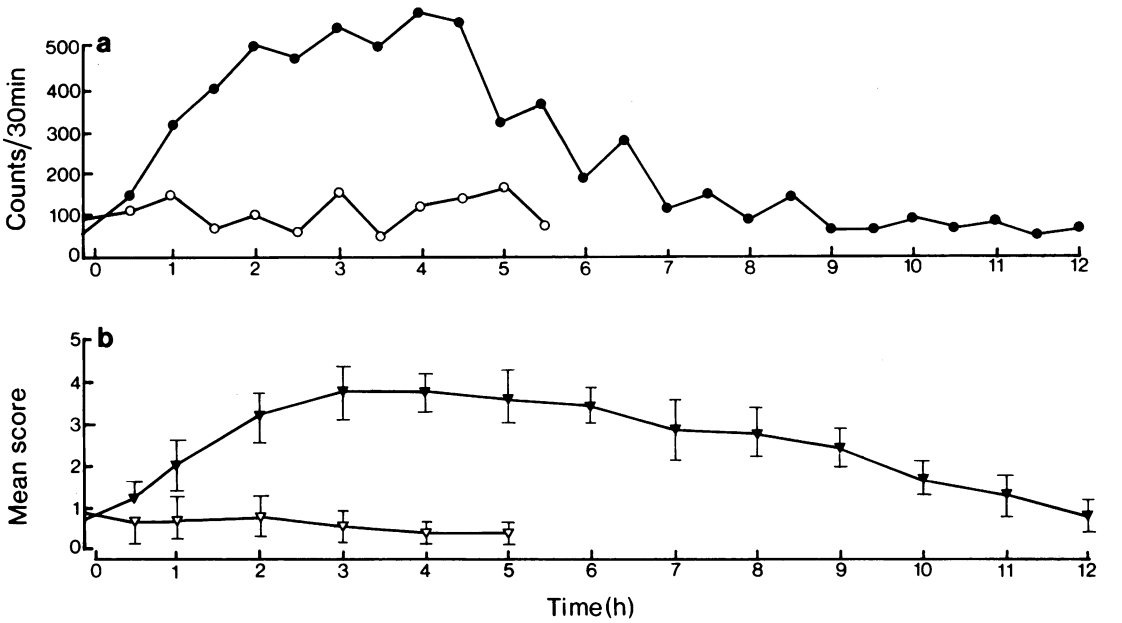

Fig. 3 Ethanol withdrawal syndrome in mice. Times on the abscissae refer to time after ethanol withdrawal. (a) Locomotor activity during ethanol withdrawal. Animex sensitivity 40 μA. Groups of 15 mice; ethanol withdrawn (●) and controls under similar environmental conditions (○). (b) Withdrawal signs during ethanol withdrawal scored as described by Goldstein (1972a). Each point represents the mean with s.e. mean of at least 15 observations. Ethanol withdrawn (▼) and controls kept under similar environmental conditions (▽).

signs of tremor and tail lift, and handling convulsions could only rarely be elicited. Piloerection was sometimes seen, but this was not common.

Acute ethanol withdrawal (withdrawal to withdrawal plus 3 hours)

Blood and brain concentrations of ethanol fell rapidly during ethanol withdrawal, so that ethanol concentrations did not differ from those in untreated mice after 3 h (Figure 2).

During this period withdrawn mice showed locomotor excitation which was often intense. In general this increased locomotor activity preceded the other signs of ethanol withdrawal which began during this period and reached a maximum after about 3 h (Figure 3).

Brain monoamine concentrations showed a rapid transient rise above pre-withdrawal values during the early period of withdrawal. This increase was greater for noradrenaline and dopamine than for 5-hydroxytryptamine, and reached a maximum within 1 h of ethanol

Table 1 Effect of chronic ethanol administration by inhalation on mouse brain monoamine concentrations

	Period of ethanol administration		
Monoamine	*3 days*	*7 days*	*10 days*
Noradrenaline	100 ± 11.8% (NS)	128 ± 10.3% (NS)	147 ± 13.6% ($P < 0.05$)
Dopamine	71 ± 12.1% (NS)	115 ± 12.4% (NS)	138 ± 12.1% ($P < 0.05$)
5-hydroxytryptamine	106 ± 14.2% (NS)	121 ± 13.5% (NS)	130 ± 7.7% ($P < 0.02$)

Monoamine concentrations are expressed as means with s.e. mean of percentages compared to control means as 100%. Absolute values for control concentrations were: noradrenaline, 0.76 ± 0.05 μg/g; dopamine, 1.32 ± 0.04 μg/g; 5-hydroxytryptamine, 0.93 ± 0.03 μg/g. These are means with s.e. mean of at least five determinations of concentrations per wet weight of brain tissue. *P* values in parentheses were calculated with Student's *t* test; NS indicates not significant at the $P < 0.1$ level.

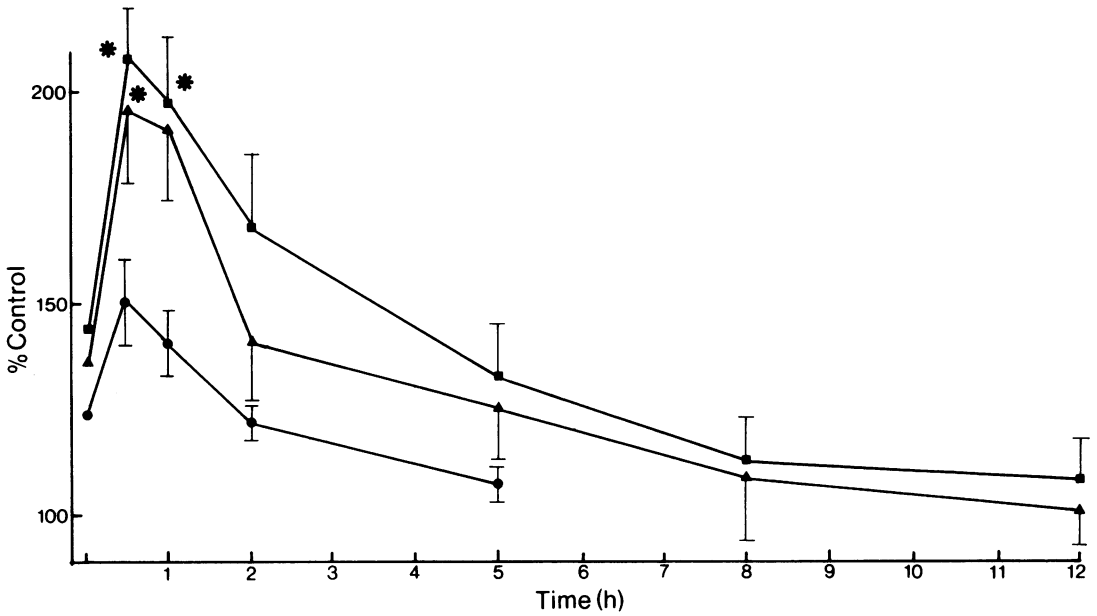

Fig. 4 Mouse brain monoamine concentrations during ethanol withdrawal. Noradrenaline (■), dopamine (▲) and 5-hydroxytryptamine (●). Points represent the mean with s.e. mean of at least five determinations. Time on the abscissae indicates the time after ethanol withdrawal. * Indicates a monoamine concentration significantly ($P < 0.1$) above pre-withdrawal values.

withdrawal. After this time, monoamine concentrations decreased and reached pre-withdrawal values 2 h after withdrawal (Figure 4).

Subacute ethanol withdrawal (withdrawal plus 3 h to withdrawal plus 24 hours)

During this period of withdrawal, ethanol was almost undetectable in the blood and brain of withdrawn mice. Withdrawn mice showed locomotor excitement until 5 or 6 h after withdrawal. After this time locomotor depression gradually supervened. The subjective signs of ethanol withdrawal (tremor, piloerection, handling convulsions and tail lift) persisted during this period. The duration of these changes was variable, but in most instances withdrawn mice showed no gross difference from untreated controls after 12-15 h (Figure 3).

Brain monoamine concentrations fell during this period, and had regained pre-treatment control values after 8 hours. These changes are also shown in Figure 4.

Modification by drugs of the ethanol withdrawal syndrome

The injection of ethanol (2 g/kg, i.p.) at the start of ethanol withdrawal postponed the locomotor excitation associated with the early phase of ethanol withdrawal. The changes in brain monoamines associated with withdrawal were also postponed. Returning withdrawn mice to a high concentration of ethanol vapour was also effective in both these respects. The administration of ethanol at this time also inhibited the development of the other signs of ethanol withdrawal. Results for the changes produced by injected ethanol are shown in Figure 5.

Inhibition of tyrosine hydroxylase by the administration of α-methyltyrosine methyl ester (300 mg/kg, i.p.) 4 h before withdrawal reduced brain catecholamine concentrations and prevented the transient rise in noradrenaline and dopamine associated with ethanol withdrawal. This treatment did not affect the locomotor depression and ataxia shown by mice before withdrawal, but greatly reduced the locomotor excitement shown by mice after withdrawal. However, the subjective signs of ethanol withdrawal were potentiated by the administration of α-methyltyrosine. Piloerection and handling convulsions were particularly affected. These results are shown in Figure 6.

An inhibitor of dopamine β-hydroxylase, FLA-63 (40 mg/kg, i.p.), when administered 4 h before withdrawal greatly reduced noradrenaline concentrations in the brains of treated mice. Brain dopamine concentrations were slightly decreased.

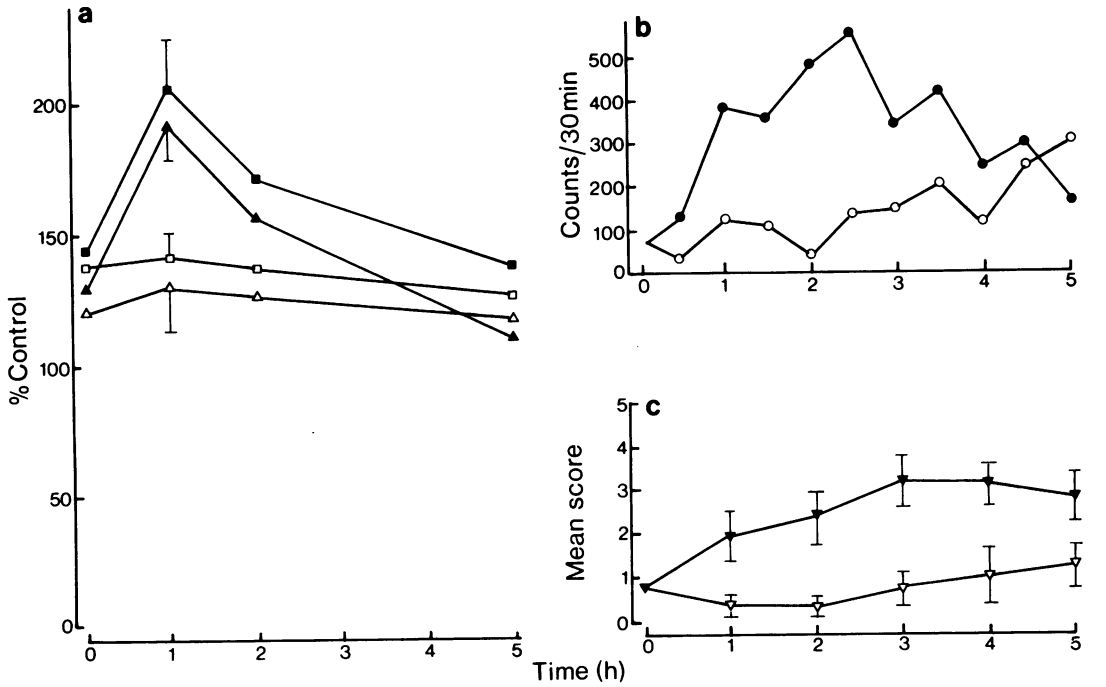

Fig. 5 Effect of ethanol (2 g/kg, i.p.) administered at the start of ethanol withdrawal. Time on the abscissae refers to time after ethanol withdrawal. (a) Mouse brain catecholamine concentrations. Ethanol withdrawal—noradrenaline (■) and dopamine (▲). Ethanol withdrawal plus ethanol administration: minus noradrenaline (□) and dopamine (△). Each point represents the mean of at least five determinations. Vertical bars represent s.e. mean. (b) Locomotor activity in groups of 15 mice. Animex sensitivity 40 μA. Ethanol withdrawal (●). 'Ethanol withdrawal' plus ethanol administration (○). (c) Withdrawal score as mean with s.e. mean for groups of 15 mice. Ethanol withdrawal (▼). 'Ethanol withdrawal' plus ethanol administration (▽).

The transient increase in brain noradrenaline associated with withdrawal was prevented by this treatment: the equivalent change in brain dopamine concentration was reduced (Figure 7). Before withdrawal, this treatment appeared to increase locomotor depression and ataxia, and after withdrawal, the phase of locomotor excitement was reduced. Piloerection and handling convulsions were increased during withdrawal by FLA-63 (Fig. 7), but this treatment, when given to otherwise untreated mice, caused behavioural changes similar to those seen in this phase of ethanol withdrawal.

Discussion

The results confirm that dependence on ethanol can be produced in mice when the drug is administered by inhalation. During the chronic administration of ethanol there is evidence for the development of metabolic and perhaps pharmacological tolerance. Withdrawal of ethanol from mice which have developed tolerance and dependence leads to a characteristic withdrawal syndrome.

During both acute and chronic administration of ethanol, changes occur in brain monoamine concentrations. Administration of ethanol acutely has been shown by a number of workers to reduce the concentrations of noradrenaline and 5-hydroxytryptamine in brains of experimental animals, but this has not always been confirmed by others (for review see Feldstein, 1971). Our results show a transient fall in the concentrations of noradrenaline, 5-hydroxytryptamine and dopamine after acute ethanol administration. One factor which cannot be discounted here is the environmental stress of exposure to ethanol vapour. However, we have found that, under the conditions obtaining in this laboratory, the injection of ethanol (2 g/kg i.p.) to mice has a

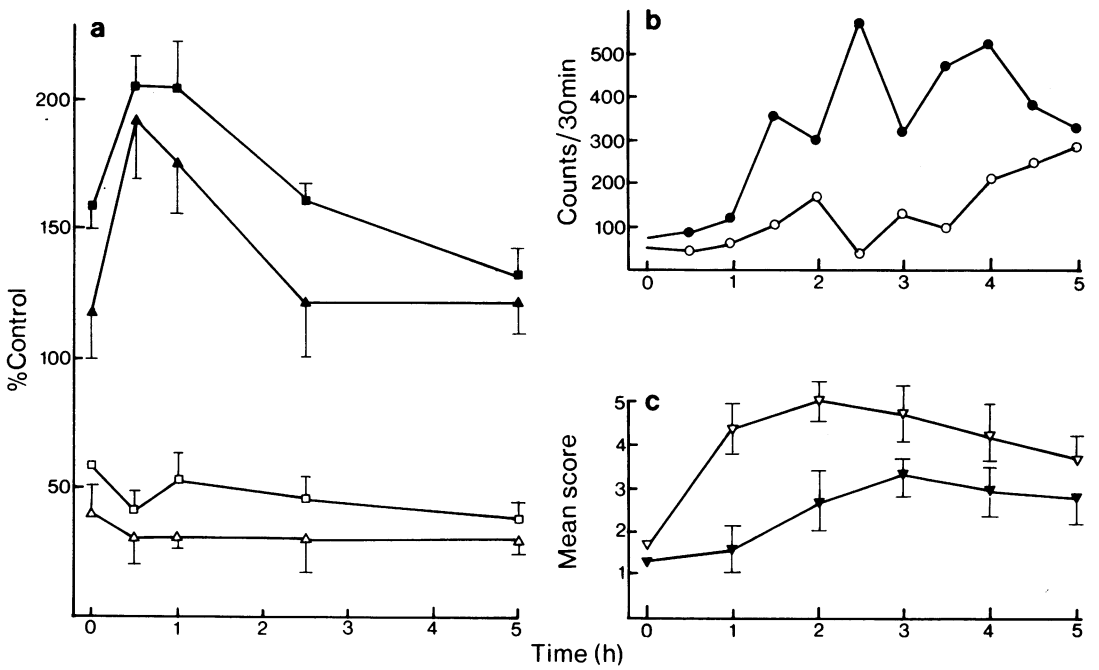

Fig. 6 Effect of administration of α-methyl tyrosine methyl ester (300 mg/kg, i.p.) 4 h before ethanol withdrawal on changes occurring during ethanol withdrawal. Time on the abscissae refers to time after ethanol withdrawal. (a) Mouse brain catecholamine concentrations. Ethanol withdrawal — noradrenaline (■) and dopamine (▲). α-Methyltyrosine pretreatment plus ethanol withdrawal: noradrenaline (□) and dopamine (△). Each point represents the mean with s.e. mean of at least three determinations. (b) Locomotor activity in groups of 15 mice. Animex sensitivity 40 μA. Ethanol withdrawn (●). α-Methyltyrosine pretreatment plus ethanol withdrawal (○). (c) Withdrawal score as mean with s.e. mean for groups of 15 mice. Ethanol withdrawal (▼). α-Methyltyrosine pretreatment plus ethanol withdrawal (▽).

similar effect on brain monoamines to that produced by acute inhalation (Littleton, Ortiz & Griffiths, unpublished observations) so it seems unlikely that it is the route of administration which is responsible for this change.

During the early stages of ethanol administration there is locomotor excitation in mice. Carlsson, Engel & Svensson (1972) have recently reported that administration of the inhibitor of catecholamine synthesis, α-methyltyrosine methyl ester, can prevent the locomotor excitation produced by acute ethanol administration. This may indicate that the fall in the concentrations of monoamines seen here is directly related to the behavioural changes of acute ethanol administration in our experiments.

After the initial fall in the concentrations of brain monoamines in mice given ethanol by inhalation, the concentrations remained similar to those of controls for several days while administration of ethanol continued. Later, brain monoamine concentrations began to rise, those of noradrenaline and dopamine showing a greater increase than that of 5-hydroxytryptamine. The cause of the increases in the concentrations of monoamines in the brain is obscure. Although ethanol administration influences the metabolism of the aldehydes formed by oxidative metabolism of monoamines (Smith & Gitlow, 1967; Lahti & Majchrowicz, 1967; Walsh, Truitt & Davis, 1970) there do not appear to be any reports that ethanol directly influences the synthesis or metabolism of the amines themselves.

It seems possible, however, that the increased concentration of monoamines in the brain seen after chronic administration of ethanol may be due to a reduction in monoamine metabolism. The transient increase in monoamine concentrations early in ethanol withdrawal might possibly be related to increased synthesis of monoamines before the excitement phase of withdrawal. This conclusion is supported by the fact that inhibition

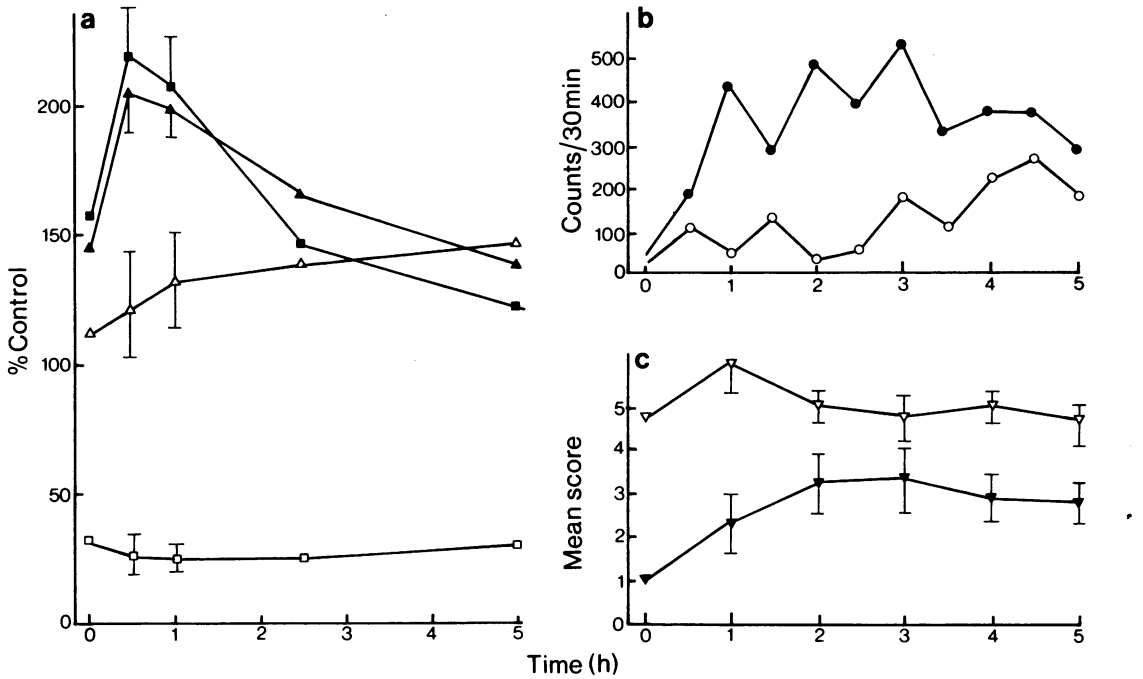

Fig. 7 Effect of administration of FLA-63 (40 mg/kg, i.p.) 4 h before ethanol withdrawal on changes occurring during ethanol withdrawal. Time on the abscissae refers to time after ethanol withdrawal. (a) Mouse brain catecholamine concentrations. Ethanol withdrawal — noradrenaline (■) and dopamine (▲). FLA-63 pretreatment plus ethanol withdrawal — noradrenaline (□) and dopamine (△). Each point represents the mean of at least five determinations. Vertical bars represent s.e. mean. (b) Locomotor activity in groups of 15 mice. Animex sensitivity 40 μA. Ethanol withdrawal (●). FLA-63 pretreatment plus ethanol withdrawal (○). (c) Withdrawal score as mean with s.e. mean for groups of 15 mice. Ethanol withdrawal (▼). FLA-63 pretreatment plus ethanol withdrawal (▽).

of catecholamine synthesis prevented the transient increase in brain catecholamine concentration and also markedly reduced the locomotor excitement normally shown in this phase of ethanol withdrawal.

The other, subjective, signs of ethanol withdrawal were potentiated by inhibition of catecholamine synthesis. This finding is consistent with that of Goldstein (1972b) who demonstrated that reserpine also potentiated the ethanol withdrawal syndrome. These results suggest that monoamines play a protective role in the later response to ethanol withdrawal. If this is true it is possible that potentiation of monoamine synthesis by the administration of precursors, such as L-DOPA or 5-hydroxytryptophan, or inhibition of monoamine breakdown by the administration of monoamine oxidase inhibitors may ameliorate the effects of ethanol withdrawal.

In our experiments, the inhibition of dopamine β-hydroxylase was more effective than inhibition of tyrosine hydroxylase in increasing the severity of withdrawal signs. However, since FLA-63 produced convulsions similar to those seen during ethanol withdrawal when given alone, we are unable to say whether noradrenaline or dopamine metabolism is of more importance in ethanol withdrawal. These results support the recently published work (McKenzie & Soroko, 1973) on the convulsant effects of FLA-63.

The small amount of relevant work on human alcoholics (Mendelson, 1971) supports the findings reported here. In alcoholic subjects allowed free access to ethanol, urinary catecholamine excretion (noradrenaline, adrenaline and metabolites) increased during ethanol intake and remained high on ethanol withdrawal. The increased urinary excretion of catecholamines during withdrawal showed a close relationship to the severity of the withdrawal syndrome; the more striking the

behavioural signs, the greater the increase in urinary catecholamines.

Our results suggest a relationship between brain monoamine metabolism and ethanol dependence, particularly between the ethanol withdrawal syndrome and changes in brain catecholamine metabolism occurring at this time. However, it is still possible that brain catecholamines are not directly involved in the processes of ethanol dependence and withdrawal. The findings outlined above could be explained if normal catecholamine synthesis were required for non-specific arousal and for any protective response to non-specific stress. Obviously, further work is needed to elucidate the nature of these changes in brain monoamines and their relationship to ethanol dependence and withdrawal. Meanwhile, it is hoped that model systems of this sort will prove useful in such experimentation.

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References

BROWNLEE, G. & SPRIGGS, T.L.E. (1965). Estimation of dopamine, noradrenaline, adrenaline, and 5-hydroxytryptamine from single rat brains. *J. Pharm. Pharmac.*, **17**, 429-433.

CARLSSON, A., ENGEL, J. & SVENSSON, T.H. (1972). Inhibition of ethanol-induced excitation in mice and rats by α-methyl-*p*-tyrosine. *Psychopharmacologia*, **26**, 307-312.

CURZON, G. & GREEN, A.R. (1970). Rapid method for determination of 5-HT and 5-HIAA in small regions of rat brain. *Br. J. Pharmac.*, **39**, 653-655.

FELDSTEIN, A. (1971). In: *The Biology of Alcoholism*, Vol. I, Biochemistry, ed. Kissin, B. & Begleiter, H. pp. 127-159, New York, London: Plenum Press.

FREUND, G. (1969). Alcohol withdrawal syndrome in mice. *Arch. Neurol.*, **21**, 315.

GOLDSTEIN, D.B. (1972a). Relationship of alcohol dose to intensity of withdrawal signs in mice. *J. Pharmac. exp. Ther.*, **180**, 203-215.

GOLDSTEIN, D.B. (1972b). Effect of reserpine on elicited convulsions after alcohol withdrawal in mice. *5th Int. Congress on Pharmacology*, San Francisco 1972. Volunteer Abstracts p. 85 No. 501.

GOLDSTEIN, D.B. & PAL, N. (1971). Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science*, **172**, 288-290.

GRIFFITHS, P.J., LITTLETON, J.M. & ORTIZ, A. (1973). A method for the induction of dependence to ethanol in mice. *Br. J. Pharmac.*, **47**, 669-670P.

LAHTI, R.A. & MAJCHROWICZ, E. (1967). The effects of acetaldehyde on serotonin metabolism. *Life Sci.*, **6**, 1399-1406.

LAVERTY, R. & TAYLOR, K.M. (1968). The fluorimetric assay of catecholamines and related compounds: improvements and extensions to the hydroxyindole technique. *Anal. Biochem.*, **22**, 269-279.

LIEBER, C.S., RUBIN, E., DeCARLI, L.M., MISRA, P. & GANG, H. (1970). Effects of pyrazole on hepatic function and structure. *Lab. Invest.*, **22**, 615.

MARSHALL, E.K. Jr & OWENS, A.J. Jr (1955). Rate of metabolism of ethyl alcohol in the mouse. *Proc. Soc. exp. Biol. Med.*, **89**, 573.

McKENZIE, G.M. & SOROKO, F.E. (1973). Inhibition of the anticonvulsant activity of L-DOPA by FLA-63 a dopamine β-hydroxylase inhibitor. *J. Pharm. Pharmac.*, **25**, 76-77.

MENDELSON, J.H. (1971). In: *The Biology of Alcoholism* Vol. I, Biochemistry, ed. Kissin, B. & Begleiter, H. pp. 513-544, New York, London: Plenum Press.

SMITH, A.A. & GITLOW, S. (1967). In: *Biochemical Factors in Alcoholism*, ed. Maickel, R.P. pp. 53-59, Oxford: Pergamon Press.

WALSH, M.J., TRUITT, E.B. & DAVIS, V.E. (1970). Acetaldehyde mediation in the mechanism of ethanol-induced changes in norepinephrine metabolism. *Molec. Pharmac.*, **6**, 416-424.

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