J. Pharm. Pharmacol. 1988, 40: 197–202
Received November 16, 1987

The Anxiolytic and Anxiogenic Actions of Ethanol in a Mouse Model

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Abstract—The administration to mice of ethanol in the drinking water for 7 days modified exploratory activity (rearings/line crossings) in an anxiety testing box separated into white and black sections with an interconnecting door. During ethanol intake mice exhibited reduced anxiety responding, shown as increased rearings and line crossings in the white section, to which the mice are normally averse, with corresponding decreased behaviour in the black section. When naive mice were presented with a choice between normal drinking water and drinking water containing ethanol, they consumed sufficient of the latter to secure a full anxiolytic response, making up the total volume of fluid required by also drinking the former. A 48 h withdrawal from a 14 day treatment with ethanol caused a reversed profile of exploratory behaviour, directed preferentially at the black section of the test box, and indicative of an anxiogenic response. Diazepam, tiapride or clonidine given twice daily during withdrawal from ethanol could each secure a reduction in the withdrawal anxiogenesis. It is concluded that the simple model of anxiety described in the mouse may be useful for eludicating the mechanisms involved in the anxiolytic and anxiogenic potential of ethanol and may aid the search for novel agents having potential to suppress withdrawal anxiogenesis.

It is generally accepted that the anxiolytic actions of the benzodiazepines are mediated via an action on a benzodiazepine-GABA-receptor-chloride channel complex (see reviews by Guidotti et al 1983; Braestrup et al 1983). It is hypothesized that the ionophore system is an oligomeric complex, composed of at least three interacting components sensitive to agents acting at the GABA recognition sites, the benzodiazepine receptors and a site(s) sensitive to barbiturates and other depressants of the central nervous system. Ethanol is an important example of the latter compounds and whilst ethanol has important actions on other transmitter systems (see Balldin et al 1985), it is clear that ethanol shares some common actions with the benzodiazepines, e.g. anticonvulsant and muscle relaxant properties, and has cross tolerance (see review by Ticku 1983).

In a putative model of anxiety in the mouse using a black and white test box system, mice normally demonstrate an aversion to the brightly lit white area. An anxiolytic agent such as diazepam will antagonize the aversion during treatment with an anxiogenic component revealed by increased exploratory activity in the black section following its withdrawal (Costall et al 1987; Barry et al 1987). The present studies were designed to investigate firstly, whether ethanol administration and withdrawal has a comparable action to diazepam in the mouse test. Secondly, the aim was to determine the usefulness of the mouse model in assessing drug action to attenuate the behavioural consequences of ethanol withdrawal, using diazepam, tiapride and clonidine, agents selected on the basis of their clinical use in the treatment of drug-induced withdrawal states (Tseng et al 1975; Bonnaffoux et al 1981).

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Materials and Methods

Animals

Naive male albino B.K.W. mice, approximately 35 g, were used in all experiments. Ten mice were normally housed in each cage and kept on a 12 h light/dark cycle with lights off at 0800 h.

Test for changes in anxiety responding

The apparatus used for the detection of changes in exploratory behaviour consisted of an open-topped box (45 × 27 × 27 cm high) lined into 9 cm squares, two-fifths painted black and illuminated under a dim red light (1 × 60 W) and partitioned from the remainder of the box which was painted white and brightly illuminated with a 60W light source located 17 cm above the box. An opening 7·5 × 7·5 cm located at floor level in the centre of the partition allowed access between the black and white areas.

Tests were conducted between 1300 and 1800 h in a quiet darkened room illuminated with a red light. Animals which had been subject to various drinking regimes including the intake of ethanol (see below) were taken for testing either during ethanol intake or following its withdrawal with or without concomitant drug treatment (see below). Mice were taken from a dark holding room in a dark container to the dark testing room where, after a period of adaptation to the environment of the test room, they were placed individually into the centre of the white, brightly lit area of the test box and their behaviour observed over a 5 min period by remote video recording. Three behaviours were noted (a) the number of exploratory rearings in the white and black sections, (b) the number of line crossings in the white and black areas and (c) the time spent in the white and black areas.

Experimental design

Animals were used once only in treatment groups of 5.

Where drug treatments were combined with ethanol withdrawal, the behaviour of appropriate vehicle control animals was assessed on each day of testing. Non-treated mice, allowed normal water intake, were used as control animals on each day of testing.

Results were analysed using Single-Factor Analysis of Variance and where appropriate followed by Dunnett's procedure for comparing all treatments with control.

Treatment groups

The effect of ethanol treatment. Ethanol was provided in the drinking water for 14 days. Five groups of mice received (a) drinking water without additives (control), drinking water containing (b) 1% (c) 4% and (d) 8% w/v ethanol and the 5th group of mice, (e), had free choice between either normal drinking water or drinking water containing 8% w/v ethanol. The exploratory activity of mice in the anxiety test box was measured on the 7th day of ethanol intake and following its withdrawal after 14 days of administration (replacement by normal drinking water for 48 and 96 h). The body weight of mice was recorded on day 1 and 14, during and 48 and 96 h following all treatments and the volume of drinking water/ethanol consumed daily by each group of animals was recorded.

The effect of clonidine, diazepam and tiapride on the behavioural consequences of ethanol withdrawal. Mice were presented with ethanol (8% w/v) in the drinking water for 14 days. On the morning of withdrawal (0800 h) animals were treated (i.p.) with diazepam (10·0 mg kg^{-1}), or tiapride (40·0 mg kg^{-1}) or clonidine (0·01 mg kg^{-1}); these treatments were repeated at 2000 h on the same day, at 0800 and 2000 h on the following day and at 0800 h on the 3rd day to cover the period of ethanol withdrawal. Animals were then tested in the anxiety testing box on the 3rd day 8 h after administration of the last dose of drug.

Drugs

Drinking water containing ethanol (J. Burroughs Ltd) was freshly prepared each day. Tiapride HCl (SESIF) and clonidine HCl (Boehringer Ing.) were prepared in distilled water and diazepam (Roche) was dissolved in the minimum quantity of polyethylene glycol and made up to volume with distilled water. Doses were administered intraperitoneally in a volume of 1 mL/100 g and expressed as the base.

Results

General observations

Under present test conditions, control vehicle treated mice showed a characteristic profile of (a) an approximate 2-fold increase in rearings in the black section (39·3 ± 5·2/5 min) compared with the white area (22·6 ± 3·0/5 min), (b) increased line crossings in the black section (53·1 ± 5·7/5 min) compared with the white (32·2 ± 4·2/5 min) to display a preference for exploratory activity in the black section of the

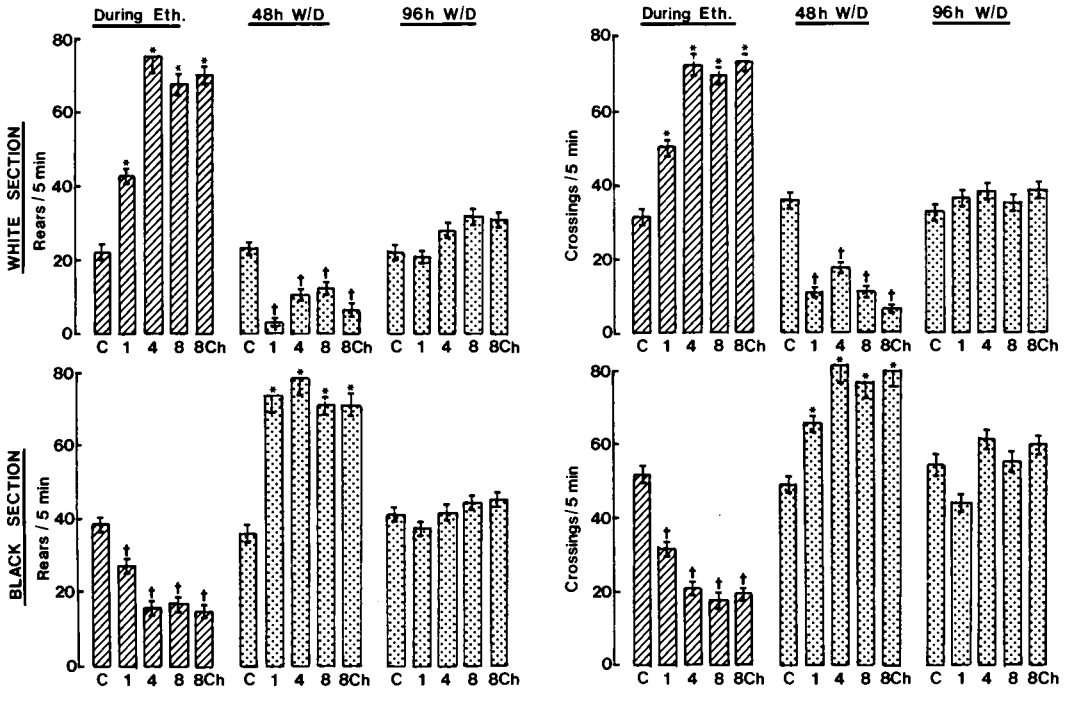

FIG. 1. Effects of ethanol intake on rearing behaviour and line crossings in the white and black sections of a box separated into light (white illumination) and dark (red illumination) compartments having an interconnecting 'door'. Measurements were made from remote video recordings every min and were cumulated over a 5 min period. Mice were presented with drinking water containing a fixed quantity of ethanol (indicated as 1, 4 and 8% w/v) or were allowed free choice between normal drinking water or that containing 8% w/v ethanol (indicated 8 Ch). Mice were tested on the 7th day (During Eth.) of a 14 day period of ethanol intake and then at 48 h and 96 h following withdrawal (W/D). C indicates the response of normal, non-treated mice. The data for each treatment was obtained from different groups of mice (n = 5), control = 25. s.e.m.s given. Significant increases in responding are indicated as $^{*}P < 0{\cdot}001$, significant decreases as $\dagger P < 0{\cdot}001$ (one-way ANOVA followed by Dunnett's *t*-test).

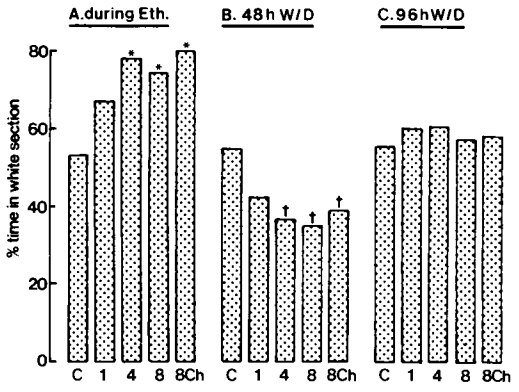

FIG. 2. Effects of ethanol intake on the time spent in the white area of a white and black box separated into light (white illumination) and dark (red illumination) compartments having an interconnecting 'door'. Measurements were made from remote video recordings every min and were cumulated over a 5 min period. Mice were presented with drinking water containing a fixed quantity of ethanol (indicated as 1, 4 and 8% w/v) or were allowed free choice between normal drinking water or that containing 8% w/v ethanol (indicated 8 Ch). Mice were tested A. on the 7th day (during Eth.) of a 14 day period of ethanol intake and then at B. 48 h and C. 96 h following withdrawal (W/D). C indicates the response of normal, non-treated mice. The data for each treatment was obtained from different groups of mice (n = 5), control = 25. S.e.m.s on original data less than 13%. Significant increases or decreases in responding are indicated as $*P<0.001$ and $\dagger P<0.001$, respectively (one-way ANOVA followed by Dunnett's t-test).

test area. It should be noted that mice spent an approximately equal time in each section. The preference for an increased exploratory activity in the black section is possibly induced by the aversive properties of the brightly lit area. Preliminary studies have established that the changes in preference for the white or black areas are most readily shown by measures of the time spent in each section and the rearings and line crossings (see Costall et al 1987; unpublished data), and such data are presented in the subsequent results. Also, since vehicle treated animals gave responses indistinguishable from those of non-treated animals ($P>0.05$), only the responses obtained from non-treated animals are given as control data in the ethanol-drug interaction studies. It is emphasized that any one group of mice were only used on a single occasion in the anxiety test situation.

Modification of exploratory behaviour by fixed intake of ethanol

Mice were found to readily accept 1% w/v ethanol in the drinking water and when tested 7 days after the start of the experiment, mice demonstrated an exploratory behaviour opposite to that observed in control mice receiving normal drinking water. Thus, the ethanol-treated mice showed a significant increase in rearings and line crossings in the white area (to 191 and 159%) with a reduction in the black area (by 29 and 38%). The same anxiolytic profile of behavioural responding was observed in mice which had imbibed 4 and 8% w/v ethanol in the drinking water. However, at these concentrations of ethanol, the intensity of increased rearings and line crossings in the white section to 336 and 230% of control values was remarkable, and was approximately twice that which could be obtained using maximally effective doses of the anxiolytic agents diazepam and tiapride alone (see Figs 1, 3). Since the intake of 4 and 8% w/v ethanol had comparable effects, this was considered to be maximal.

The changes in rearings and line crossings in the white section occurred concomitant to an increase of some 40% in time spent in the white area (Fig. 2). However, given the much greater percentage increases in rearings and line crossings, such changes represent genuine increases in exploratory behaviour during the experimental period.

Mice which had been drinking 1, 4 or 8% w/v ethanol solutions daily for 14 days were abruptly withdrawn from the treatment by replacing the ethanol solutions with normal drinking water, and their behaviour in the black:white test box was then assessed 48 and 96 h after withdrawal. 48 h following withdrawal mice exhibited marked reductions in both rearings and line crossings in the white area (by 87 and 70%) and marked increases in these behaviours in the black area (to 216 and 167%), to reveal an anxiogenic profile. The reduction or enhancement of exploratory behaviour in the white and black areas, respectively, is associated with a decrease/increase in the time spent in the white/black sections, although the latter constituted only modest changes of 20–30%. There was no significant difference in the degree of withdrawal anxiogenesis exhibited by the mice receiving 1, 4 or 8% w/v ethanol. 96 h after ethanol withdrawal, the behaviour of mice had returned to normal, control values (Figs 1, 2).

Table 1. Fluid intake and body weight of mice during and following ethanol intake in drinking water. Mice were allowed free access to drinking water (control), drinking water containing ethanol (fixed treatments), or to drinking water/ethanol solution in the free choice situation. Each cage contained 10 mice and the mean values calculated from measurement of the group intake of fluid with s.e.m.s given (intake calculated from data for 14 days). The body weights are the mean values of 5–20 determinations, with s.e.m.s given. Weight changes did not achieve significance ($P>0.05$, Student's t-test).

Treatment	Total fluid intake (mL/24 h)	Ethanol intake (g kg^{-1}/24 h)	Weight (g)			
			Day 1	Day 14	48 h	96 h
			Ethanol intake		Ethanol withdrawal	
Control	4·4±0·3	—	34·8±3·2	36·3±3·1	36·8±2·9	37·8±3·4
Fixed treatments:						
1% w/v Ethanol	5·2±0·4	1·20±0·1	35·8±3·4	38·9±3·6	39·4±3·6	39·8±3·6
4% w/v Ethanol	4·8±0·4	4·43±0·3	35·5±3·1	38·1±3·4	37·7±3·1	38·2±3·2
8% w/v Ethanol	4·1±0·4	7·57±0·6	33·8±3·6	36·4±3·4	35·4±3·2	35·6±3·4
Free choice:						
Drinking water/	3·8±0·3	—	34·7±2·9	38·2±3·2	37·8±3.4	40·4±3·6
8% w/v Ethanol	1·1±0·05	2·03±0·2				

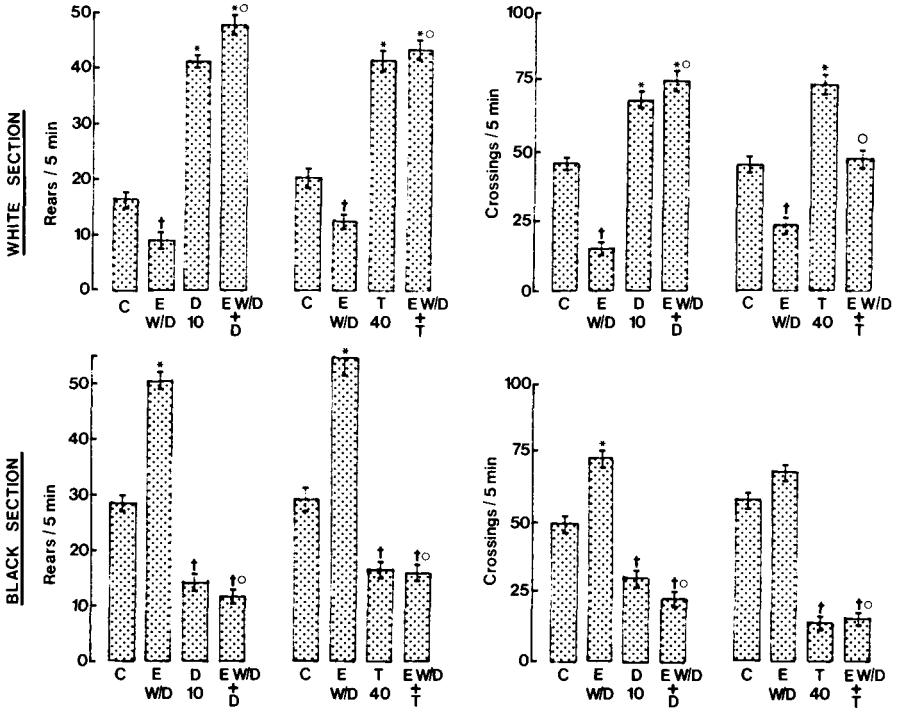

FIG. 3. The effect of diazepam (D) and tiapride (T) on the changes in exploratory behaviour following ethanol (E) withdrawal (W/D). Exploratory rearings and line-crossings per 5 min period were measured from remote video recordings in the white and black sections of a box separated into light (white illumination) and dark (red illumination) compartments having an interconnecting 'door'. Data were obtained from control mice (C, no treatment), mice receiving a 14 day intake of 8% w/v ethanol in the drinking water and withdrawn for 48 h (E W/D), mice receiving the ethanol treatment but administered diazepam (10·0 mg kg^{-1} i.p.) and tiapride (40·0 mg kg^{-1} i.p.) twice daily on the day the ethanol was withdrawn, on the following day, and on the 3rd day as a single injection followed by testing 8 h later (E W/D + D; E W/D + T). Two further groups of mice received the diazepam (D) and tiapride (T) treatments alone. n = 5. S.e.m.s given. Significant increases or decreases in responding compared to C are indicated as $*P < 0{\cdot}001$ and $\dagger P < 0{\cdot}001$ respectively. Antagonism of the effect of ethanol by diazepam or tiapride significant to $^{\circ}P < 0{\cdot}001$ (all analyses by one-way ANOVA followed by Dunnett's *t*-test).

Modification of exploratory behaviour by free-choice drinking of ethanol

Naive mice presented with a choice of drinking water or drinking water containing 8% w/v ethanol (positioning about the cage changed randomly) drank both solutions in the ratio of approximately 4:1 ($3{\cdot}8 \pm 0{\cdot}4$ and $1{\cdot}1 \pm 0{\cdot}1$ mL/24 h respectively) (Table 1). The voluntary intake of the ethanol solution (equivalent to 0·25 mL ethanol/100 g/24 h) was accomplished on the first day of intake and persisted at this level for the 14 days of treatment. When assessed on the 7th day of treatment mice showed a profile and intensity of anxiolytic activity in the test box indistinguishable from that of mice receiving the 'no-choice' ethanol regime (4 or 8% w/v), i.e. marked increases in rearing and line crossings and modest increases in the time spent in the white section with corresponding reductions in the black section. Following withdrawal of the voluntary intake of ethanol, mice showed a profile of anxiogenic activity comparable with that of mice which has been withdrawn from a fixed ethanol regime, i.e. 48 h after withdrawal marked reductions in rearings and line crossings and modest reductions in the time spent in the white section were recorded, with increased behaviour in the black area. Again, as for mice subject to fixed ethanol intakes, the withdrawal anxiogenesis for those mice taking ethanol by choice had waned by 96 h of its withdrawal when rearings/line crossings and time spent in the white and black areas of the testing box had returned to control values (Figs 1 and 2).

Modification by diazepam, tiapride and clonidine of the changes in exploratory behaviour following ethanol withdrawal

Doses of diazepam (10 mg kg^{-1}), tiapride (40 mg kg^{-1}) and clonidine (0·01 mg kg^{-1}) were selected on the basis of preliminary studies, lower doses proving ineffective in antagonizing the intense anxiogenesis following ethanol withdrawal. These doses were administered twice daily on the day of and following ethanol withdrawal (14 days treatment) and at 0800 on the 3rd day which was 8 h before the animal's behaviour was assessed in the black and white test box.

The administration of diazepam and tiapride alone lead to enhanced rearings and line crossings in the white section with a decreased incidence of such behaviour in the black area (Fig. 3). The changes in rearings and line crossings were greater than could be expected from the modest increase in the time spent in the white section (Fig. 5). The administration of diazepam or tiapride following ethanol withdrawal antagonized both the reduced rearings/line crossings in the white section and the increased rearings/line crossings in the black section seen as a consequence of previous, continued intake of ethanol (Fig. 3).

Treatment with clonidine alone led to increased rearings in the white section, to 147%, but without modification of line crossings or time spent in the white area, or any change in behaviour in the black area of the test box (Figs. 4, 5). Nevertheless, the administration of clonidine after ethanol withdrawal was found to antagonize the reduction in

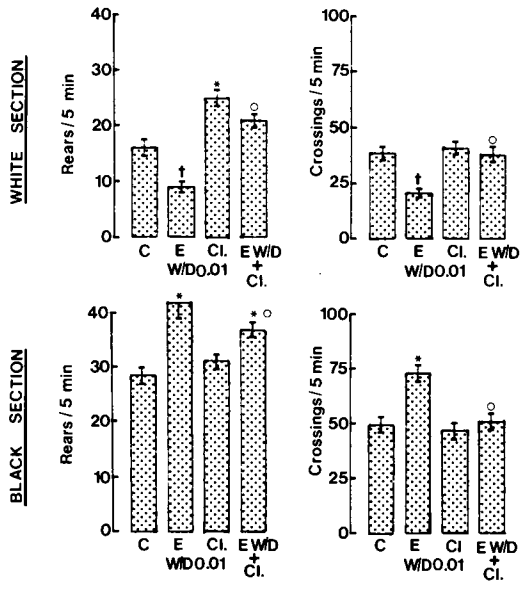

FIG. 4. The effect of clonidine (Cl.) on the changes in exploratory behaviour following ethanol (E) withdrawal (W/D). Exploratory rearings and line crossings/5 min were measured from remote video recordings in the white and black sections of a box separated into light (white illumination) and dark (red illumination) compartments having an interconnecting 'door'. Data were obtained from control mice (C, no treatment), mice subject to a 14 day intake of 8% w/v ethanol in the drinking water and withdrawn for 48 h (E W/D), mice receiving the ethanol treatment but administered clonidine (0·01 mg kg^{-1} i.p.) twice daily on the day ethanol was withdrawn, on the following day, and on the 3rd day as a single injection followed by testing 8 h later (E W/D + Cl.). A further group of mice received the clonidine treatment alone (Cl. 0·01). n = 5. S.e.m.s given. Significant increases or decreases in responding compared to C are shown as $*P < 0{\cdot}001$ and $\dagger P < 0{\cdot}001$, respectively. Significant antagonism of the effect of ethanol by clonidine is indicated as $^{\circ}P < 0{\cdot}001$ (all analyses by one-way ANOVA followed by Dunnett's *t*-test).

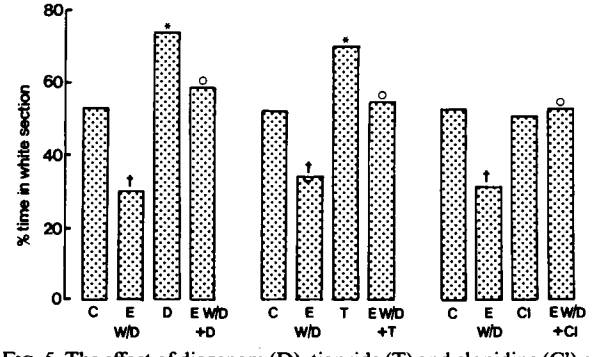

FIG. 5. The effect of diazepam (D), tiapride (T) and clonidine (Cl) on the changes in time spent in the white area of the test box following ethanol (E) withdrawal (W/D). Time spent in the white area per 5 min period was measured from remote video recordings in the white and black sections of a box separated into light (white illumination) and dark (red illumination) compartments having an interconnecting 'door'. Data were obtained from control mice (C, no treatment), mice receiving a 14 day intake of 8% w/v ethanol in the drinking water and withdrawn for 48 h (E W/D), mice receiving the ethanol treatment but administered diazepam (10·0 mg kg^{-1} i.p.), tiapride (40·0 mg kg^{-1} i.p.) and clonidine (0·01 mg kg^{-1} i.p.) twice daily on the day the ethanol was withdrawn, on the following day, and on the 3rd day as a single injection followed by testing 8 h later (E W/D + D; E W/D + T, E W/D + Cl). Three further groups of mice received the diazepam (D), tiapride (T) and clonidine (Cl) treatments alone. n = 5. S.e.m.s on original data less than 12%. Significant increases or decreases in responding compared to C are indicated as $*P < 0{\cdot}001$ and $\dagger P < 0{\cdot}001$, respectively. Antagonism of the effect of ethanol withdrawal (E W/D) by diazepam, tiapride or clonidine significant to $^{\circ}P < 0{\cdot}01$ (all analyses by one-way ANOVA followed by Dunnett's *t*-test).

rearings/line crossings in the white section and the increased rearings/line crossings in the black section. Indeed, the values for exploratory behaviour after ethanol-clonidine treatment were indistinguishable from those of non-treated control animals (Figs 4, 5). Thus, the withdrawal anxiogenesis seen after ceasing 14 days' intake of ethanol was shown to be antagonized by diazepam, tiapride and clonidine.

Discussion

The chronic intake of ethanol in the drinking water of mice for a period of 7 days lead to a modification of the exploratory behaviour in a testing box divided into two interconnecting compartments painted black and white, the mice being taken from an environment which renders them averse to the white, brightly lit environment. An important change in behaviour was seen as an increased time spent in the white section with a decrease in the black area. Concomitant with such changes were increased exploratory rearings and line crossings in the white area and decreased in the black. The increase in exploratory rearings in the white section was approximately twice that which could be expected from the increased amount of time spent in this section, to indicate a genuine increase in exploratory behaviour. Line crossings, a measure of locomotor activity, were also increased to a value above that which could have been expected from an increase in the amount of time spent in the white area, but the increase was more modest than recorded for changes in rearing.

The behavioural change of increased exploratory activity in the white section caused by ethanol is interpreted in terms of a decrease in the aversive properties associated with a brightly lit environment of the nocturnal rodent (Crawley & Goodwin 1980). This profile of change in exploratory activity between the white and black sections has previously been reported for benzodiazepines and substituted benzamides (Costall et al 1987), the former having an established anxiolytic efficacy in the clinic (Hollister 1982), anxiolytic activities also being reported for the substituted benzamides (Standish-Barry et al 1983; Hasegawa et al 1984). Interestingly, in the animal model, the behavioural consequences during persistent ethanol intake were found to be even more marked than those of maximally effective doses of diazepam or tiapride to increase exploratory behaviour in the white section. The use of fixed and free-choice ethanol regimes established that the dose of ethanol required to produce a maximal anxiolytic effect in the mouse was in the range 40 to 70 mg/day.

However, ethanol is known to act in low doses to enhance spontaneous motor activity and to reduce activity at higher doses (see review by Pohorecky 1977), effects which may directly modify measures of exploratory behaviour. Indeed, in the rat, the attenuation of the effects of punishment by ethanol was accompanied by motor incoordination and ataxia (Vogel et al 1980). However, in the present studies, changes in motor performance in their own right are unlikely to interfere with or determine the anxiolytic effects of ethanol. Firstly, the ethanol treatment failed to cause any overt changes in locomotor activity, e.g. excitement or sedation. Secondly, if the effects of ethanol in the anxiety test box reflected a general stimulant action, then exploratory behaviour should have been increased in both the black and

white sections, and clearly this was not the case. Whilst dependence liability of ethanol has also been hypothesized to relate to behavioural arousal occurring with low to moderate doses (Waller et al 1986), from the present study we propose that the voluntary intake of ethanol may also partially reflect a consequence of an anxiolytic action.

The continued intake of ethanol by the mice may be encouraged by the consequences of its withdrawal. Thus, 48h after the discontinuation of a 14 day period of ethanol intake (either fixed or free choice) the profile of anxiolytic action was reversed to one of anxiogenesis in the black and white test box. The profile of enhanced exploratory activity in the black section and reduced activity in the white area, interpreted as anxiogenesis, has also been recorded following the withdrawal of diazepam and the acute administration of the β-carboline derivative FG7142 (Costall et al unpublished data). The withdrawal of high doses of diazepam in man is associated with an anxiogenic response (see review by Ayd 1982) and the acute administration of the β-carboline derivative FG7142 is also known to cause anxiogenesis in man and in the rat (see review by Dorow et al 1983; and Pellow & File 1984, 1986). Therefore, the profile of exploratory behaviour in the mouse model caused by ethanol withdrawal is consistent with an anxiogenic response.

The profile of anxiogenesis induced by ethanol withdrawal was not observed if diazepam, tiapride or clonidine were administered during the withdrawal period and the nature of the interaction between ethanol and diazepam could be interpreted at the benzodiazepine-GABA-receptor-chloride channel complex. Thus, there is considerable evidence to indicate that diazepam may have 'agonist' action at the benzodiazepine recognition site to facilitate GABA transmission (see review by Haefely 1983) (FG7142 may have 'inverse agonist' action at the benzodiazepine receptor to have actions opposite to that of diazepam). Ethanol can also influence the GABA receptor complex at a different site, but to enhance GABA transmission (see reviews by Liljequist & Engel 1982; Braestrup et al 1983; Ticku 1983). Thus, the benzodiazepine may effectively 'substitute' for the lack of ethanol. There is no evidence that tiapride can interact directly at the benzodiazepine-GABA receptor complex but its actions are particularly interesting since anxiogenesis does not follow its withdrawal in the mouse model (Barry et al 1987).

Clonidine was included in the present study as an agent shown to reduce the autonomic/motor disturbances associated with morphine withrawal in rats, monkeys and humans (Tseng et al 1975; Gold et al 1978; Katz 1985). At the dose used, clonidine administered alone failed to modify exploratory behaviour in the black and white sections of the test box, failing to present a profile of anxiolytic activity. Nevertheless, clonidine was effective in antagonizing the profile of anxiogenesis caused by ethanol withdrawal in the mouse model and it is interesting that a clinical study has also shown that clonidine is effective in alleviating subjective anxiety in ethanol withdrawal (Wilkins et al 1983). There are no reports that clonidine can directly interact at the benzodiazepine-GABA receptor ethanol-sensitive complex, and the actions of clonidine may reflect an ability to stimulate at presynaptic α-adrenoceptors at an unspecified site.

In summary, the findings of the present study are that ethanol, given in the drinking water to mice, provides an anxiolytic effect which is reversed to anxiogenesis on abrupt withdrawal; the anxiogenesis is not associated with the development of motor abnormalities (see Goldstein 1973; Ritzman & Tabakoff 1976; Majchrowicz 1985) and is antagonized by diazepam, tiapride and clonidine. It is interesting to speculate that the development of anxiogenesis following ethanol withdrawal in man may be a primary effect (see also Conger 1956) which contributes to the difficulty in withdrawing ethanol, and that an understanding of the nature of the interaction between diazepam, tiapride, clonidine and ethanol may help to elucidate the perturbation in the system(s) causing anxiogenesis, and may aid in the development of more effective treatments for certain withdrawal symptoms.

Acknowledgements

The authors gratefully acknowledge gifts of drugs from SESIF (France), Roche Products Ltd. and Boehringer.

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