Psychopharmacology (1995) 122: 369–373

ORIGINAL INVESTIGATION

R. Spanagel · A. Montkowski · K. Allingham · T. Stöhr
M. Shoaib · F. Holsboer · R. Landgraf

Anxiety: a potential predictor of vulnerability to the initiation of ethanol self-administration in rats

Received: 25 May 1995 / Final version: 19 September 1995

Abstract Anxiolytic effects of ethanol have been proposed to be important factors in the initiation of ethanol consumption. To examine this hypothesis, drug-naive Wistar rats were tested in the elevated plus-maze to determine their initial level of anxiety. Based on their response, we separated the animals into anxious and non-anxious groups. After that, animals went through an oral ethanol self-administration procedure. Rats that were initially classified as anxious showed a significantly ($P < 0.01$) higher intake and preference for ethanol during the initiation phase of the voluntary drinking procedure than non-anxious animals. In another experiment, intraperitoneal (IP) injections of ethanol (0.5–1.5 g/kg) produced dose-dependent anxiolytic effects in rats when tested in the elevated plus-maze procedure. Blood ethanol levels following IP injections during the plus-maze test were similar to those reached during the oral ethanol self-administration procedure, which shows that the rats indeed drank sufficient amounts of ethanol to experience its anxiolytic effects. These findings indicate that the basal level of anxiety plays an important role in vulnerability to alcohol drinking.

Key words Anxiety · Elevated plus-maze · Ethanol · Oral self-administration · Tension-reduction hypothesis · Individual differences

Introduction

The reinforcing effects of drugs of abuse are seen as the major factor leading to drug-seeking behaviour and to a subsequent addictive state. In the case of ethanol, however, the demonstration of acute reinforcing effects in animal models has been difficult to achieve (Bozarth 1990). A variety of other factors may also contribute to the vulnerability to ethanol drinking; in particular, the anxiolytic effects of ethanol are seen as a possible motivation for the consumption of this drug. The tension-reduction hypothesis predicts that individuals who are innately anxious or stressed while in an undrugged state, are more sensitive to the anxiolytic effects of ethanol, and therefore show a higher predisposition for ethanol drinking (Cappell and Herman 1972; Pohorecky 1981, 1990; Wilson 1988). Although clear experimental evidence for the tension-reduction hypothesis is still lacking, a high comorbidity has been found for several anxiety disorders and alcohol abuse in clinical and epidemological studies (Bibb and Chambless 1986; George et al. 1990; Kushner et al. 1990; Schuckit and Hesselbrock 1994).

To our knowledge, the study of Stewart and coworkers (1993) is one of the few which has examined the relationship between anxiety and ethanol drinking in ethanol-preferring and non-preferring lines of rats. Although that study indicates a higher degree of anxiety in ethanol-preferring as compared to non-preferring rats, conclusions are limited, since a higher degree of anxiety could also be a consequence of selective breeding over generations. Furthermore, those results are confounded by those of another study (Päivärinta and Korpi 1993), which examined anxiolytic effects of ethanol in rats under a different context. This study showed that ethanol-preferring and non-preferring rats exhibited similar behavioural characteristics, i.e. reduced anxiety induced by ethanol in the elevated plus-maze paradigm.

In the present study, we sought to examine the relationship between anxiety and ethanol-drinking behaviour in drug-naive Wistar rats by using the elevated plus-maze test (Pellow et al. 1985; Reibaud and Böhme 1993). Single-housed animals were tested for their

R. Spanagel (✉) · A. Montkowski · K. Allingham
M. Shoaib · F. Holsboer · R. Landgraf
Max Planck Institute of Psychiatry, Clinical Institute,
Department of Neuroendocrinology, Kraepelinstraße 2–10,
80804 Munich, Germany

initial level of anxiety. Using different selection criteria for anxious behaviour, rats were then divided into two groups – anxious and non-anxious – and subsequently tested in a voluntary oral ethanol-drinking paradigm.

Materials and methods

Animals

Male Wistar rats (Max Planck Institute of Psychiatry, Martinsried, Germany) weighing 200–220 g were housed individually for at least 10 days before plus-maze testing in plastic cages in a climatically controlled colony room. Animals were maintained on a 12-h light/dark cycle (lights on: 7 a.m.–7 p.m.) with food and water available ad libitum. At the end of the experiment the animals were killed with an overdose of halothane (Hoechst, Frankfurt, Germany). The experiments were approved by the Committee on Animal Care and Use of the relevant local governmental body.

Elevated plus-maze procedure

The plus-shaped maze was made of grey PVC plastic and consisted of two arms which were open to the environment (open arms 50 × 10 cm) and two arms with side and end walls (50 × 10 × 40 cm). The arms were connected by a central area (10 × 10 cm) and the plus maze was elevated from the floor to a height of 75 cm. A video camera was mounted vertically over the plus-maze and a trained observer blind to treatment conditions scored behaviour from a monitor in an adjacent room over a 5-min period.

At the beginning of a test session a rat was placed in the central area of the maze facing to one of the closed arms and then allowed it to move freely among the open and closed arms. The following measures were scored: the number of entries into open and closed arms and the time spent in open and closed arms. An arm entry was defined as two forepaws into an arm. Based on the ratio entries into open arms/total entries and time spent in open arms/total time in both types of arms, the animals were divided into two groups: anxious and non-anxious. To consider an animal as anxious, the two parameters measured (ratio of entries into and time spent on open arms) had to correlate. Thus animals with levels below 45% for entries and below 30% for the time spent were considered as anxious. In the non-anxious group, levels above 55% for the entries and above 40% for the time spent in open arms were used as selection criteria. A total number of 50 animals were tested. From this group, we separated 12 animals per group using the criteria described above.

For the purpose of testing ethanol-induced anxiolytic effects, rats received intraperitoneal (IP) ethanol injections (0.5–1.5 g/kg; 12.5% v/v ethanol solution). Immediately after injection, the rats were placed back into their home cages for 10 min before the 5-min plus-maze test. One day after the plus-maze test, the selected animals went through the oral ethanol self-administration procedure.

Determination of ethanol preference and intake

Only anxious and non-anxious animals as judged by their performance in the elevated plus-maze were tested for ethanol preference. A standardized test procedure for ethanol preference was used in which two bottles containing increasing concentrations of ethanol solution and water, respectively, were continuously made available as a free choice to the animals. The 24-h consumption of ethanol, water and food was measured daily at 9 a.m. Body weight was recorded every third day. The bottle positions were changed randomly daily according to a predetermined schedule, so that a position habit did not develop. The solution of ethanol was increased in concentration after 4 days as follows: day 1–4: 2%; day 5–8: 4% (v/v solutions) (Shoaib and Almeida 1994).

Measurement of blood ethanol levels

A separate group of rats ($n = 10$) were fitted with chronic IV catheters in the jugular vein. One day after recovery, rats underwent the ethanol self-administration procedure as described above. Blood samples were taken for each concentration at different time points (8 p.m.; 12 p.m.; 4 a.m.; 8 a.m.) and were collected in heparinized tubes. After centrifugation, the supernatant fractions were immediately used for ethanol determination. Ethanol was measured by a fully automated NAD-ADH enzyme spectrophotometric system (Hitachi). Blood ethanol levels were also determined in rats 10 min after an injection of ethanol (0.5 g/kg; IP).

Statistics

The data were analyzed by an analysis of variance (ANOVA) to compare the influence of different doses of ethanol on anxious behaviour in the elevated plus-maze. A two-way ANOVA for repeated measures was used to compare ethanol drinking in "anxious" and "non-anxious" animals over time. All results are expressed as the mean ± SEM. Differences were considered significant if $P < 0.05$.

Results

Drug-naive animals ($n = 50$) were tested in the plus-maze for their basal anxiety level. The subsequent separation into "anxious" and "non-anxious" rats resulted in statistically well differentiated groups. Thus, a two-way ANOVA revealed a significant effect of preselection according to initial anxiety level (for open/total entries: $[F(1, 16) = 22.47; P < 0.001; n = 12]$ and for open/total time in arms: $[F(1, 16) = 57.1; P < 0.001; n = 12]$) (Fig. 1).

"Anxious" and "non-anxious" rats exhibited a different drinking behaviour in the free-choice ethanol self-administration procedure. Thus, a two-way ANOVA for repeated measures revealed that "anxious" rats drank significantly more ethanol during the initiation phase. In detail, during the choice between 2% ethanol solution and water (days 1–4) and 4% ethanol solution and water (days 5–8), anxious rats differed significantly from non-anxious rats in their daily ethanol intake (days 1–4: $[F(1, 24) = 22.44; P < 0.001]$, days 5–8: $[F(1, 24) = 32.34; P < 0.001]$) and ethanol preference (days 1–4: $[F(1, 24) = 20.94; P < 0.001]$, days 5–8: $[F(1, 24) = 42.86; P < 0.001]$) (Fig. 2).

Figure 3 shows the effects of ethanol on the behavioural performance of the rats in the elevated plus-maze expressed as the proportion of open arm entries to total arm entries and of time spent in open arms to total time spent in both types of arms. Ethanol given acutely (0.5–1.5 g/kg; IP) increased the time spent in open arms

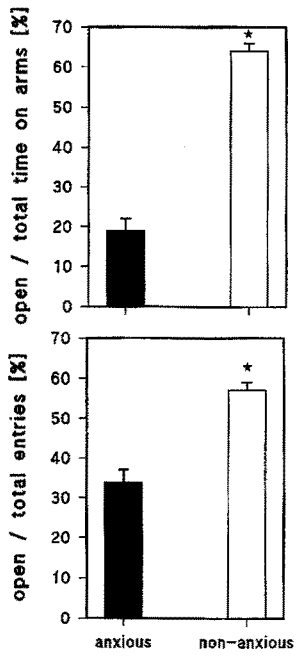

Fig. 1 Separation into anxious and non-anxious rats according to the elevated plus-maze parameter open/total time on arms and open/total entries in percent (means + SEM). Asterisks indicate significant differences between the two groups ($P < 0.001$; $n = 12$ per group)

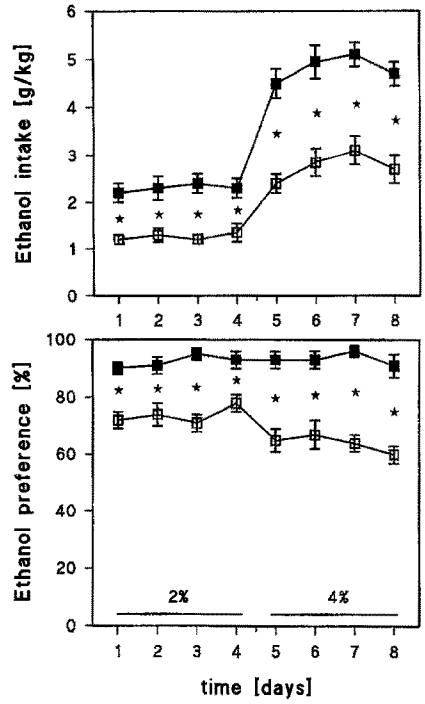

Fig. 2 Comparison of the daily ethanol intake and ethanol preference of anxious (■) and non-anxious (□) rats during the initiation drinking phase. The data points represent the mean absolute ethanol consumption, as g/kg (±SEM), and mean ethanol preference, as percent of ml ethanol intake/total fluid intake (±SEM), during day 1–8 of free choice between increasing ethanol concentrations (2–4% v/v) and water. *Asterisks* indicate significant differences between anxious and non-anxious animals ($P < 0.01$; $n = 12$ per group)

[$F(3, 27) = 5.4$; $P < 0.01$; $n = 6$–8]. The number of open arm entries was also significantly enhanced [$F(3, 27) = 6.7$; $P < 0.01$; $n = 6$–8]. It should be mentioned that at a dose of 1.0 g/kg ethanol, one out of eight rats and at a dose of 1.5 g/kg ethanol, two out of eight rats, respectively, fell off the plus-maze.

Blood ethanol concentrations in animals that underwent the ethanol drinking procedure were compared to blood ethanol concentrations in animals that received 0.5 g/kg ethanol (IP). There was a high degree of variation among animals and different time points of determination. However, we found in the ethanol self-administering animals blood ethanol concentrations (0–30 mg/dl; mean ± SE 22.5 ± 15.8 mg/dl by a daily ethanol consumption of 4.81 ± 0.15 g/kg ethanol) which were in the range of blood ethanol concentrations following IP injection of 0.5 g/kg ethanol (30–40 mg/dl; mean ± SE 37.0 ± 5.4 mg/dl).

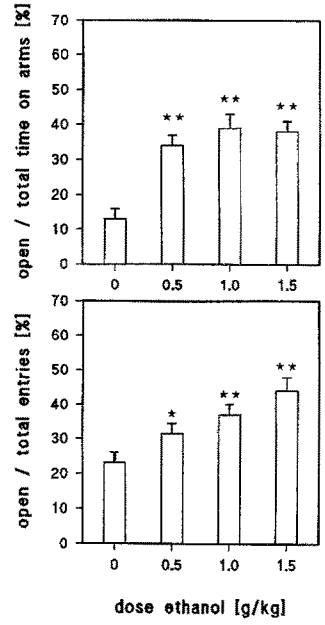

Fig. 3 Effects of different doses of ethanol (0.5, 1.0, 1.5 g/kg; 12.5% v/v solution; IP) on anxious behaviour of rats measured in the elevated plus-maze test. Values are means + SEM of plus-maze measures: open/total time on arms and open/total entries, both in percentages. *Asterisks* indicate significant differences compared with the saline control group ($^{*}P < 0.05$ and $^{**}P < 0.01$; $n = 6$–8)

Discussion

In the present study, we examined the relationship between anxiety and ethanol consumption in rats. Depending on the rat's individual basal behaviour on the elevated plus-maze, which varied widely between individuals even of the same Wistar strain, we separated animals into an anxious and a non-anxious group. Vulnerability to ethanol consumption in these preselected groups was then tested by subjecting the animals to a voluntary free-choice ethanol drinking procedure. Anxious rats exhibited a higher ethanol intake and preference in comparision to non-anxious rats during the acquisition phase of ethanol drinking. It is, therefore, suggested that the degree of anxiety may underlie, at least in part, the initial motivation to drink alcohol. Furthermore, it is important to note that the blood ethanol concentrations found in ethanol self-administering animals were in a similar range as those following IP injections of ethanol during the plus-maze test.

This finding demonstrates that the ethanol self-administering animals reached blood ethanol levels that were apparently capable of inducing anxiolytic-like effects in these animals.

The linkage between anxiety and alcohol drinking has been a topic of discussion for many decades. The strongest evidence for such an interaction comes from epidemiological surveys, family studies and field studies in humans (Cappell and Herman 1972; Wilson 1988; Schuckit and Hesselbrock 1994). These studies led to the tension-reduction hypothesis, which predicts that individuals who are chronically anxious or stressed while in an undrugged state have an innate sensitivity to the anxiolytic effects of ethanol and therefore show a greater vulnerability to drinking ethanol. However, basic research studies in laboratory animals attempting to validate this hypothesis have produced many conflicting results. Thus, in spite of numerous studies on the interaction of various stressors and ethanol drinking behaviour, it is not clear whether or not stress increases ethanol consumption (Pohorecky 1981; Caplan and Puglisi 1986; Volpicelli et al. 1990; Wolffgramm 1990). Furthermore, it is also unclear whether the degree of anxiety, which might depend, among other things, on pre- and postnatal stress experience (Pohorecky 1981, 1990) affects ethanol consumption. There is only one study that explicitly examines this issue in ethanol-preferring and non-preferring lines of rats (Stewart et al. 1993). This study demonstrated that rats of these two lines differed in behavioural tests of anxiety and in their response to ethanol treatment, whereby ethanol-preferring rats exhibited a higher degree of anxiety than ethanol non-preferring rats (Stewart et al. 1993). Our results are in line with these findings, and both studies provide evidence in support of the tension-reduction hypothesis.

Several points need attention. Firstly, the observed behavioural differences between ethanol-preferring and non-preferring rat lines in the Stewart et al. (1993) study are likely to be related to neurochemical characteristics, e.g. differences in serotoninergic systems (Wong et al. 1990; Zhou et al. 1991), which may be associated with selective breeding for high and low oral ethanol consumption. Secondly, we found significant differences in ethanol drinking behaviour between preselected anxious and non-anxious rats in the early acquisition phase of alcohol drinking. The ethanol self-administration paradigm used in our study (Shoaib and Almeida 1994) was chosen to avoid taste aversion which occurs at higher ethanol concentrations (i.e. > 6% v/v ethanol solutions) (Cicero 1980). Taste aversion might confound results in the particular question of interest, since the major focus of our study was on the initiation of alcohol drinking. Although rats do not become physically dependent upon ethanol and usually show no signs of motor impairment when a two-bottle choice procedure with low concentrations is used (Meisch and Lemaire 1993), it should be emphasized that the rats in our study consumed almost 5 g/kg per day of ethanol and reached blood alcohol levels during self-administration which were in the range of a 0.5 g/kg ethanol injection, a dose which clearly induced anxiolytic-like effects in the elevated plus-maze test (see Fig. 3). Thirdly, it is of note that the elevated plus-maze test probes a special form of anxiety (Handley and McBlane 1993; File et al. 1994); therefore, the use of other animal models of anxiety might lead to different results.

Both clinical and epidemiological studies have demonstrated that most anxiety disorders are causally related to alcohol abuse (Schuckit and Hesselbrock 1994). Thus, it has been found that subjects with phobic disorders have a considerably elevated risk of developing secondary alcohol use disorders (Robins et al. 1984). The frequency of secondary alcohol problems is probably best established for agoraphobia with panic attacks (ICD-10) and for panic disorder with agoraphobia (DSM-III-R) (George et al. 1990; Otto et al. 1992). However, for panic disorders without agoraphobia as well as for generalized anxiety disorders, the relationship to alcohol abuse is less clear. Recent findings from the National Comorbidity Survey (Wittchen et al. 1994) could not demonstrate a significant correlation between these anxiety disorders and alcohol use disorders. Taken together, these data suggest various pathways in which anxiety and alcohol might interact. In light of this conclusion, it would appear that further investigations on the interaction of anxiety and alcohol in laboratory animals are warranted.

In summary, anxious rats showed a significantly higher intake and preference for ethanol during the initiation phase of ethanol drinking than non-anxious animals. This increased predisposed vulnerability to alcohol among individuals may be one mechanism leading to increased alcohol consumption and eventually to alcohol dependence.

Acknowledgements We wish to thank Christine Bartl for her technical assistance and Dr. Obermaier for the determination of the blood ethanol concentrations. This work was supported by the BMFT FKZ: 01EB9419.

References

Bibb J, Chambless DL (1986) Alcohol use and abuse among diagnosed agoraphobics. Behav Res Ther 24:49–58

Bozarth MA (1990) Evidence for the rewarding effects of ethanol using the conditioned place preference method. Pharmacol Biochem Behav 35:485–487

Caplan MA, Puglisi K (1986) Stress and conflict conditions leading to and maintaining voluntary alcohol consumption in rats. Pharmacol Biochem Behav 24:271–280

Cappell H, Herman CP (1972) Alcohol and tension reduction: a review. Q J Stud Alcohol 33:33–64

Cicero TJ (1980) Alcohol self-administration, tolerance and withdrawal in humans and animals: theoretical and methodologi-

cal issues. In: Rigter H, Crabbe JC (eds) Alcohol tolerance and dependence. Elsevier, Amsterdam, pp 1–51
File SE, Zangrossi H, Sanders FL, Mabbutt PS (1994) Raised corticosterone in the rat after exposure to the elevated plus-maze. Psychopharmacology 113:543–546
George DT, Nutt DJ, Dwyer BA, Linnoila M (1990) Alcoholism and panic disorder: is the comorbidity more than coincidence? Acta Psychiatr Scand 81:97–107
Handley SE, McBlane JW (1993) An assessment of the elevated X-maze for studying anxiety and anxiety-modulating drugs. J Pharmacol Toxicol Methods 29:129–138
Kushner MG, Sher KJ, Beitman BD (1990) The relation between alcohol problems and the anxiety disorders. Am J Psychiatry 147:685–695
Meisch RA, Lemaire GA (1993) Drug self-administration. In: Van Haaren F (ed). Methods in behavioural pharmacology. Elsevier, Amsterdam, pp 257–300
Otto MW, Pollak MH, Sachs GS, O'Neil CA, Rosenbaum JF (1992) Alcohol dependence in panic disorder patients. J Psychiatr Res 26:29–38
Päivärinta P, Korpi ER (1993) Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. Pharmacol Biochem Behav 44:127–132
Pellow S, Chopin P, File SE, Briley M (1985) Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 14:149–168
Pohorecky LA (1981) The interaction of alcohol and stress: a review. Neurosci Biobehav Rev 5:209–229
Pohorecky LA (1990) Interaction of ethanol and stress: research with experimental animals – an update. Alcohol Alcohol 25:263–276
Reibaud M, Böhme GA (1993) Evaluation of putative anxiolytics in the elevated plus-maze test. Methods Neurosci 14:230–239
Robins LN, Helzer JE, Weisman MM, Orvaschel H, Gruenber E, Burke JD, Reiger DA (1984) Lifetime prevalence of specific psychiatric disorders in three sites. Arch Gen Psychiatry 45:949–958
Schuckit MA, Hesselbrock V (1994) Alcohol dependence and anxiety disorders: what is the relationship? Am J Psychiatry 151:1723–1734
Shoaib M, Almeida OFX (1994) Interrelations between the positive reinforcing effects and aversive stimulus properties of alcohol in rats. Neuropsychopharmacology 10:126
Stewart RB, Gatto GJ, Lumeng L, Li T-K, Murphy JM (1993) Comparison of alcohol-preferring (P) and nonpreferring (NP) rats on tests of anxiety and for the anxiolytic effects of ethanol. Alcohol 10:1–10
Volpicelli JR, Ulm RR, Hopson N (1990) The bidirectional effects of shock on alcohol preference in rats. Alcohol Clin Exp Res 6:913–916
Wilson GT (1988) Alcohol and anxiety. Behav Res Ther 26:369–381
Wittchen H-U, Zhao S, Kessler RC, Eaton WW (1994) DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. Arch Gen Psychiatry 51:355–362
Wolffgramm J (1990) Free choice ethanol intake of laboratory rats under different social conditions. Psychopharmacology 101:233–239
Wong DT, Threlkeld PG, Lumeng L, Li TK (1990) Higher density of serotonin-1A receptors in the hippocampus and cerebral cortex of alcohol-preferring P rats. Life Sci 46:231–235
Zhou FC, Beldsoe S, Lumeng L, Li TK (1991) Immunostained serotonergic fibers are decreased in selected brain regions of alcohol-preferring rats. Alcohol 8:425–431