AN EXPERIMENTAL MODEL OF ALCOHOL-INDUCED ANXIETY AND DEPRESSIVE BEHAVIOUR IN RATS

Stefka Valcheva-Kuzmanova¹, Miroslav Eftimov¹, Krasimir Kuzmanov²

¹Department of Preclinical and Clinical Pharmacology and ²Vivarium, Medical University of Varna

ABSTRACT

Behavioural symptoms of alcohol abuse often mimic various psychiatric disorders including anxiety and depression.

PURPOSE: The aim of the present study was to create a model of alcohol-induced anxiety- and depressive-like behaviour in female Wistar rats using a subchronic (14 days) daily alcohol exposure.

MATERIAL AND METHODS: Ethanol was applied at a daily dose of 8 g/kg. The following behavioural tests were used: open field test (OFT) for investigation of locomotor activity, elevated plus maze test (EPM) and social interaction test (SIT) for measurement of anxiety and forced swim test (FST) for estimation of depression-like behaviour.

RESULTS: Ethanol induced a significant reduction (p<0.01) in both horizontal and vertical locomotor activity of rats. In the ethanol group, the decreased time spent in the open arms of the EPM (p<0.05) and the decreased social interaction time (p<0.05) were consistent with anxiety-like effect of alcohol. The increased immobility time in the FST (p<0.01) showed that ethanol induced depression-like behaviour in rats.

CONCLUSION: The findings of the present study demonstrated that subchronic daily treatment of female Wistar rats with a relatively high dose of alcohol induced anxiogenic and depression-like behaviour. This animal model could be used for testing the anxiolytics and antidepressants for the treatment of alcohol-induced behavioural changes.

Key words: alcohol, experimental model, anxiety, depression, rats

INTRODUCTION

Epidemiological studies indicate significant co-morbid expression of alcoholism, anxiety, and depression (4). These co-morbid conditions are predominantly manifested in alcoholic women than men and are particularly high during and/or following alcohol withdrawal. Alcohol use and abuse appear to be related to neuroadaptive changes at functional, neurochemical, and structural levels.

Over the past decades, intensive research has been devoted to a variety of neurobiological aspects of depression and anxiety (10). Animal models seem to be a useful tool in biomedical sciences (1). They can provide some clues to association of alcohol and mood disorders and thus suggest effective interventions (11). Furthermore, such models are particularly useful in situations when the experiment can not be performed in humans because of ethical and other considerations.

The aim of the present study was to create a model of alcohol-induced of anxiety- and depression-like behaviour in female Wistar rats using a subchronic (14 days) daily alcohol exposure.
MATERIAL AND METHODS

Experimental substances

Ethanol 96% (Geya 99, Bulgaria) was used in the experiment.

Animals

Female Wistar rats (n=20; b. m. 200±20 g) were used throughout the study. The animals were housed in plastic cages in a well-ventilated room maintained at 22±1 °C and on a 12/12 light/dark cycle.

All procedures concerning animal treatment and experimentation were in accordance with the national laws and policies and in conformity with the international guidelines (EEC Council Directive 86/609, IL 358, 1, December 12, 1987).

Experimental procedure

The rats were divided in two groups. Ethanol-group rats (n=10) received ethanol for 14 days at a daily dose of 8 g/kg (as a 20% solution, given as two oral gavages of 24 mL/kg at 8 a.m. and 4 p.m.). Control rats (n=10) received the same volume of distilled water.

On the 15th day, 16 hours after the last ethanol exposure, the behavioural tests were performed one hour one after the other. Locomotor activity was investigated in the open field test (OFT), anxiety was measured in the elevated plus-maze test (EPM) and social interaction test (SIT). Depression-like behaviour was assessed in the forced swim test (FST).

Open field test (OFT)

OFT test is a common measure of exploratory behaviour and general activity in rodents (3). It was performed for 5 min in an arena (100×100×40 cm) painted white except for 6 mm blue lines that divided the floor into 5×5 equal size squares. Behaviours recorded were the following: crossings (the number of lines crossed with the four paws) and rearings (the number of times the animal stood on its hind limbs).

Elevated plus-maze (EPM)

EPM test is one of the most widely used tests to evaluate anxiety-like behaviours (14). EPM apparatus consists of two opposite open arms (50×10 cm) and two opposite arms enclosed by 40 cm high walls. The arms are elevated 50 cm from the floor. They are connected by a central 10×10 cm square and thus the maze forms a ‘plus’ shape. Each rat was placed in the central square with the head facing the open arm of the EPM and its behaviour was observed for 5 min. The total time spent in the open arms was recorded. The decrease of the total time spent in the open arms was a measure of anxiety-like behaviour.

Social interaction test (SIT)

SIT test is used to assess anxiety-like behaviours. Rats were tested for 5 min in the square arena (100×100×40 cm) of the open field apparatus under conditions of high light and unknown test partner to create a high level of anxiety (16). The two partners were matched by weight (difference of no more than 10 g). The rats were gently placed at the opposite corners of the arena. In each group, there were five pairs of rats. The following behaviours were observed and scored: sniffing, nipping, grooming, following, mounting, kicking, jumping on and crawling under or over the partner. Passive contact (sitting or lying next to each other) was not considered social interaction. Anxiety-like behaviours were defined as the decrease of the total time spent in interaction with the partner.

Forced swim test (FST)

In FST, immobility and swimming are distinguished as mutually exclusive behavioural states. Swimming behaviour is defined as movement throughout the cylinder. Immobility is defined when no additional activity is observed other than that required to keep rat’s head above the water. The increased immobility time is a measure of depression-like behaviour.

The method of Porsolt et al. (15) was used to assess rat immobility as a measure of depression-like behaviour. Each rat was placed in a glass cylinder pool (17 cm in diameter and 50 cm in height) for 5 min. The cylinder was filled with 30 cm of water (21±1 °C) to ensure that the animal could not touch the bottom of the cylinder with its hind paws or its tail. The test was performed in two sessions with a 24h interval. The results from the second session were recorded.

Statistical analysis

Results were presented as mean±SEM. Data were tested by Student’s t-test. A level of p<0.05 was
An experimental model of alcohol-induced anxiety and depressive behaviour in rats

considered significant. GraphPad Prism statistical software was used.

RESULTS

**Open field test (OFT)**

Ethanol caused a significant reduction (p<0,01) in rat spontaneous locomotor activity as assessed by the number of crossings (horizontal activity) (Fig. 1A) and number of rearings (vertical activity) (Fig. 1B).

**Elevated plus-maze (EPM)**

The elevated plus-maze data showed that ethanol induced a significant reduction (p<0,05) in the time spent in the open arms from 48,7±17,9 sec for the control group to 9,9±6,1 sec for the ethanol one, respectively (Fig. 2).

**Social interaction test (SIT)**

Ethanol significantly reduced (p<0,05) the time of social contacts between the test partners from 48,5±7,6 sec for the control group to 26,4±4,3 sec for the ethanol one (Fig. 3).

**Forced swim test (FST)**

In the FST, the immobility time for the animals from the ethanol group (105,5±7,7 sec) was significantly higher (p<0,01) than that of the animals from the control one (63,3±12,1 sec).

---

**Fig. 1.** Horizontal (A) and vertical (B) locomotor activity of rats treated subchronically with distilled water (control) or ethanol at a dose of 8,0 g/kg (ethanol) **p<0,01 vs control

**Fig. 2.** Time spent in the open arms of EPM by rats treated subchronically with distilled water (control) or ethanol at a dose of 8,0 g/kg (ethanol) *p<0,05 vs control

**Fig. 3.** SI time of rats treated subchronically with distilled water (control) or ethanol at a dose of 8,0 g/kg (ethanol) in SIT *p<0,05 vs control
DISCUSSION

In the present study, female rats treated subchronically with relatively high doses of ethanol demonstrated different behaviour in comparison with water-treated control rats. At the time of withdrawal from alcohol (16 h after the last alcohol treatment), ethanol-treated rats had decreased locomotor activity in the OFT. The OFT is a common measure of exploratory behaviour and general activity in rats, where both the quality and quantity of the activity can be measured. This test is also commonly used to assess the sedative, toxic, or stimulant effects of compounds (3). In the present study, the decreased locomotor activity of ethanol-treated animals might be partly due to neurotoxic effect of alcohol on neuronal cells.

Both, the decreased time spent in the open arms of the EPM and the decreased social interaction time were consistent with an anxiety-like effect of ethanol (7,12). The increased immobility time in the FST indicated that alcohol induced a depression-like behaviour in rats.

Alcohol use and abuse appear to be related to neuroadaptive changes at functional, neurochemical, and structural levels. Although the exact mechanism by which ethanol exerts its effect is still a matter of debate, studies during the last decade showed that ethanol, affecting several neurotransmitter systems, differentially modifies processes of neurotransmission in the central nervous system. After chronic ethanol consumption, reduced GABAergic and increased glutamatergic functions have been observed and associated with neurochemical mechanisms underlying the development of alcohol-induced anxiety, tolerance and dependence (9). Cortical norepinephrine (NE) is reduced in rats following alcohol administration. These results implicate cortical NE as a major player in alcohol-induced depression (2).

Acute and chronic ethanol exposures modulate intracellular signaling pathways, e.g. cAMP-responsive element binding (CREB) protein in the brain. A study of the downstream effectors of CREB identifies several important CREB-related genes such as neuropeptide Y (NPY), brain-derived neurotrophic factor (BDNF), and corticotrophin-releasing factor (CRF) that may play a crucial role in the behavioural effects of ethanol (8). Neuropeptides play an important role in alcohol-induced depression and anxiety (13). NPY suppresses anxiety-like behaviour. Alcohol exposure is associated with a reduced NPY expression and an increased CRF one (17).

Literature data show that alcohol-induced depression-like behaviour in Wistar rats is associated with a hippocampal BDNF reduction (5). BDNF, like most neurotrophins, is responsible for neuronal survival, development and plasticity. Human studies show a significant reduction of peripheral BDNF levels in several psychiatric disorders including major depression and alcohol dependence (6).

Brain chromatin remodeling due to histone covalent modifications may also be involved in mediating the behavioural effects and neuroadaptive changes that occur during ethanol exposure (8).

CONCLUSION

The findings of the present study indicate that subchronic daily treatment of female Wistar rats with a relatively high dose of alcohol induces anxiogenic and depression-like behaviour. This animal model can be used for testing the anxiolytics and antidepressants for the treatment of alcohol-induced behavioural changes.

REFERENCES

1. Borsini, F., J. Podhorna, D. Marazziti. Do animal models of anxiety predict anxiolytic-like effects of
An experimental model of alcohol-induced anxiety and depressive behaviour in rats


