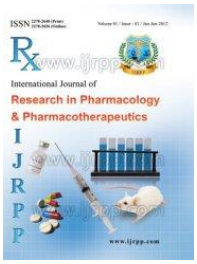
International Journal of Research in Pharmacology & Pharmacotherapeutics



ISSN Print: 2278-2648 ***IJRPP |Vol.4 | Issue 3 | Jul – Sep - 2015***
ISSN Online: 2278-2656 ***Journal Home page: www.ijrpp.com***

Research article ***Open Access***

Effect of verapamil and nimodipine in reversing the ethanol withdrawal induced anxiety in rats

R. Saravanan*, V. Venkataramanan, G. Karthikeyan

Department of Pharmacology, Annapoorana Medical College and Hospital, Salem, Tamilnadu, India

***Corresponding Author: R. Saravanan**

Email: drsarvan.ssss@gmail.com

ABSTRACT

BACKGROUND AND OBJECTIVE

Anxiety that occurs during alcohol withdrawal may be the precipitating factor for relapse of alcoholism. Earlier studies have demonstrated calcium channel activity peaks during alcohol withdrawal and calcium channel antagonists were found to decrease behavioral manifestations of alcohol withdrawal syndrome. The objective of the study was to evaluate the anxiolytic potential of calcium channel antagonists, verapamil and nimodipine during ethanol withdrawal in rats.

METHODOLOGY

Male wistar albino rats were fed with 10% ethanol liquid diet for 4 weeks. Ethanol was withdrawn 8 hours before test procedure. Verapamil (20mg/kg) and nimodipine (10mg/kg) were administered orally. Elevated plus maze (EPM) and Open field test (OFT) were used to assess the anxiolytic activity.

RESULT

In the EPM model, the time spent in the open arm and closed arm was increased and decreased respectively in rats treated with both verapamil and nimodipine as compared to control. Number of rearing in open arm and closed arm were also increased which shows increased exploratory behavior in rats. In OFT model, the time spent in central squares and peripheral squares was increased and decreased respectively in rats treated with verapamil and nimodipine as compared to control. Number of entries into the central area and the number of lines crossed in central and peripheral areas were also increased in drug treated rats as compared to control. Number of rearing was significantly increased in the central areas but not in the peripheral areas.

CONCLUSION

Verapamil and Nimodipine significantly reduced ethanol withdrawal induced anxiety. Calcium channel antagonists could be effectively used as alternative to benzodiazepines in ethanol withdrawal induced anxiety.

KEY WORDS: Verapamil, Nimodipine, Ethanol withdrawal, Anxiety

INTRODUCTION

Alcohol is a psychoactive substance with dependence-producing properties has been widely used in many cultures for centuries. According to WHO, in 2012, about 3.3 million deaths, or 5.9% of all global deaths, were attributable to alcohol consumption. In 2012, 139 million DALYs (disability-adjusted life years), or 5.1% of the global burden of disease and injury, were attributable to alcohol consumption. About 30% of Indians consume alcohol, out of which 4-13% are daily consumers and up to 50% of daily consumers

fall under the category of hazardous drinking. Ethanol has many neuronal effects, including decreases in the actions of the excitatory amino acid, N-methyl-D-aspartate[1] (NMDA), potentiation of the chloride flux induced by gamma-aminobutyric acid (GABA) agonists[2] and blockade of calcium uptake. Acute alcohol administration inhibits voltage dependent L-type, N-type, and T-type calcium channels[3]. Chronic alcohol consumption has effects opposite to that of acute alcohol consumption and leads to increased depolarization-stimulated calcium influx[4]. Chronic alcohol use leads to an increase in a group of calcium channels called L-type calcium channels, resulting in an increased calcium uptake[5,6]. The calcium channel activity which is increased during chronic alcohol intake, peaks during acute withdrawal and has been hypothesized to have effects of alcohol withdrawal. It is also shown that calcium channel blockers can reduce physical symptoms of alcohol withdrawal in rats and humans[7,8]. On cessation of chronic ethanol intake, the neurotransmitters previously suppressed by alcohol are no longer suppressed. They rebound, resulting in alcohol withdrawal syndrome consisting of anxiety, irritability, agitation, tremors, seizures, and delirium tremens.

Benzodiazepines (BZD) are currently the drug of choice for management of alcohol withdrawal[9,10]. However, BZDs can be used only as a short course therapy, because of risk of development of dependence and abuse[11,12]. Chronic BZD administration facilitated the subsequent development of ethanol dependence in rats[13]. BZD can also cause adverse effects like memory impairment, drowsiness, lathergy, ataxia, diplopia, confusion[14,15,16]. There is growing evidence that calcium channels may play an important role in ethanol-dependence. Administration of dihydropyridine calcium channel blockers decreased the behavioral effects of ethanol withdrawal syndrome[5,17]. The number of dihydropyridine-sensitive binding sites in the CNS was increased by chronic ethanol administration and thought to represent voltage-sensitive calcium channels[5,18]. This was a functional increase, because dihydropyridine compounds action on phosphatidyl inositol turnover and on neurotransmitter release were also increased[5]. The dihydropyridine sensitive sites increase with long-term ethanol administration is most likely to be an adaptive response to its acute effects in decreasing calcium influx. Nitrendipine and nimodipine were found to abolish all the spontaneous seizures and reduced or prevented seizures following ethanol withdrawal. Verapamil significantly decreased seizure episodes and both flunarizine and verapamil lowered mortality. The dihydropyridines calcium channel antagonists were considerably more effective in the withdrawal syndrome than benzodiazepine diazepam but had little effect on pentylenetetrazol seizures, against which diazepam gave good protection[19]. Hence this study was undertaken to evaluate the effect of verapamil and nimodipine on ethanol withdrawal induced anxiety in rats.

MATERIALS AND METHODS

ANIMALS

Male wistar albino rats weighing 200 – 250 gm were used in the study. They were housed in clean, clear polypropylene cages in groups of three in each cage maintained at 12 hour light and dark cycle. They were given 10% ethanol liquid diet, standard pellet diet (Laboratory animal feeds, VRK nutritional solutions, Sangli, Maharashtra, India) and water ad libitum for 4 weeks. Ethanol was withdrawn 8 hours before starting the experiment.

EXPERIMENTAL DESIGN

The animals were given 10% ethanol containing liquid diet for a period of 4 weeks. The test procedure was performed 8 hours after withdrawing ethanol. The test drugs were administered in doses based on earlier studies. Drug solution was prepared just before administration. In this study, drugs/vehicle was administered orally (p.o) 1 hour before the experimental procedure. The study was carried out after obtaining approval by the Institutional Animals Ethics Committee. Twenty four rats were used in this study. They were divided into four groups of six animals each. The treatment schedule was as follows –

Group I- Control, received potable water for the whole study period and no drug treatment.

Group II – Ethanol control, received ethanol containing liquid died for 4 weeks and no drug treatment.

Group - III – Verapamil group, received ethanol containing liquid died for 4 weeks and Verapamil (20mg/kg) p.o 1 hour before test procedure.

Group IV – Nimodipine (10mg/kg) p.o 1 hour before test procedure.

Two models were used in this study to assess anxiety in rats – elevated plus maze (EPM) and open field test (OFT). The test was carried out 1 hour after administration of the drugs on the 10th day. The apparatus in each model was wiped with 10% ethanol after trial with each rat to eliminate possible bias due to odour of previous animal.

ELEVATED PLUS MAZE (EPM)

The elevated plus maze was widely used as a standard model to assess anxiety in rats[20]. The Elevated plus maze apparatus has two closed arms of 50×10×40 cm with an open roof and two open arms of 50×10 cm, arranged around a central square of 10×10 cm, such that the platform of both open arms and closes arms are opposite to each other[21]. The platforms of elevated plus maze are raised 50 cm from the ground. The drugs were administered to the rats 45 minutes before the experimental procedure. On the 10th day, the rats were placed in the central square facing one of the closed arms[22]. The number of entries, time spent and the number of rears in all four arm (open/closed) was recorded for a period of 5 min[23]. An entry is counted only when all four paws of the rat are in the arm.

OPEN FIELD TEST (OFT)

The apparatus consists of a square arena 96 x 96 cm^2 with 60 cm high walls. The floor of open field apparatus is divided into 25 small squares. Nine squares in the middle were defined as the central squares and sixteen squares along the walls were defined as the peripheral squares[24]. On the 10th day, 45 minutes after drug administration, the rat was placed in a corner of the open field apparatus and the time spent, the number of squares crossed and rearing in the central squares and periphery was observed for a period of 5 minute. The open field was illuminated with 40W bulb from a height of about 100 cm.

STATISTICAL ANALYSIS

All values are expressed as mean ± SEM. Data was analyzed using one-way ANOVA. Post-hoc comparisons were performed by applying Bonferroni test. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out by using SPSS for Windows (SPSS 17.0).

RESULTS

ELEVATED PLUS MAZE (EPM)

In the EPM model, the time spent in the open arm by the rats treated with verapamil (124.50±7.57s) and nimodipine (132±8.08s) was significantly ($P<0.05$) increased with respect to sham control (54.83±8.60s). The time spent in closed arm by verapamil and nimodipine treated rats was significantly ($P<0.05$) decreased with respect to control. Number of rearing in open arm and closed arm were also significantly increased in rats treated with verapamil and nimodipine. There was no significant change in the number of entries into the arms in any of the drug treated groups (Table 1). The significance of the results was indicative of increased exploratory behavior of rats, which shows that verapamil and nimodipine decreased the ethanol withdrawal induced anxiety.

Table 1: Effect of lansoprazole on anxiety in rats in elevated plus maze model

Group / Drug	Number of entries		Time spent in seconds (s)		Number of rears	
	Open arm	Closed arm	Open arm	Closed arm	Open arm	Closed arm
1 / Control – without ethanol	2.67±0.33	2.83±0.31	149.33±8.95	150.66±8.95	8.67±1.05	16.17±1.56
2 / Sham control – with ethanol	2.67±0.56	2.50±0.34	54.83±8.60	245.17±8.61	1.67±0.67	8.67±0.56
3 / Verapamil (20mg/kg)	3.33±0.21	3.83±0.47	124.50±7.57*	175.5±7.57*	9.83±1.14*	16.33±1.82*
4 / Nimodipine (10mg/kg)	3.33±0.42	3.33±0.42	132.00±8.08*	168.00±8.08*	10.67±1.67*	19.00±1.63*

Values are expressed as mean ±SEM, n = 6 in each group

* $P<0.05$ as compared to control. (ANOVA followed by Bonferroni's test)

OPEN FIELD TEST (OFT)

In OFT model, the time spent in central squares by the rats treated with verapamil (54.00±4.54s) and nimodipine (45.00±4.34s) was significantly (P<0.05) increased as compared to sham control (15.00±2.78s). The time spent in peripheral squares by the rats treated with verapamil (246.00±4.55s) and nimodipine (255.00±4.34s) was significantly (P<0.05) decreased as compared to control (285.33±2.82). Number of entries into the central area and the number of lines crossed in central and peripheral areas were also significantly increased in verapamil and nimodipine treated group as compared to control. Number of rearing was significantly increased in the central areas but not in the peripheral areas which is not shown in table (Table 2). The result shows that rats treated with verapamil and nimodipine significantly decreased ethanol withdrawal induced anxiety.

Table 2: Effect of lansoprazole on anxiety in rats in open field model

Group/Drug	Number of entries in the centre	Time spent in seconds (s)		Number of squares crossed	
		Centre	Periphery	Centre	Periphery
1 / Control – without ethanol	8.33±0.71	52.16±3.34	247.83±3.34	25.83±2.09	43.50±4.81
2 / Sham control – with ethanol	2.33±0.60	15.00±2.78	285.33±2.82	8.83±1.19	20.83±1.51
3 / Verapamil (20mg/kg)	7.67±0.67 *	54.00±4.54*	246.00±4.55*	26.67±2.56*	33.50±3.86*
4 / Nimodipine (10mg/kg)	8.83±1.16 *	45.00±4.34*	255.00±4.34*	26.67±2.15*	36.83±4.35*

Values are expressed as mean ±SEM, n = 6 in each group

* P<0.05 as compared to control. (ANOVA followed by Bonferroni's test)

DISCUSSION AND CONCLUSION

Withdrawal of ethanol is well known to produce withdrawal symptoms like anxiety, tremor, irritability and seizure etc. Elevated plus maze and open field test has been used to demonstrate anxiety behavior in rodents. In EPM avoidance of open arms, preference for closed arms and a decrease in rears by the animal indicates anxiety[20,25]. In OFT, preference to peripheral areas and decrease in rears indicates anxiety. Termination of ethanol intake in rats precipitate withdrawal induced anxiety which reduces the exploratory behavior of the animal demonstrated by decrease in time spent, number of entries and rearing in open arm. Whereas drugs that attenuate ethanol withdrawal induced anxiety increase the time spent, number of entries and rearing in open arm of EPM. Similarly in open field test, increase in time spent and number of entries in central areas indicates reduced anxiety. Several previous studies have reported that withdrawal of ethanol following chronic ingestion produces anxiety[26,27]. Calcium channel antagonists, verapamil, nitrendipine and nimodipine were used in the earlier studies to alleviate ethanol withdrawal induced seizure[19]. In the present study, we tested the anxiolytic potential of verapamil and nimodipine in ethanol withdrawal induced anxiety. In the EPM model, the time spent in the open arm was increased in rats treated with verapamil and nimodipine. This suggests the anxiolytic effect of calcium channel antagonist in ethanol withdrawal induced anxiety. The exploratory activity of rats were also increased which is shown by the increase in number of rearing in verapamil and nimodipine treated group. In OFT model, the time spent in central squares was increased in rats treated with verapamil and nimodipine. The number of entries into the central area and the number of lines crossed in central and peripheral areas were also increased in verapamil and nimodipine treated group suggestive of increased exploratory behavior in rats. Increased exploratory behavior is a sign of decreased anxiety in rats. Since benzodiazepines on long term use have potential to develop dependence and abuse, calcium channel antagonists could be an alternative for ethanol withdrawal anxiety.

REFERENCES

[1]. Lovinger DM, White G, Weight FF, 1989. Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science., 243(4899):1721-1724

[2]. Ticku MK, Lowrimore P, Lehoullier P, 1986. Ethanol enhances GABA-induced 36Cl-influx in primary spinal cord cultured neurons. Brain Research Bulletin., 17:123-126

[3]. Dildy-Mayfield J.E, Mihic S.J, Liu Y, Deitrich R.A, Harris R.A, 1992. Actions of long chain alcohols on GABA A and glutamate receptors: relation to in vivo effects. Br J Pharmacol., 118:378-384

[4]. Grant AJ, Koski G, Treistman SN, 1993. Effect of chronic ethanol on calcium currents and calcium uptake in undifferentiated PC12 cells. Brain research., 600:280-284

[5]. Dolin S, Little H, Hudspith M, Pagonis C, Littleton J, 1987. Increased dihydropyridine-sensitive calcium channels in rat brain may underlie ethanol physical dependence. Neuropharmacology., 26:275-279

[6]. Messing RO, Carpenter CL, Diamond I, Greenberg DA, 1986. Ethanol regulates calcium channels in clonal neural cells. Proc. Natl. Acad. Sci. USA., 83:6213-15

[7]. Koppi S, Eberhardt G, Haller R, König P, 1987. Calcium channel blocking agent in the treatment of acute alcohol withdrawal – caroverine versus meprobamate in a randomized double blind study. Neuropsychobiology., 17:49-52

[8]. Bone GH, Majchrowicz E, Martin PR, Linnoila M, Nutt DJ, 1989. A comparison of calcium antagonists and diazepam in reducing ethanol withdrawal tremors. Psychopharmacology., 99:386-388

[9]. Romach and Sellers, 1991. Management of the alcohol withdrawal syndrome. Annu Rev Med., 42:323-40.

[10]. Daeppen JB, Gache P, Landry U, Sekera E, Schweizer V, Gloor S, et al. 2002. Symptom-triggered vs schedule fixed doses of benzodiazepine for alcohol withdrawal: a randomized treatment trial. Archives of internal medicine., 162:1117-1121

[11]. Schuster CL and Humphries RH, 1981. Benzodiazepine dependency in alcoholics, Conn. Med., 1981 45:11

[12]. O'brien CP 2005. Benzodiazepine use, abuse, and dependence. Jour. Clin. Psychiatry., 66(2):28-33

[13]. Martijena ID, Lacerra C, Bustos SG, Molina VA, 2001. Chronic benzodiazepoine administration facilitates the subsequent development of ethanol dependence. Brain res., 891:236-246

[14]. George KA and Dundee JW, 1977. Relative amnesic actions of diazepam, flunitrazepam and lorazepam in man. Br J Clin Pharmacol., 4(1):45-50

[15]. Lister RG, 1985. The amnesic action of benzodiazepines in man. Neurosci Biobehav Rev. Spring., 9(1):87-94

[16]. Heinala, Pieponen T and Heikkinen H, 1990. Diazepam loading in alcohol withdrawal: Clinical pharmacokinetics. International Journal of Clinical Pharmacology, Therapy and Toxicology., 28:211-217

[17]. Littleton JM, Little HJ, Whittington MA, 1990. Effect of dihydropyridine calcium channel antagonists in ethanol withdrawal; doses required, stereo specificity and actions of BAY K8644. Psychopharmacology., 100(3):387-392

[18]. Dolin SJ and Little HJ, 1989. Are changes in neuronal calcium channels involve in ethanol tolerance. J. Pharmaco. Exp. Ther.. 250:985-991

[19]. Little HJ, Dolin SJ, Halsey MJ, 1986. Calcium channel antagonists also decrease the ethanol withdrawal syndrome. Life Sci., Dec 1;39 (22):2059-65

[20]. Pellow S, Chopin P, File SE, Briley M, 1985. Validation of open:closed arm entries in elevated plus maze as a measure of anxiety in the rat. J Neurosci Methods., 14:149-67

[21]. Handley SL and Mithani S, 1984. Effects of α-adrenoreceptor agonists and antagonists in a maze-exploration model of 'fear'-motivated behaviour. Naunyn- Schmeideberg's Arch Pharmacol., 327(1):1–5

[22]. Rodgers RJ, Dalvi A 1997. Anxiety, defence and the elevated plus maze. Neurosci Biobehav Rev., 21(6): 801– 10

[23]. Walf AA and Frye CA, 2007. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat. Protoc., 2(2): 322 – 328

[24]. Fernandez F, 2002. N/OFQ exerts anxiogenic actions in an open field. J Undergrad Res., 3:10

[25]. Fernandes C and File SE, 1996. The influence of open arm ledges and maze experience in the elevated plus maze. Pharmacol. Biochem. Behave., 54:31-40

[26]. Kokare DM, Chopde CT, Subhedar NK, 2006. Participation of alpha-melanocyte stimulating hormone in ethanol induced anxiolysis and withdrawal anxiety in rats. Neuropharmacology., 51(3):536-45

[27]. Gatch MB, Wallis CJ, Lal H, 2000. Effects of ritanserin on ethanol withdrawal-induced anxiety in rats. Alcohol. 21(1):11-17.