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Effect of 5-HT deplete on ethanol induced anxiolysis in EPM test.

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Abstract: Based onthe anxiolytic effects of p-CPA were revealed in the most used animal behavior models Moreover, depletion of 5-HT induced by p-CPA or 5, 6-dihydroxytryptamine a neurotoxin destroying serotonergic neurons.Anxiolytic and stimulant effectsof ethanol have been related to the serotonergic system.Therefore, present investigationdetermine the effect of ethanol in serotonin depleted condition on anxiety, separate groups of rats were administered with p-CPA (300 mg/kg, i.p.) for 3 days and 4th day challenged with saline (10 ml/kg, i.p.) or ethanol (1g/kg, i.p.) 30 min thereafter subjected to EPM test and anxiety related indices were measured for 5 min.The administration of p-CPA is effective in the reduction of anxiety through depletion of serotonin and the data of the present study showed thepotentiation of the open arm activity with sub-anxiolytic dose of ethanol (1 g/kg, i.p.) by p-CPA. Therefore, increase in 5-HT transmission might counter regulate ethanol induced traits on anxiety and reduction in 5-HT transmission might enhance these effects on EPM.

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I. INTRODUCTION

The anti-anxiety or tension-reduction properties of alcohol have been demonstrated in a variety of behavioral situations in both animals and humans. Acutely, ethanol exerts a number of different behavioral effects including, hypothermia, ataxia, amnesia, locomotors stimulation, anxiolysis, and an anticonvulsant effect. It also possesses reinforcing properties that can lead to long-term abuse (Neurobiology of Addiction, by George F. Koob and Michel Le Moal).

Ethanol was the standard drug for (self) treatment of feelings of discomfort, tension, anxiety and stress. The anxiolytic property of ethanol has been demonstrated in a variety of behavioral situations in both humans and animals. (Bedford et al., 1978; Polivyand et al., 1976s). Anxiety is generally accepted to be involved in the development of alcohol dependence and relapse. This may partly be due to the anxiolytic effect of ethanol being detectable in both men and animals (Labuda et al., 2000; Prunell et al., 1994). Prolonged ethanol (alcohol) consumption leads to the development of tolerance and dependence. This induces long lasting changes in the brain neuronal systems leading to adversebehavioral consequences. Considerable evidences suggest that the anxiolytic effect of ethanol may be one of the factors that promote alcohol consumption (Henniger et al., 2002; Langen et al., 2004).Studies of antianxiety-like actions of pharmacological agents in animals undergoing ethanol withdrawal are challenging, in part because of the co-occurrence of potentially confounding signs, such as tremor, suppressed activity/locomotion, and seizures.Nonetheless, the results of many previous reports show that withdrawal from chronic ethanol treatment results in anxiety-like behavior in both human beings (Koob et al., 1997; Meyer et al., 1986; Naranjo et al., 1985) and animal models (Baldwin et al., 1991; Criswell et al., 1993; File et al., 1989; Knapp et al., 1998; Moy et al., 1997, 2000). Ethanol withdrawal anxiety is one of the leading causes for its reinstatement and dependence (Baldwin et al., 1991; Koob et al., 2003; Roelofs et al., 1985).A dual role for 5-HT has been suggested by (Graeff et al., 1966; Jequier et al., 1967) were the first to show that the administration of p-CPA depletes in a specific manner the synthesis of 5-HT by inhibiting the release of tryptophan-hydroxylase, which is involved in the formation of 5-HTP. (Chaput et al., 1990) reported that 350 mg of p-CPA (during 2 days, with a daily injection) reduced the dorsal hippocampal 5-HT concentration by about 95%. The evidence that pretreatment (during 3 days, with a daily injection in most cases) with p-CPA may modulate animals' emotional reactivity first arose from the study of (Tenen et al., 1967). WHO demonstrated the efficacy of this 5-HT depleter in counteracting the disruption of drinking induced by stress since this initial experimentmore than 30 studies have investigated the behavioral effect of p-CPA in anxiety models. Seventy per cent of these studies revealed an anxiolytic-like action of p-CPA whereas, only 9% showed the opposite effect. Although some learning paradigms some authors (Oakley et al., 1992; Treit et al., 1991)

have suggested that pharmacological variables.Such as the route of administration or the doses used may account for some of this variation. For example Treit (1991) suggested that the outcome of an administration of 5-HT_{14} receptor agonists into the central nervous system is more reliable than peripheral application. However, detailed examination of the literature indicates that neither route of administration or drug dose can satisfactorily explain these inconsistencies.

II. LITERATURE REVIEW

Animalliterature demonstrating an increase in aversive behavior with increased 5-HT function and an anxioytic effect of reducing serotonergic function (Chopin et al., 1987; Iverson et al., 1984). Thus increased anxiety might be associated with increased endogenous 5-HT, whereas anxiolysis tends to be associated with decreased endogenous 5-HT.5-HT2 receptor antagonists, including some with both 5-HT1A agonist and 5-HT2 antagonist action, can selectively decrease acute alcohol reinforcement (Roberts et al., 1998). On the other hand, the success of the SSRIs in the treatment of anxiety disorders suggests that an increase in serotonergic tone may be anxiolytic in humans. Therefore, exact function of 5-HT in regulating anxiety still remains obscure.

In animal studies, central serotonergic deficiency correlates with high alcohol intake (Prisco et al., 1995).This supports the classical hypothesis of alcohol addiction assuming that an increased 5-HT function reduces alcohol consumption (Higgins et al., 1995; Sellers et al., 1992). However, there is a dearth of reports exploring the effects of various 5-HT receptor ligands on ethanol induced anxiolysis.

III. MATERIALS

1.1. Subjects

Adult male Sprague Dawley rats weighing between 200-250g was used respectively. All animals were on a 12:12-h light/dark cycle (lights on 0700 h) in a temperature-controlled (24±1°C) environment and behavioral assessment was conducted during the light cycle between 0900 h and 1400 h to minimize diurnal steroid fluctuations. Each experimental group had a separate set of animals (n=6), and an individual animal was tested once only to avoid 'one-trial tolerance' to anxiolytic efficiency of drugs including ethanol (Bertoglio and Carobrez, 2002) in EPM test. Animals were brought to the experimental room 12 h prior to the start of the experiment to minimize nonspecific stress-induced steroid increase.

All procedures were carried out under strict compliance with ethical principles and guidelines of the Committee (IAEC/Pharmacol/05/2010-11) for the Purpose of Control and Supervision of Experimental Animals, Ministry of Environment and Forests, Government of India, New Delhi,and approved by Institutions animal ethics committee. Every effort was made to reduce the suffering of animals during experiments

1.2. Drugs

Sr. no	Drugs	Category	Company	Solvent	Route
1	Ethanol	Drug of abuse	Lobachem	0.9% saline	i.p
2	p-CPA	5-HT depleter	Sigma	0.9% saline	i.p

Table 1.1. Listof drugs used

1.2.1. Drugs solutions and administrations

Ethanol was injected intraperitoneally (i.p.).Ethanol was diluted with 0.9% saline to a concentration of 8% w/v for i.p. injection p-CPA injected by i.p route.

IV. METHODS

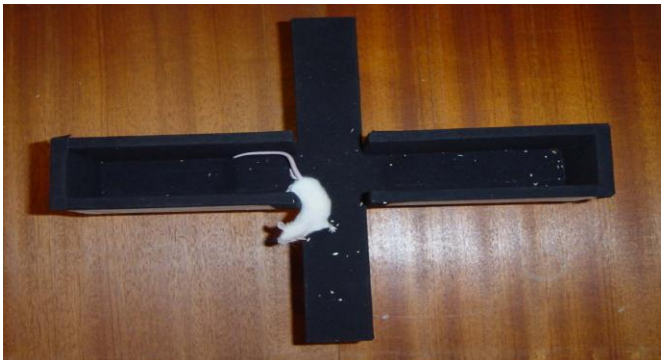

Fig.4.1. Elevated plus maze apparatus

Good example of a model based on the study of unconditioned responses to less intense threatening situations. Anxiogenic or anxiolytic effects were assessed based on the frequency of open arms entries as well as time spent in the open arms. The plus maze was made up of plexiglas painted black. The plus maze consisted of

opposite facing two open (50×10 cm) and two enclosed arms (50×10×40 cm) connected by a central platform (10×10 cm), thus making a plus sign. The whole maze is raised 60 cm above the floor.ratweretested on the plus maze in a room with low, indirect incandescent lighting (100-W lamp, fixed 2 m above the maze floor) and very low noise levels On the day of testing, rat were placed singly at the center of the maze, head facing an open arm and allowed to explore for 5 min.The number of entries into the open arms, the time spent in open arms as well as the number of closed-arm entries were recorded for 5 min by a digital video tracking device (V. J. instruments, India), connected to computer outside in order to reduce the disturbance to animals behavior during test session. Moreover, closed-arm entries were considered as an index of general activity levels than the total arm entries (Dawson et al., 1995; Pellow et al., 1985; Lister, 1987;Rao et al., 2003; Rodgers and Johnson, 1995). An entry was registered only when all four paws of the animal were placed on the arm. The maze was wiped clean with damp cotton and dried after testing each rat. Anxiogenic or anxiolytic effects were assessed based on the frequency of open arms entries as well as timespent in the open arms (Pellow et al., 1985). Decrease in time spent on the open arms and a low frequency of open arms entries relative to control animals were considered as an increase in anxious behavior. Separate groups of animals were used for each treatment and each subject tested were given a single 5 min trial. All animals were tested between 0900–1400 h to minimize circadian influences

4.3.1. Calculation of Behavioral parameters on EPM

a. % open arms time spent (% OAT): (seconds spent on the open arms of the maze/300) × 100;

b. % open arm entries (% OAE): (the number of entries into the open arms of the maze/number of entries into open + closed arms) × 100;

c. Number of closed arms entries were recorded (Motor activity was assessed by recording the distance travelled by animal in cm).

4.4. Chronic ethanol administration

Ethanol was administered to rats for prolonged period as described previously with some modifications (Miller et al., 1980; Lal et al., 1988; Jung et al., 2000; Hirani et al., 2002; Kokare et al., 2006). Briefly, rats were assigned to different treatment groups and housed individually in polypropylene cages. Initially they received nutritionally balanced liquid diet (PROTINEX, Novartis, India) for two days to allow adaptation to novel food. Water was available ad libitum. From third day onwards, ethanol was added to the liquid diet of some groups (final concentration 6% v/v, ethanol-fed), while isocaloric amount of ethanol was substituted with dextrose in the liquid diet of remaining groups (pair-fed control) and had free access to it for 1, 3, 5, 7 or 10 days. Fresh aliquot (100 ml/rat) of ethanol containing liquid diet was introduced in the respective cages each morning at 0800 h. Diet of pair-fed groups was unchanged but was restricted so that consumption matched the mean amount of the ethanol-containing diet consumed. The dietary consumption and body weight of each animal was monitored daily (0800 h) for all the groups. The average daily ethanol consumption was found to be 10.56 g/kg. Body weights of ethanol-fed rats were not different in comparison to pair-feds during initial drinking phase or throughout the course of the experiment. The animals were subjected for 5 min to elevated plus maze test at different time intervals, but individual animal was tested only once to avoid 'one trial tolerance' to drug effect (Bertoglio and Carobrez, 2002).

4.6. STATISTICAL ANALYSIS

The data were analyzed by parametric tests (Sharma et al., 2007; Umathe et al., 2008), significant differences between treatment groups were determinedby using one-way ANOVA followed by the Newman-Keuls test. All values are expressed as mean±SEM of 6 rats in each group. P value less than 0.05 was considered statistically significant in all of the cases.

V. EXPERIMENTAL DESIGN

5.1 Effects of acute ethanol in EPM test

Rats were randomly divided in different groups (n=6) and injected withsaline (10 ml/kg, i.p.) or ethanol (8% w/v in saline; 0.5-2.5 g/kg, i.p.). 30 min after i.p. or 15 min after intra-CeA injection, individual animal was placed on the central platform of EPM test and allowed to explore for 5 min as described above. Total numbers of entries into the open and closed arms, as well as time spent in open arms were monitored.

Effect of 5-HT depleter P-CPA on ethanol-induced anxiolysis

Determine the effect of ethanol in serotonin depleted condition on anxiety, separate groups of rats were administered with p-CPA (300 mg/kg, i.p.) for 3 days and 4th day challenged with saline (10 ml/kg, i.p.) or

ethanol (1g/kg, i.p.) 30 min thereafter subjected to EPMtest and anxiety related indices were measured as described above for 5 min.

VI. Results

6.1. Effectsof acute ethanol in EPM test

One way ANOVA revealed that acute treatment of ethanol have significantly affected the open arm behavior of rats after comparing with respective control groups on EPM [ethanol (%OAT, $F(5,30)=349.5$, $P<0.0001$; %OAE, $F(5,30)=178.0$, $P<0.0001$)]. Post hoc test indicates that ethanol (1, 1.5 & 2 g/kg, i.p.)significantly enhances the preference of the rat for open arm as evident from increase in both the indices i.e, %OAT ($P<0.001$) and %OAE ($P<0.001$), without affecting the number of closed arm entries [ethanol $F(5,30)=1.430$, $P>0.05$]. However, treatment of higher dose of ethanol (2.5 g/kg, i.p.)shows reduced %OAT and %OAE ($P<0.001$) along with decrease in closed arm entries ($P<0.05$), as compared to saline treated rats. Ethanol (0.5 g/kg, i.p.)treatment in this dose was devoid of any effect on anxiety indices on EPM ($P>0.05$). These results are depicted in Fig. 6.1.

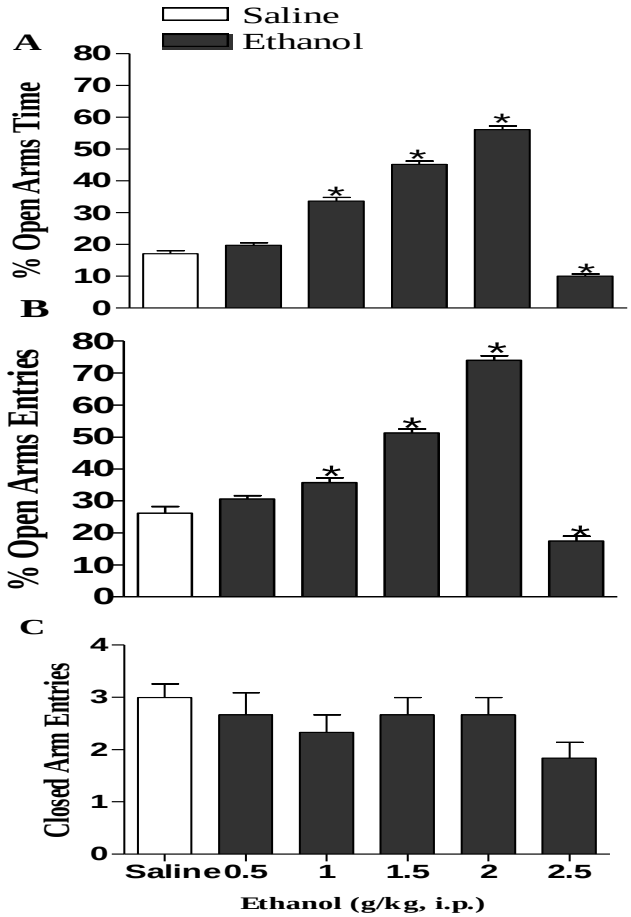

Fig.6.1. Effect of acute ethanol (g/kg, i.p.; 8% w/v in saline), anxiety related indices were measured in EPM. Each bar represents mean±SEM of data from 6 rats. *$P<0.001$, vs respective control (One way ANOVA followed by Newman-Keuls Test).

Effect of acute p-CPA alone and effect of p-CPA on ethanol-induced anxiolysis

As shown in Fig.6.6., the acute administration of p-CPA (300 mg/kg, i.p.) for 3 day haveincreased open arm behavior and also prior administration of p-CPA (300 mg/kg, i.p.) for 3 days and ethanol (1 g/kg, i.p.) was administered after 3rd day p-CPA administration, thereafter 30 min animal was placed on EPM for 5 min. The interaction revealed potentiation of open arm activity with sub-anxiolytic dose of ethanol (1 g/kg, i.p.) [P-CPA (%OAT $F(3,15)=86.77$, $P<0.0001$; %OAE $F(3,15)=49.77$, $P<0.0001$]. No relevant difference in closed arm entries were observed [p-CPA $F(3,15)=0.3571$, $P>0.05$ NS].

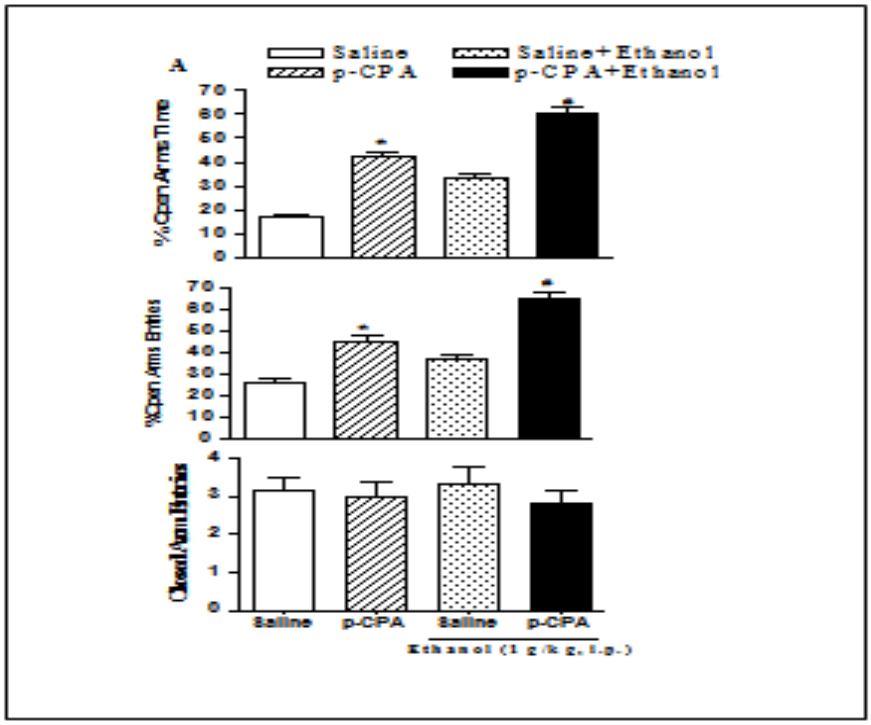

Fig. 6.6. Effect of p-CPA (mg/kg, i.p.) alone or in combination with ethanol (1 g/kg, i.p.), on behavior of rat in the EPM test. Separate groups of rats were administered p-CPA for 3 days and 15 min later challenged with ethanol (1 or 2 g/kg, i.p.) 0n 4rd day. 30 min thereafter, individual rat was placed in the center of the arm to explore the EPM for 5 min. Each bar represents mean±SEM of data from 6 rats. *P<0.001, vs respective control (One way repeat measure ANOVA followed by Newman-Keuls Test).

VI. Discussion

The anxiolytic effect of p-CPA treatment in the present study is in agreement with the data obtained in previous reports (Crawley 1981; Lopez-Rubelcava 1996).The anxiolytic effects of p-CPA were revealed in the most used animal behavior models (Gibson et al. 1994; Johnston and File 1986; Lopez-Rubelcava 1996). Moreover, depletion of 5-HT induced by p-CPA or 5,6-dihydroxytryptamine a neurotoxin destroying serotonergic neurons, produced an elevation of tyrosine hydroxylase activity as well as its amount and even mRNA coding for tyrosine hydroxylase in the locus coeruleus (Crespi et al. 1980, Sturtz et al. 1994). The administration of

p-CPA is effective in the reduction of anxiety through depletion of serotonin and the data of the present study showed thepotentiation of the open arm activity with sub-anxiolytic dose of ethanol (1 g/kg, i.p.) by p-CPA. Therefore, it is reasonable to hypothesize that increase in 5-HT transmission might counter regulate ethanol induced traits on anxiety and reduction in 5-HT transmission might enhance these effects on EPM.

VII. Summary and Conclusion

Anxiety is an exaggerated feeling of apprehension, uncertainty, and fear. It is an unpleasant state of tension with an anticipation of imminent danger and 29% of people in their lives suffer from anxiety disorder. Chronic ethanol consumption and its withdrawal lead to anxiety in both humans and experimental animals. Although several neurotransmitters and their receptors system have been proposed to be involved in pathogenesis of alcoholism, the basis of anxiety following prolonged ethanol consumption and also on withdrawal is not clearly understood. The present study provides first functional evidence reduction of anxiety through depletion of serotoninin acute ethanol induced anxiolytic effects. This effect of ethanol was independent of any motor effects as revealed from locomotor count.

Three days treatment of p-CPA by i.p. route potentiated the sub-anxiolytic effect of ethanol (1 g/kg, i.p.) in EPM. Therefore, it is reasonable to hypothesize that increase in 5-HT transmission might counter regulate ethanol induced traits on anxiety and reduction in 5-HT transmission might enhance these effects on EPM.

In conclusion, finally it can be suggested that ethanol increase in 5-HT transmission that leads to anxiety hence p-CPA is effective in the reduction of anxiety through depletion of serotonin and the data of the

present study showed thepotentiation of the open arm activity with sub-anxiolytic dose of ethanol (1 g/kg, i.p.) by p-CPA.mightbe attributed to increase in tolerance to ethanol antianxiety effect and withdrawal induced anxiety.

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