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Differential effects of catecholamine antagonists on ethanol-induced excitation in mice

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Abstract. Catecholamine antagonists were assessed for their effects on ethanol-induced motor excitation. Motor excitation was measured in male Swiss-Webster mice using an open-field apparatus. Mice were treated with several doses of ethanol and at each dose, mice were pretreated with pimozide, a dopamine D2 antagonist, Schering 23390, a dopamine D1 antagonist, phenoxybenzamine, a noradrenergic alpha-1 antagonist, or yohimbine, a noradrenergic alpha-2 antagonist. Each mouse was subjected to only one dose regimen, and all injections were given IP. Ethanol produced an increase in locomotor activity. The degree to which pimozide attenuated ethanol excitation decreased with increasing ethanol dosage. At the highest dose of ethanol, pimozide increased ethanol excitation. Schering 23390 attenuated ethanol-induced excitation only at doses which affected motor activity per se. Phenoxybenzamine produced a dose-dependent reduction in ethanol excitation. Yohimbine had its greatest effects at the medium dose (4.0 mg/kg). These observations seem to indicate a role for both the dopamine D2 receptor and the noradrenergic alpha-1 receptor in ethanol-induced motor excitation.

Key words: Ethanol – Dopamine – Norepinephrine – Locomotor activity – Drug antagonists

The measurement of locomotor activity in the open-field has been widely employed to assess the effects of ethanol alone and in combination with other drugs (Carlsson et al. 1972). Studies examining the effects of ethanol alone have demonstrated that ethanol possesses both excitatory and depressant properties which appear to be dose dependent (Friedman et al. 1980; Frye and Breese 1981; Strombom and Liedman 1982; Smoothy and Berry 1985; Durcan and Lister 1988).

Little is presently known about the neural substrate of ethanol-induced motor excitation. Previous research has suggested that the catecholamines (CA) are involved in the mediation of ethanol-induced motor excitation (Liljequist and Carlsson 1978; Menon et al. 1987). For instance, Carlsson et al. (1972) reported that when CA synthesis was inhibited in NMRI mice using the tyrosine hydroxylase inhibitor alpha-methyl-*p*-tyrosine (AMPT), ethanol-induced motor activation was not observed. This effect was also noted by Friedman et al. (1980) in the inbred mice strains C57 Bl and Balb. AMPT was also reported to attenuate euphoria and stimulation induced by alcohol in human subjects (Ahlenius et al. 1973). The suppression of ethanol-induced motor stimulation by AMPT was partially reversed by administering the dopamine (DA) precursor L-dopa in concentrations which by themselves did not influence motor activity (Engel et al. 1974). However, the relative importance of norepinephrine (NE) and/or DA was not assessed.

Further investigation of the neural mechanisms of ethanol excitation suggested that both NE and DA may play a role in mediating this behavioral phenomenon. For instance, Dudek et al. (1984) found that the DA agonist apomorphine attenuated ethanol-induced motor activity in selected strains of mice. Liljequist et al. (1981) found that both DA and NE receptor antagonists suppressed ethanol-induced stimulation.

The above studies did not assess the relative contribution of individual receptor types to ethanol induced motor activation. Recent research (Kebabian and Calne 1979; Clark and White 1987) on specific DA receptors has led to the identification of at least two subtypes. These DA receptor subtypes have been categorized as D1 and D2 (Kebabian and Calne 1979). Investigations into the behavioral functions of D1 and D2 DA receptors revealed that both receptor subtypes may play a role in mediating motor activity (Hoffman and Beninger 1985; Beninger et al. 1990) and reinforcement (Nakajima 1986; Koechling et al. 1988; Beninger et al. 1990; Wise and Colle 1984). Despite the apparent functional similarity of the two DA receptor subtypes in some behaviors, both antagonistic and synergistic interactions between these

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two DA receptors have been postulated (Clark and White 1987; Beninger et al. 1990).

Similar investigations to those described above were also conducted on the neurotransmitter NE. The division of NE receptors into alpha and beta subtypes has been accepted for some time. Previous research (Hayashida and Smith 1971; Matchett and Erickson 1977) concerning the involvement of NE receptors in ethanol activation suggested that the alpha NE receptor rather than the beta receptor was involved in ethanol-induced stimulation.

Further studies have shown that the NE alpha receptor consists of at least two subtypes, alpha-1 and alpha-2 (Berthelson and Pettinger 1977; Young and Kuhar 1980). Durcan et al. (1989) showed that two highly selective alpha-2 antagonists, atipamezole and idazoxan, had no effect on ethanol-induced motor excitation. However, alpha-1 agonists were found (Menon et al. 1987) to increase ethanol-induced excitation in selectively bred mice. This would suggest that the alpha-1 rather than the alpha-2 receptor may be involved in ethanol-induced motor excitation.

The present study was designed to investigate the contribution of both DA and NE to ethanol-induced excitation. This study attempted to explore the relative contributions of both the D1 and D2 DA receptors and the alpha-1 and alpha-2 receptors for NE to ethanol-induced excitation. Schering 23390, a selective D1 antagonist, and pimozide, a D2 antagonist, were employed to assess the role of DA in ethanol-induced excitation. Phenoxybenzamine, an alpha-1 antagonist, and yohimbine, an alpha-2 antagonist, were used to examine the involvement of NE in ethanol-induced excitation.

Experiment 1

Materials and methods

Subjects were Swiss-Webster mice, weighing approximately 26–28 g at the time of testing. The mice were housed four to a cage in a room regulated for temperature and humidity with 12 h lights on and 12 h lights off, and with free access to standard lab chow and water.

A 20% (v/v) ethanol solution was prepared by diluting 95% ethanol with tap water. Mice were injected IP with fluids in volumes of 5, 7.5 and 10 ml/kg to yield ethanol doses of 0.8, 1.2 or 1.6 g/kg. Pimozide was dissolved in 0.3% tartaric acid solution and Schering 23390 was dissolved in saline. Both were injected IP in a volume of 10 ml/kg body weight. Mice were treated 30 min prior to testing with either pimozide (0.0, 0.0321, 0.0625, 0.125, 0.25 and 0.5 mg/kg) or Schering 23390 (0.0, 0.0125, 0.025, 0.05 and 0.1 mg/kg) and then 30 min later injected contralaterally with ethanol (0.0, 0.8, 1.2 and 1.6 mg/kg). Groups of six mice were treated with one of each of the dose regimens. Immediately following the second set of injections each animal was placed in an open-field apparatus for a 7-min time period. The open-field consisted of a glass cylinder 22 cm in diameter and 25 cm in height. The floor of the cylinder was divided into four equal quadrants by two intersecting lines. A score was assigned each time an animal crossed a line with all four legs. The test room was illuminated with fluorescent light. Animals were randomly assigned to each drug regimen, and tested in random order. Animals in the pimozide condition were tested between 1 p.m. and 4 p.m., animals in the other treatment conditions were tested between 8 a.m. and 1 p.m.

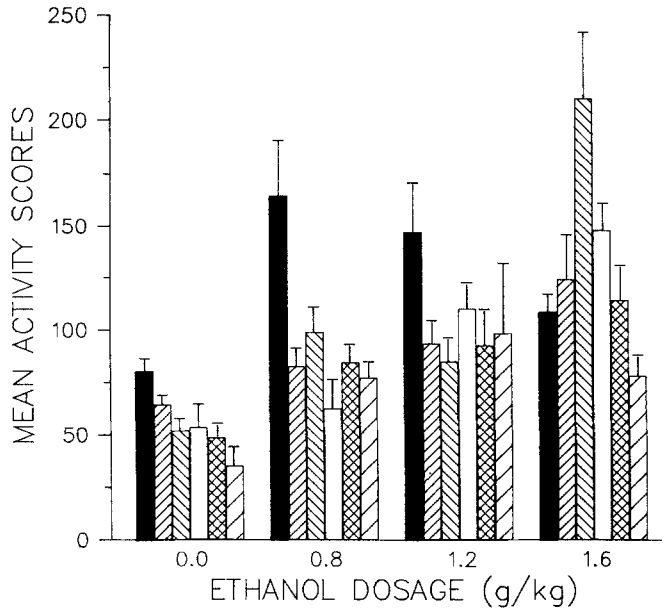

Fig. 1. Mean activity for animals ($n=6$ per group) pretreated with pimozide (mg/kg) at each dose of ethanol. ■ 0.0; ▨ 0.0312; ▧ 0.0625; □ 0.125; ▩ 0.25; ▨ 0.5

Results

Data were analysed using a two-way independent measures analysis of variance (ANOVA). Post hoc differences between groups were examined using Tukey's Honestly Significant Difference (HSD) test.

Ethanol treatment resulted in a significant increase in locomotor activity for the 0.8 g/kg ($P<0.01$) and the 1.2 g/kg ($P<0.05$) dose. Pimozide pretreatment (Fig. 1) produced no statistically significant changes in motor activity for any of the doses tested. Increases in locomotor activity observed following 0.8 g/kg ethanol were significantly attenuated by all doses of pimozide ($P<0.05$). At the 1.2 g/kg ethanol dose, only the 0.0625 mg/kg dose of pimozide produced an attenuation ($P<0.05$), while the other pimozide doses had no effect. In contrast, this latter dose resulted in a significant ($P<0.01$) augmentation of motor activity following 1.6 g/kg ethanol. This increased locomotor activity was above control levels and also greater than the activity induced by the other two ethanol doses. Only 0.5 mg/kg pimozide in combination with 1.6 g/kg ethanol was lower than ethanol alone ($P<0.05$).

Statistical analysis of these data revealed a significant main effect for both ethanol [$F(3,99)=24.63$, $P<0.001$] and pimozide [$F(5,99)=6.174$, $P<0.001$] and a significant interaction [$F(15,99)=3.76$, $P<0.001$].

The effects of Schering 23390 on ethanol-induced motor activity were analysed separately from pimozide, as these experiments were conducted at a different time of day.

Ethanol produced an increase ($P<0.01$) in locomotor activity at all three doses (0.8, 1.2 and 1.6 mg/kg) compared to vehicle-treated animals. Schering 23390 at doses of 0.05 and 0.1 mg/kg reduced activity ($P<0.01$) when administered alone and also reduced ethanol-

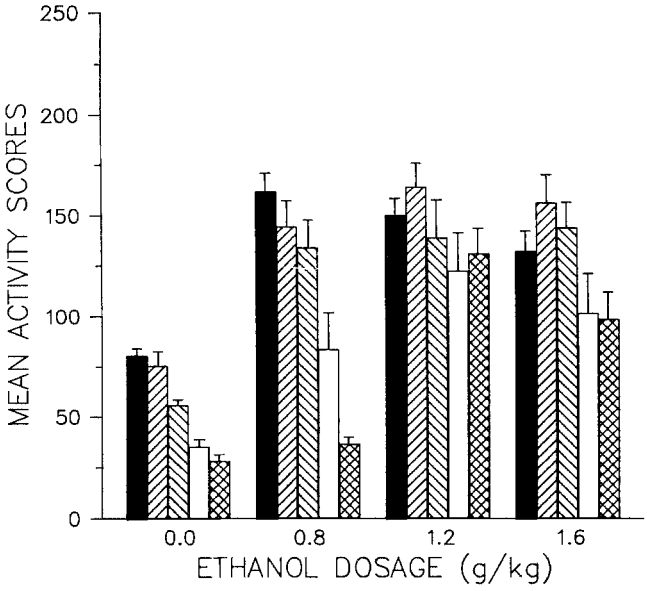

Fig. 2. Mean activity for animals ($n=6$ per group) pretreated with Schering 23390 (mg/kg) at each dose of ethanol. ■ 0.0; ▨ 0.0125; ▧ 0.025; □ 0.05; ▩ 0.1

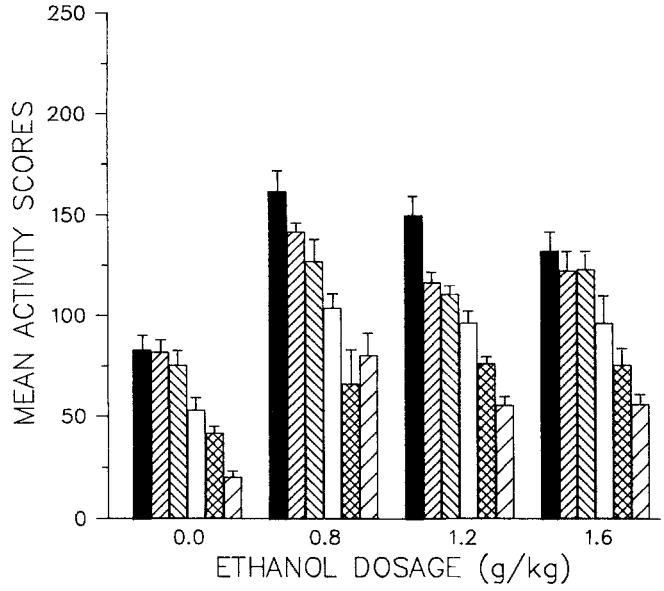

Fig. 3. Mean activity for animals ($n=6$ per group) pretreated with phenoxybenzamine (mg/kg) at each does of ethanol. ■ 0.0; ▨ 1.0; ▧ 2.0; □ 5.0; ▩ 10.0; ▨ 15.0

induced activation following 0.8 g/kg ethanol ($P<0.01$) (Fig. 2).

There was no effect of any dose of Schering 23390 on motor activity induced by 1.2 and 1.6 g/kg ethanol. Schering 23390 at doses of 0.0125 and 0.025 mg/kg neither affected activity per se nor showed any significant effect on ethanol-induced activation. Only those Schering 23390 doses which by themselves depressed motor activity were effective in attenuating ethanol-induced motor activation.

A two-way ANOVA revealed a significant main effect for ethanol [$F(3,82)=69.62$, $P<0.001$] and Schering 23390 [$F(4,82)=30.07$, $P<0.001$]. This analysis also showed a significant interaction between ethanol and Schering 23390 [$F(12,82)=3.43$, $P<0.001$].

Experiment 2

Materials and methods

Phenoxybenzamine was dissolved in a few drops of glacial acetic acid and this solution was further diluted with a 5.5% glucose solution. Yohimbine was dissolved in a few drops of 2 N HCl and made up to volume with saline. All injections were administered IP at a volume of 10 ml/kg. Phenoxybenzamine (0.0, 1.0, 2.0, 5.0, 10.0 and 15.0 mg/kg) and yohimbine (0.0, 0.0125, 0.025, 1.0, 4.0 and 8.0 mg/kg) were injected 30 min prior to testing. All other procedures were the same as in the first experiment.

Results

Significant increases ($P<0.01$) in motor activity were observed following the administration of all three doses (0.8, 1.2 and 1.6 g/kg) of ethanol.

Phenoxybenzamine pretreatment alone decreased locomotor activity at 5.0, 10.0 and 15.0 mg/kg ($P<0.01$) (Fig. 3). In addition, these doses also attenuated ethanol-induced motor excitation at all three ethanol doses tested. In contrast, 1.0 and 2.0 mg/kg phenoxybenzamine had no effect on locomotor activity when administered alone. Pretreatment with 2.0 mg/kg phenoxybenzamine was observed to attenuate ethanol-induced excitation, but only at 0.8 and 1.2 g/kg ethanol ($P<0.01$). The lowest dose of phenoxybenzamine (1.0 mg/kg) was seen to significantly attenuate ethanol-induced activity only at 1.2 g/kg ethanol ($P<0.01$).

Statistical analysis revealed a significant main effect for phenoxybenzamine [$F(5,103)=95.33$, $P<0.001$] and ethanol [$F(3,103)=81.84$, $P<0.001$] and a significant interaction [$F(15,103)=2.84$, $P<0.001$].

Pretreatment with yohimbine (8.0 mg/kg) significantly ($P<0.01$) decreased activity when administered alone (Fig. 4). This dose of yohimbine also reduced ethanol excitation for all doses of ethanol ($P<0.01$). Yohimbine 4.0 mg/kg did not affect activity itself but produced a reduction in ethanol-induced excitation ($P<0.01$) at all ethanol doses. Ethanol 1.6 g/kg in combination with 0.01 mg/kg yohimbine produced a significant ($P<0.01$) augmentation in motor activity compared to treatment with either agent alone.

Significant main effects were observed for yohimbine [$F(5,103)=77.51$, $P<0.001$] and ethanol [$F(3,103)=115.54$, $P<0.001$]. The interaction between these two variables was also significant [$F(15,103)=8.89$, $P<0.001$].

Discussion

Ethanol was observed to increase locomotor activity at all test doses, with the lowest ethanol dose producing the greatest stimulation of motor activity and the highest dose resulting in the smallest increase in motor activity.

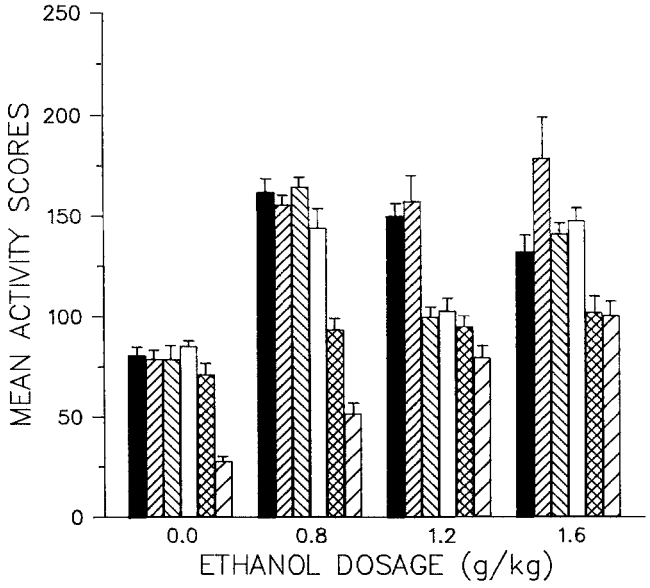

Fig. 4. Mean activity for animals ($n=6$ per group) pretreated with yohimbine (mg/kg) at each dose of ethanol. ■ 0.0; ▨ 0.0125; ▧ 0.025; □ 1.0; ▩ 4.0; ▨ 8.0

These findings are consistent with previous literature in that administration of low doses of ethanol have been repeatedly shown to result in behavioral activation (Engel et al. 1974; Friedman et al. 1980; Liljequist et al. 1981; Strombom and Liedman 1982; Smoothy and Berry 1985; Durcan and Lister 1988).

The present data provide additional support for the notion that the CA mediate the motor stimulatory effects of ethanol (Engel et al. 1974; Stroembom et al. 1977; Friedman et al. 1980; Liljequist et al. 1981; Menon et al. 1987).

Specifically, our results suggest roles for both DA, particularly via the D2 receptor, and NE, possibly via the alpha-1 receptor, in the mediation of ethanol-induced locomotor activation. These conclusions are based on the observations that at low ethanol doses both pimozide and phenoxybenzamine showed the greatest attenuation of ethanol-induced motor activation. The D1 receptor antagonist Schering 23390 was also observed to attenuate ethanol-induced locomotor activation; however, this effect was seen only at doses of Schering 23390 which, when administered alone, depressed locomotion.

The involvement of the alpha-2 receptor in ethanol excitation remaines unclear from the present data. Even though yohimbine attenuated ethanol activation, which was most apparent at the medium dose (4.0 mg/kg), the selectivity of this drug for the alpha-2 receptor has been questioned (Colpaert 1984; Dwoskin et al. 1988), as have many other drugs in the past. There are now indications that yohimbine may also act as an antagonist (Dwoskin et al. 1988) or agonist (Colpaert 1984) for serotonergic receptors. We are now using a more selective alpha-2 antagonist in order to determine the role of the NE alpha-2 receptor in the mediation of ethanol-induced excitation.

It has been suggested that forward locomotion in laboratory animals may be a reflection of the psychomotor stimulant properties of psychoactive drugs (Wise and Bozarth 1987). According to these authors, these stimulant properties are suggested to be homologous with the reinforcing properties of a drug and thus can serve as a predictor of whether the drug would be reinforcing or self-administered. They also suggested that these two homologous phenomena are mediated by dopamine (DA) in all reinforcing drugs.

We obtained support for the prediction of the psychomotor stimulant theory of addiction with regard to pimozide's interaction with ethanol. However, the present results concerning the actions of phenoxybenzamine on ethanol-induced excitation would appear to be in conflict with this notion. The proposed notion that the reinforcing properties of all addictive agents are DA mediated would not predict an effect for any NE manipulations. Our results therefore do not support the theory that the DA circuitry alone mediates the psychomotor stimulant properties of all addictive agents.

An unexpected finding was that relating to the groups of mice receiving the highest dose of ethanol in combination with selected doses of pimozide, Schering 23390 and yohimbine but not phenoxybenzamine. Pretreatment with these three antagonists and 1.6 g/kg ethanol resulted in increased locomotor activity levels which were above those induced by ethanol alone. This increased motor activity was most apparent for pimozide and yohimbine at their respective lower doses. It is possible that ethanol at this dose may have attenuated or prevented the motor retardation effects of the three antagonists. This finding appears to be incongruent with the psychomotor stimulant theory of addiction, which would have predicted a decreased and not an increased level of motor activity. Clearly the effect of ethanol at the high dose is far more complex than can be explained by this theory.

In conclusion, ethanol-induced motor excitation was attenuated most prominently by pimozide and phenoxybenzamine. This effect was most apparent at the two lower doses of ethanol. These results seem to suggest that ethanol-induced excitation may be partially mediated by DA via the D1 receptor and by NE, possibly via the alpha-1 antagonist. From the present data it seems that there are qualitative differences between DA and NE in their interaction with ethanol. The differential involvement of these two transmitters in the mediation of this behavior is now being examined. The first step is to investigate the facilitation observed for pimozide at the high dose of ethanol. Further research is needed to determine the degree of interaction, if any, of these systems in the mechanisms underlying ethanol-induced motor activation.

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