Abstract — Aims: To compare the effect of an antagonist of the mGlu5 glutamate receptor, 2-methyl-6-(phenylethynyl)pyridine (MPEP) on a test for anxiety and on the volitional consumption of ethanol. Methods: The test for anxiety was placement of a Sprague-Dawley rat for a 5 min observation period in an elevated plus-maze. Volitional consumption of ethanol in a two-choice paradigm was determined for male and female myers high ethanol-preferring rats after a 10-day ‘step-up’ test of 3–30% v/v ethanol vs water used to determine each rat’s preferred concentration of ethanol. Each rat received a 4-day baseline period, 3-days of drug injection b.i.d., and a 4-day post-treatment period and then rotated to a different dose of drug or vehicle. Results: The effects of MPEP on elevated plus-maze activity were not significant at doses up to 3.0 mg/kg subcutaneously 60 min. before observation. There was a dose-dependent, 0.3, 1.0, 3.0 mg/kg, decrease in consumption of preferred concentrations of ethanol, along with a decrease in the proportion of ethanol consumed to total fluids consumed. The 3.0 mg/kg b.i.d. dose of MPEP reduced consumption by 57%, proportion by 45%, and food intake by 10%. Conclusions: MPEP did not appear to have an anti-anxiety effect, but volitional drinking in a genetic model was reduced. The mGlu5 receptor may provide a target for drug action to reduce the consumption of ethanol.

INTRODUCTION

The different receptors for glutamic acid provide multiple binding sites for the development of drugs by the pharmacologist. The AMPA receptor is a ligand-gated Na⁺ channel, the NMDA receptor is a ligand-gated channel that allows both Na⁺ and Ca²⁺ to enter the cell. Finally, there is a series of metabotropic glutamate receptors all in the 7-transmembrane G-protein coupled receptor superfamily that are divided into three families. Competitive, non-competitive and glycine-site antagonists of the NMDA glutamate receptor will reduce consumption of ethanol by the Myers high ethanol-preferring (mHEP) rat (McMillen et al., 2004). In contrast to a channel ionophore receptor, the metabotropic glutamate receptors are linked by G-proteins to catalytic enzymes for second messenger systems. Agonists and antagonists with preference for one or another of these receptors are becoming available.

The mGlu5 metabotropic glutamate receptor is in the Group I mGlu receptor family that activates phospholipase C through Gₛₐ₅₁₁ and releases calcium from internal stores (reviewed by Conn and Pinn, 1997). This receptor appears to be located post-synaptically in dopaminergic terminal areas (Romano et al., 1995) and would have an effect similar to the NMDA glutamate receptor in that intracellular Ca²⁺ concentrations would rise after stimulation of either receptor. The NMDA and mGlu5 glutamate receptors have many important interactions with dopaminergic function and can change motor behaviour (Breyssse et al., 2002), fear and anxiety responses (Spooren et al., 2000; Schulz et al., 2001), nicotine self-administration (Paterson et al., 2003), prevent neurotoxicity of amphetamines (McMillen et al., 1992), and other forms of neurotoxicity (Bruno et al., 2000) among a long list of effects on the central nervous system.

Antagonists at different acceptor sites on the NMDA receptor reduce the volitional consumption of ethanol by genetic drinking rats (McMillen et al., 2004). Both the NMDA and mGlu5 glutamate receptors increase intracellular Ca²⁺. This suggests that an antagonist of the mGlu5 Type I group receptor should reduce the consumption of ethanol. The following experiments tested the ability of a highly preferential mGlu5 receptor non-competitive antagonist, 2-methyl-6-(phenylethynyl)pyridine (MPEP; Spooren et al., 2001), for its ability to reduce the volitional consumption by the Myers high ethanol-preferring (mHEP) rat in a 24 h access paradigm. The data were compared to the ability of MPEP to alter the activity of the Sprague-Dawley rat in the elevated plus-maze, a measure of potential anti-anxiety activity (File, 1990).

MATERIALS AND METHODS

Subjects and screening

Ten male Wistar rats were purchased from Harlan Sprague-Dawley (Frederick, MD) and used for the experiment on the elevated plus-maze. The mHEP rats, six male and six female, were from the F13 generation of breeding in the East Carolina University colony. The progenitor rat strains for this line were three male alcohol preferring P rats obtained from T.-K. Li of the Indiana University Alcohol Research Center and three female Sprague-Dawley rats purchased from Harlan Sprague-Dawley, and selected based on an ethanol drinking screen. These rats consume copious amounts of ethanol, even in the presence of palatable alternatives (Myers et al., 1998), and the line is maintained by breeding male and female rats from different litters. The rats were initially housed with continuous access to food and water and maintained on a 12 h on/12 h off light schedule. All procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by East Carolina University’s Institutional Animal Care and Use Committee.

At 60 days of age, each male mHEP rat was placed in a suspended stainless steel cage with three drinking tubes mounted on the front. After a 1-day adaptation, one tube was filled with...
None of the doses of MPEP altered the activity of the Wistar rats in the elevated plus-maze (Table 1). There were no differences in the latency to the first entry of an open arm, open arm duration or frequency of entry. Furthermore, there was no difference in the total frequency of entries ($F_{3,35} = 0.62$, NS), which indicates that even the highest dose tested had neither a sedating nor an activating effect on locomotion. As the table indicates, the rats spent most of the time in the closed arms of the plus-maze.

In contrast, MPEP showed a dose–response for the reduction of ethanol consumption by the mHEP rat as shown in Table 2. The amount consumed each day decreased by 45.5% after the 1.0 mg/kg dose ($F_{2,9} = 8.10$, $P < 0.01$) and by 57.6% after the 3.0 mg/kg dose ($F_{2,9} = 28.95$, $P < 0.0001$). The effect of the highest dose persisted into the post-treatment period. There was an effect of injection of saline vehicle ($F_{2,9} = 7.86$, $P < 0.05$), but no effect was seen after the lowest dose of MPEP ($F_{2,5} = 0.30$, NS). The decline in the amount consumed was mirrored by decreased proportions of ethanol consumed as a fraction of total fluids consumed. After the 3.0 mg/kg dose there was a 45% decrease in proportion ($F_{2,9} = 15.17$, $P < 0.0001$). Vehicle did not produce a significant effect on proportion (28% decrease, $F_{2,9} = 2.31$, NS). Note that the baseline data for proportions of ethanol solutions as a fraction of total fluid consumed were all close to 0.5 because of the screening and selection of concentrations for each rat. This allows for an increase or a decrease in proportion and consumption with treatment.

Table 3 shows the effect of injection of MPEP on food intake. The doses of 1.0 and 3.0 mg/kg reduced food intake significantly, but <10% of baseline. At the highest

### RESULTS

**Open arm entry, duration on open arms, and total frequency of arm entries** were recorded and analysed by ANOVA followed by Dunnett’s *t*-test (Zar, 1984). Statistical analyses were performed with GB-STAT, Dynamic Microsystems, Silver Spring, MD.
dose, the effect did not persist into the post-treatment period in contrast to the significant decline in the consumption of ethanol.

DISCUSSION

MPEP was expected to have an anxiolytic-like effect on the elevated plus-maze based on experiments by others (Spooren et al., 2000; Kuhn et al., 2002). The doses used in the current study were similar to those found to increase open arm activity in the plus-maze and social interactions by the rat (Spooren et al., 2000). The drug also was able to block the expression of conditioned fear by the rat when administered in this dose range (Kuhn et al., 2002). Three differences in the present study were that Wistar rats and not Sprague-Dawley rats were the experimental subjects, the drug was administered s.c. and not p.o.s., and the rats were handled briefly for 3 days prior to testing as is the practice in this laboratory. It is not clear whether any of these differences may account for the difference in results.

In contrast to the elevated plus-maze results, MPEP had a clear dose–response effect on the consumption of ethanol. Although a handling effect due to the injection of saline vehicle occurred (Table 2), the lowest dose of MPEP did not have a significant effect. The percent decline in consumption was much greater after the 1.0 and 3.0 mg/kg doses of MPEP. That the proportion of total fluids consumed as solutions of ethanol declined, demonstrates an effect on the selection of ethanol rather than a general decline in fluid intake. This result indicates a pharmacological interaction that has caused the rats to accept less ethanol.

These effects, including that of vehicle, are similar to that reported for antagonists of the NMDA glutamate receptor (McMillen et al., 2004). Different laboratories have obtained different results with MPEP in different animal models. McGeehan and Olive (2003) used mice for drug-induced conditioned place preference. They demonstrated an effect on cocaine-induced conditioning, but conditioning induced by either morphine or ethanol was unaffected. This is in contrast to the present results, which examined the selection and consumption of ethanol in a more naturalistic setting with 24 h access to solutions. Backstrom et al. (2004) used conditioning cues to reinduce lever pressing for ethanol after a period of extinction and found that MPEP would reduce reinstatement of ethanol responding. They also reported that MPEP would blunt the deprivation-induced increased response for ethanol. In contrast, MPEP was reported to reduce lever pressing for intravenous self-administration of nicotine and cocaine, but did not alter the ability of these drugs to lower thresholds for intracranial self-stimulation. Clearly, more research is needed with both MPEP and other mGlu5 receptor antagonists in order to understand the behavioural impact of antagonists at this receptor.

The present results are in harmony with previous results on NMDA glutamate receptor antagonists. Drugs that prevent binding of agonists at each of the three different sites on the NMDA receptor to reduce its activation by glutamate all caused the selection and the consumption of ethanol solutions to decline (McMillen et al., 2004). Both the NMDA receptor and the mGlu5 receptor, through very different mechanisms, lead to increased intracellular concentrations of Ca\(^{2+}\). Both receptors may be located on neurons that are also postsynaptic to the limbic dopaminergic projection (Romano et al., 1995; see reviews by Baker et al., 2003; Witkin et al., 2003). Whether or not these interactions are at the dopaminergic neurons or at the cells post-synaptic to the dopaminergic projections is not clear. It is known that MPEP, in doses from 1.0 to 9.0 mg/kg intraperitoneal, will reduce nicotine and cocaine self-administration by the rat (Paterson et al., 2003; Kenny et al., 2003), but will not prevent the enhancement of intracranial self-stimulation by these drugs (Kenny et al., 2003). However, these differential results do not clarify whether or not MPEP is acting on the pre-synaptic dopamine neuron or the post-synaptic neurons of the motive circuitry.

The numerous glutamate receptors and acceptor sites that affect their function allow for many different pharmacological interactions. The development of drugs that target specific metabotropic receptors may allow for drugs with significant therapeutic benefit without the strong effects of most antagonists of the NMDA and AMPA receptors (Spooren et al., 2001). It is apparent that diminished glutamatergic activity at the receptors linked to increased intracellular calcium can decrease the consumption of ethanol. As MPEP interferes with the self-administration of other drugs of abuse, it is possible that either this drug or another mGlu5 receptor antagonist may be of benefit as an adjunct to the psychotherapy of alcohol and substance abuse.

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REFERENCES


