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$mGlu_5$ receptors are involved in the discriminative stimulus effects of self-administered ethanol in rats

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Abstract

Recent work has identified a role for metabotropic glutamate receptor subtype 5 ($mGlu_5$) in the discriminative stimulus properties of investigator-administered ethanol. The purpose of this study was to determine if $mGlu_5$ receptors modulate the discriminative stimulus properties of self-administered ethanol. Results show that the $mGlu_5$ receptor antagonist 6-Methyl-2-(phenylethynyl) pyridine (MPEP; 10 mg/kg) inhibited the discriminative stimulus properties of consumed ethanol during a self-administration test session. Further, 10 mg/kg MPEP increased and 1 mg/kg MPEP decreased the amount of self-administered ethanol required to produce full substitution. These results indicate that $mGlu_5$ receptors are involved in the expression of the discriminative stimulus properties of self-administered ethanol.

Keywords

alcohol; drug discrimination; discriminative stimulus; ethanol intake; metabotropic glutamate receptors; self-administration

1. Introduction

Drugs of abuse produce distinct private (i.e., subjective) stimulus effects. In both humans and animals, the subjective effects of alcohol and other drugs of abuse can serve as discriminative stimuli such that the subject uses these interoceptive cues to distinguish between drug and vehicle administration. The distinctive stimulus effects produced by drugs of abuse are important factors in drug taking and relapse behaviors. Recently, metabotropic glutamate receptor subtype 5 ($mGlu_5$) was found to play a role in the discriminative stimulus properties of ethanol (Besheer and Hodge, 2005), as $mGlu_5$ receptor antagonism inhibited the stimulus properties of investigator-administered ethanol (1 and 2 g/kg).

There is growing interest in the role of $mGlu_5$ receptors in drug taking behaviors. Antagonism of $mGlu_5$ receptors has been shown to attenuate self-administration of several drugs of abuse, including ethanol (Bäckström et al., 2004; Cowen et al., 2005; Hodge et al., 2006; Lominac et al., 2006; Schroeder et al., 2005). Given that $mGlu_5$ receptor antagonism appears to reduce the subjective (discriminative stimulus) properties of ethanol (Besheer and Hodge, 2005), this may contribute to reductions in ethanol self-administration.

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To address this question, the present study was designed to assess the role of $mGlu_5$ receptors in the discriminative stimulus properties of self-administered ethanol (Hodge et al., 2001; Shelton and Macenski, 1998). This procedure, previously reported by (Hodge et al., 2001), involves training animals to discriminate the stimulus properties of investigator-administered ethanol from water using standard two-lever drug discrimination procedures. Briefly, after ethanol administration, responses on a designated lever (e.g., right) produce access to sucrose reinforcement (10% w/v), and after water administration responses on the other lever (e.g., left) produce access to sucrose reinforcement. On self-administration test days, water is administered to the animals and responses on both levers are reinforced. However, ethanol (10% v/v) is added to sucrose reinforcement, allowing for assessment of the discriminative stimulus properties of self-administered ethanol. A shift in responding from the water- to the ethanol-appropriate lever indicates that the subjective effects of the consumed ethanol are detected by the animal. Thus, we are able to evaluate whether a pretreatment compound alters the discriminative stimulus properties of the consumed ethanol. In the present study, rats were trained to discriminate the stimulus properties of ethanol (1 g/kg) from water and the $mGlu_5$ receptor antagonist MPEP was evaluated for its effect on the discriminative stimulus properties of self-administered ethanol.

2. Method

2.1. Animals

Male Long Evans rats (Harlan, Indianapolis, IN; n=13) were individually housed with water available continuously. Body weights were maintained at approximately 325 g. The experiments were conducted during the light portion of the 12-h light/dark cycle. All procedures were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, National Academy Press, 1996) and institutional guidelines.

2.2. Procedure

The behavioral chambers and ethanol discrimination training procedures are described in detail elsewhere (Besheer and Hodge, 2005). Briefly, training sessions were conducted 5 days per week (M-F). Each day, ethanol (1 g/kg) or water was administered by intragastric (i.g.) gavage, and rats were immediately placed into the chambers. After 10 min a house light was illuminated and both levers were introduced into the chamber signaling the beginning of the 15-min session. Following ethanol administration, completion of 10 responses (FR10) on the ethanol-appropriate lever resulted in the presentation of the 10% (w/v) sucrose solution. Similarly, following water administration, completion of 10 responses on the water-appropriate lever resulted in sucrose delivery. During both ethanol and water sessions, responses on the inappropriate lever were recorded but produced no programmed consequences. Testing began once the percentage of ethanol- and water-appropriate responses emitted prior to the first reinforcer, and during the entire session equaled or exceeded 80% for 8 out of 10 consecutive training sessions.

Investigator-Administered Ethanol Substitution Testing—This test session was identical to the training sessions except that completion of FR10 on either lever resulted in presentation of the sucrose reinforcer and the levers were extended for 2 min once the house light was illuminated. A cumulative dosing procedure was used to determine an ethanol substitution curve (0.1, 0.3, 1.0, 1.7 g/kg ethanol, i.g.) as previously described (Hodge et al., 2001).

Self-administered Ethanol Substitution Testing—Rats were administered water (i.g.) and then tested during separate 30-min sessions with ethanol (0 or 10% v/v) added to the sucrose

reinforcement to characterize the stimulus effects of self-administered ethanol. During these sessions, behavior was free to vary between the two levers since completion of FR10 on either lever produced access to the ethanol/sucrose solution.

Effect of $mGlu_5$ receptor Antagonism on the Stimulus Properties of Self-Administered Ethanol—Rats were administered MPEP (0, 1, 10 mg/kg i.p.) 10 min before water (i.g.) and were placed in the chambers. The first and second test sessions for all rats were saline pretreatment before a sucrose only and a sweetened ethanol test, respectively. Following these initial tests, rats were administered MPEP and tested with sucrose-only, or sweetened ethanol in separate sessions. These test sessions were interspersed with training sessions only if performance during the previous 5 training sessions met the accuracy criteria. If the criteria were not met, training continued until response accuracy was 80% or greater for 5 consecutive days. Order of exposure to each MPEP dose and reinforcer condition was randomized. MPEP (Sigma-Aldrich, St. Louis, MO) was dissolved in saline, and injected i.p. at a volume of 1 ml/kg.

2.3. Data Analysis

For the ethanol substitution test, response accuracy was expressed as the percentage of ethanol-appropriate lever presses upon delivery of the first reinforcer and response rate (responses/min) was analyzed for the entire session. Repeated measures analysis of variance (RM ANOVA) was used to analyze response rate data for the ethanol substitution test, and percent ethanol-appropriate responses and cumulative sucrose or ethanol intake for the self-administration test sessions. Ethanol (g/kg) and sucrose (ml) intake was estimated from the number of delivered reinforcers. Complete substitution for the ethanol training dose (1 g/kg) was defined as >80% responding on the ethanol-appropriate lever. Tukey tests were used for all post hoc analyses.

3. Results

3.1. Stimulus Effects of Investigator-Administered Ethanol

The discrimination training procedures established reliable stimulus control as the percentage of ethanol-appropriate responses increased as a function of cumulative ethanol test dose (data not shown). Both the 1 and 1.7 g/kg ethanol doses fully substituted for the training dose (1 g/kg), with an ED50 value of 0.67 g/kg (± 0.08 S.E.M.). Response rate was reduced by increasing ethanol doses [$F(3,36)=10.54$, $P<0.001$], with a significant rate reduction at 1.7 g/kg compared to 0.1 g/kg ethanol, $P<0.001$.

3.2. Stimulus Effects of Self-Administered Ethanol

Sucrose-Only—During sucrose-only test sessions, ethanol-appropriate responses remained low throughout the session (Fig. 1A). There was a significant main effect of time interval [$F(5,120)=12.65$, $P<0.001$], and a significant interaction, [$F(10,120)=2.53$, $P=0.008$], with greater ethanol-appropriate responses after saline at 25 min of the session relative to 1 and 10 mg/kg MPEP, $Ps<0.05$. There was no effect of MPEP treatment. While this pattern of results shows an increase in ethanol-appropriate responses, these responses remained below 40% indicating the absence of ethanol substitution. It is unclear why this shift in responding occurred since it was not observed in a previous study using the same method (Hodge et al., 2001).

Cumulative sucrose consumed throughout the sessions is illustrated in Fig. 1B. There was a significant main effect of MPEP treatment, [$F(2,120)=11.09$, $P<0.001$], time interval [$F(5,120)=285.53$, $P<0.001$], and a significant interaction [$F(10,120)=4.81$, $P<0.001$]. After the first 5 min of the session, MPEP (10 mg/kg) reduced sucrose consumption at every time interval as compared to saline, $Ps<0.001$. In parallel, a significant reduction in total session response rate

was observed [F(2,24)=7.03, P=0.004], with a significant reduction after 10 mg/kg MPEP as compared to saline, P=0.003 (data not shown). Accordingly, this 10 mg/kg MPEP-induced decrease in response rate contributed to the decrease in overall sucrose consumption.

Sweetened Ethanol—Addition of ethanol to the sucrose solution resulted in an increase in ethanol-appropriate responding. Full substitution for the ethanol training dose was observed by min 20 and 15 of the test session after treatment with saline and 1 mg/kg MPEP, respectively (Fig. 1C). However, 10 mg/kg MPEP delayed full substitution for ethanol until min 30. This was confirmed by the significant main effect of drug treatment [F(2,119)=4.38, P=0.02], time interval [F(5,119)=79.73, P<0.001], and significant interaction [F(10,119)=3.46, P<0.001]. At 15 min and 20 min into the test session, 10 mg/kg MPEP significantly reduced ethanol-appropriate responses relative to saline, Ps<0.008.

Cumulative ethanol intake (g/kg) was not altered by MPEP pretreatment (Fig. 1D). Ethanol intake increased throughout the session [F(5,120)=338.41, P<0.001]. The significant interaction [F(10,120)=7.49, P<0.001] showed that ethanol intake was significantly greater than the first 5 min by 10 min into the test session and throughout the remainder of the session after treatment with saline, 1 and 10 mg/kg MPEP, Ps<0.001; however, ethanol intake after MPEP pretreatment did not differ from saline at any time point. Indeed, MPEP did not significantly alter response rate (data not shown).

Ethanol intake (g/kg) at the time of full substitution after saline (20 min), 1 mg/kg MPEP (15 min), and 10 mg/kg MPEP (30 min) is shown in Fig. 1E. A RM ANOVA showed a significant effect of MPEP dose [F(2,24)=33.982, P<0.001], indicating that after 1 mg/kg MPEP less ethanol intake was needed to produce the ethanol-like stimulus properties of the 1 g/kg ethanol training dose as compared to saline, P<0.001. In contrast, after 10 mg/kg MPEP greater ethanol intake was needed to produce the discriminative stimulus properties of 1 g/kg ethanol training dose as compared to saline, P<0.001.

4. Discussion

Results of the present study show that the discriminative stimulus effects of self-administered ethanol substituted for investigator-administered ethanol (1 g/kg, i.g.) which is consistent with previous work (Hodge et al., 2001). The $mGlu_5$ receptor antagonist MPEP reduced the subjective properties of the consumed ethanol. That is, at the time point when self-administered ethanol fully substituted for the training dose (1 g/kg) in saline-treated animals, 10 mg/kg MPEP-treated animals did not show full substitution even though the same amount of ethanol had been consumed. Accordingly, MPEP (10 mg/kg) significantly increased the amount of self-administered ethanol required to produce full substitution for the training dose. Alternatively, a lower dose of MPEP (1 mg/kg) decreased the amount of self-administered ethanol required to produce full substitution. Overall, these findings indicate that the stimulus effects of self-administered ethanol are regulated by $mGlu_5$ receptors.

A main finding of the present study is that pretreatment with MPEP (10 mg/kg) delayed the time of full substitution for the ethanol training dose (1 g/kg). Accordingly, this 10 mg/kg MPEP-induced delay in full substitution corresponded to significantly greater ethanol intake as compared to the amount of consumed ethanol required for the expression of full substitution after saline treatment. Inhibition of the discriminative stimulus effects of self-administered ethanol by MPEP is consistent with previous results showing that the $mGlu_5$ receptor antagonist inhibited the discriminative stimulus properties of investigator-administered ethanol in rats trained to discriminate ethanol (1 g/kg) from water (Besheer and Hodge, 2005).

A possible explanation for the MPEP-induced decrease in ethanol-appropriate responses during the sweetened-ethanol test sessions is that MPEP disrupted memory processes (Simonyi et al., 2005). Memory impairment would result in the inability to distinguish between the ethanol- and water-appropriate levers. Behaviorally this would be exhibited by non-specific lever pressing (i.e., 50% ethanol-appropriate responding) throughout the test session. This pattern of responding did not occur during the sucrose-only test sessions as MPEP-treated rats responded primarily on the water-appropriate lever consistent with saline treatment. Non-specific responding also did not occur during the sweetened-ethanol test sessions, as MPEP-treated rats showed early session water-appropriate responses and late session ethanol-appropriate responses. Thus, MPEP treatment did not result in non-specific lever pressing behavior making a memory impairment explanation less tenable.

Another plausible mechanism by which MPEP may have reduced the subjective properties of the consumed ethanol is by causing a direct reduction in the self-administered dosage of ethanol, which would result in weaker or less detectable discriminative stimulus or subjective effects. However, at min 20 when 10 mg/kg MPEP prevented full substitution for ethanol, the same amount of ethanol had been consumed in all the groups. Further, total session response rate, which is directly correlated with the number of reinforcers delivered, was not affected by MPEP pretreatment. Given that MPEP has been shown to reduce ethanol self-administration (see Introduction), it is unclear why MPEP did not reduce ethanol intake in the present study. One important factor to consider is that the animals in the present work were not trained to self-administer ethanol, but rather were trained to respond for sucrose. Second, in contrast to the self-administration studies, these animals were body weight restricted leading to high response rates. For example, during the sweetened ethanol sessions, the animals had consumed approximately 1.5 g/kg by the first 10 min of the session (compare to the rat self-administration studies with ethanol intake ranges from 0.5 – 0.7 g/kg in 30 min and 1 h sessions - Bäckström et al., 2004; Schroeder et al., 2005). Third, in the self-administration studies the ethanol is not sweetened as in the present work. Taken together, these factors may contribute to the lack of an MPEP-induced reduction in ethanol intake, a point critical to the interpretation of the present findings. That is, the $mGlu_5$ receptor antagonist altered the discriminative stimulus properties of self-administered ethanol in the absence of changes in ethanol intake, which suggests a direct effect on discrimination.

Another finding of the present work is that the low dose of MPEP (1 mg/kg) enhanced the stimulus effects of self-administered ethanol. After treatment with 1 mg/kg MPEP, animals consumed significantly less ethanol at the time of full substitution as compared to ethanol intake at the time of full substitution after saline pretreatment. This result suggests that the low MPEP dose interacted with ethanol making the consumed ethanol dose more like the ethanol training dose (1 mg/kg). In contrast, interaction between the high MPEP dose (10 mg/kg) and ethanol made the consumed ethanol less like the training dose, thus the significant delay in full substitution. Together these results show a dose-dependent pharmacological interaction between $mGlu_5$ receptors and ethanol.

Evidence for a physiological interaction between ethanol and $mGlu_5$ receptors is supported by work showing ethanol-induced inhibition of $mGlu_5$ receptor function (Minami et al., 1998). Further, postsynaptic Group I mGluRs play an important role in fine-tuning iGluR synapses (Kitano et al., 2002). There may also be reciprocal interactions between Group I mGluRs and NMDA receptors because low concentrations of NMDA enhances group I mGluR-mediated responses (Alagarsamy et al., 1999). These functional interactions are of relevance to the present work given that ethanol inhibits NMDA receptors and NMDA antagonists produce ethanol-like stimulus properties (see (Kostowski and Bienkowski, 1999). Thus, a biphasic effect of $mGlu_5$ receptor antagonism may not only be a result of an interaction with ethanol, but may also involve NMDA receptors. Future work examining a wider MPEP dose range on

self- and investigator-administered ethanol, will allow us to determine whether the biphasic MPEP effect in the present study is actually a leftward shift in the self-administered ethanol response curve.

During sucrose-only test sessions, $mGlu_5$ receptor antagonism (10 mg/kg MPEP) reduced cumulative sucrose intake and response rate. An explanation for this reduction is that 10 mg/kg MPEP induced a motor impairment. However, this dose of MPEP did not reduce response rate during the sweetened ethanol test sessions and did not reduce responding for sucrose reinforcement in our previous work (Besheer and Hodge, 2005). This latter point may not completely exclude a motor impairment given that the test session in that work was 2 min in duration versus 30 min in the present study. Indeed, in the present work, decreased sucrose intake did not emerge until after 5 min into the session. Another plausible explanation for a decrease in sucrose consumption is that MPEP reduced motivation for sucrose reinforcement. Indeed, $mGlu_5$ receptor antagonism has been shown to reduce the reinforcing function of food as measured by MPEP-induced decreases in break points (Paterson and Markou, 2005).

In conclusion, results from the present study show that $mGlu_5$ receptors modulate the discriminative stimulus properties of self-administered ethanol. This finding is of importance given that preclinical evidence supports a role for $mGlu_5$ receptors in ethanol self-administration and relapse (Bäckström et al., 2004; Cowen et al., 2005; Hodge et al., 2006; Schroeder et al., 2005). Further, the results from the present work build on our previous finding that the $mGlu_5$ receptor antagonist MPEP inhibited the discriminative stimulus properties of investigator-administered ethanol (Besheer and Hodge, 2005). Given the importance of a drug's stimulus effects in priming and maintaining self-administration, these data define a specific behavioral mechanism by which $mGlu_5$ receptor antagonism might decrease ethanol self-administration.

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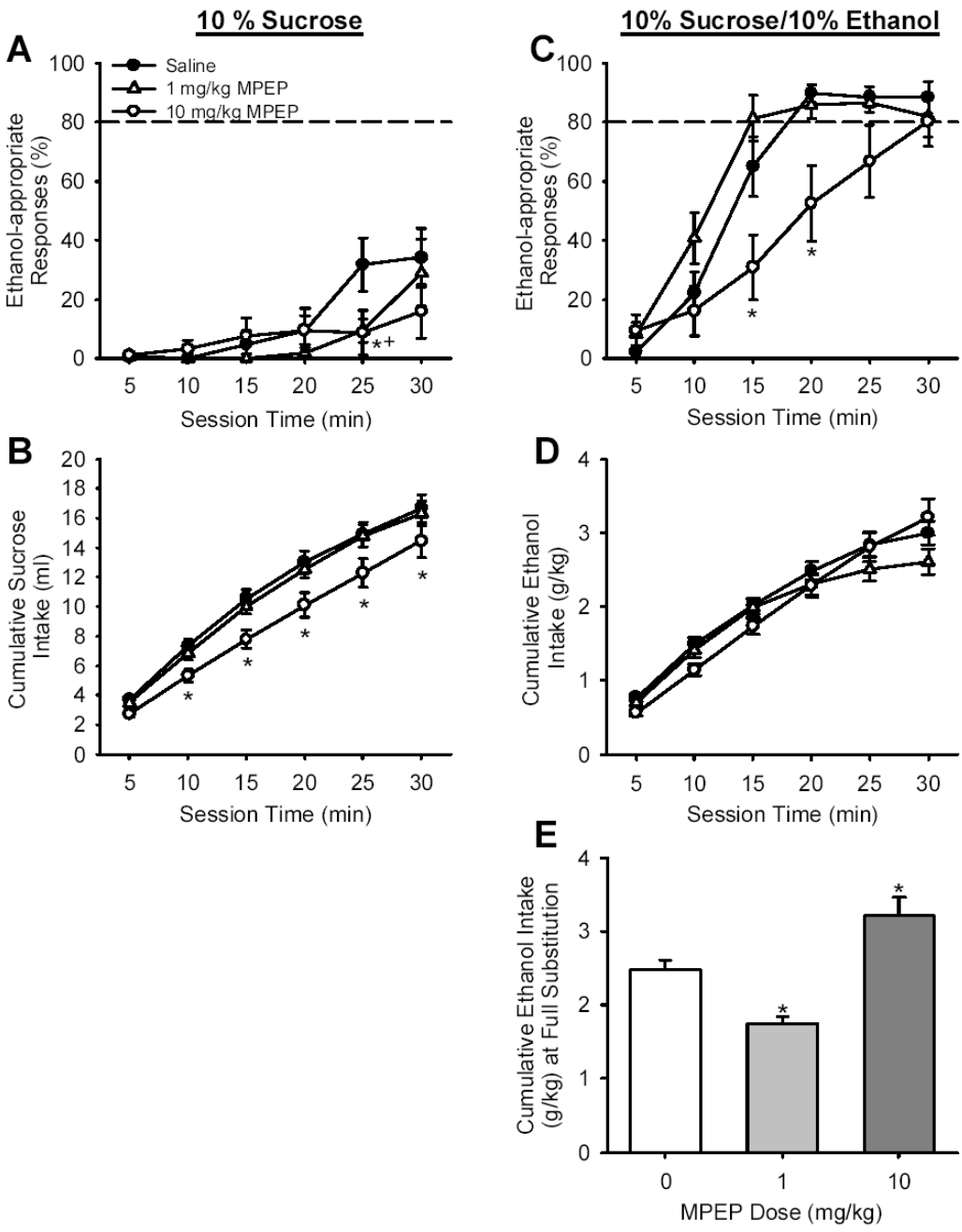

Figure 1. Sucrose-only test sessions (10% sucrose)

Mean (± S.E.M.) percentage of ethanol-appropriate responses (Panel A), and cumulative sucrose consumed (mean ml ± S.E.M.; Panel B) at each MPEP dose. Sweetened-ethanol test sessions (10% Sucrose/10% Ethanol): Mean (± S.E.M.) percentage of ethanol-appropriate responses (Panel C), cumulative ethanol intake (mean g/kg ± S.E.M.; Panel D) and cumulative ethanol intake (mean g/kg ± S.E.M.) at the time of full substitution for the ethanol training dose (1 g/kg; Panel E) at each MPEP dose. + $P<0.05$ saline vs. 1 mg/kg MPEP; * $P<0.05$ saline vs. 10 mg/kg MPEP. *Horizontal dashed line* indicates threshold for ethanol substitution (i.e., >80%).