The Metabotropic Glutamate 5 Receptor Antagonist 3-[(2-Methyl-1,3-thiazol-4-yl)ethynyl]-pyridine Reduces Ethanol Self-Administration in Multiple Strains of Alcohol-Preferences Rats and Regulates Olfactory Glutamatergic Systems

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Received June 2, 2005; accepted July 12, 2005

ABSTRACT

The metabotropic glutamate 5 receptor (mGlu5) receptor has been implicated as having a role in pain modulation, anxiety, and depression, as well as drug-seeking behavior. In the present study, we examined the effect of the selective mGlu5 receptor antagonist 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) on operant ethanol self-administration by two strains of rats, the Fawn-Hooded (FH) rat and the inbred alcohol-prefering (iP) rat. MTEP (2 mg/kg i.p.) caused a significant reduction in responding for ethanol by both strains of rats; however, in the iP rats, MTEP also induced apparent sedation at this dose, although still reduced alcohol responding at lower doses. Chronic MTEP (2 mg/kg/day) caused a significant reduction in ethanol consumption by FH rats in a two-bottle preference test; however, chronic treatment with this dose had no effect on anxiety-like behavior or depressive-like behavior in FH rats, suggesting the dose used was subthreshold for anxiolytic or antidepressive-like effects. Finally, repeated dosing with MTEP (2 mg/kg i.p.) caused significant reductions in expression of the mRNA encoding the NR1 subunit of the N-methyl-D-aspartate receptor and the GluR2 subunit of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptor in the cingulate cortex. A significant decrease in NR1 expression also occurred in the piriform cortex. Chronic MTEP also caused a significant decrease in mGlu5 gene expression and a significant increase in dopamine transporter and dopamine D2-like receptor binding within the olfactory tubercle. Collectively, these data suggest that MTEP can reduce alcohol-seeking behavior in different rodent models of alcoholism, and this effect is associated with regulation of cortical glutamate systems, particularly those in olfactory-related regions.

These studies were supported by the National Health and Medical Research Council, Australia (program Grant 236805), of which A.J.L. is a Senior Research Fellow and M.S.C. is a C.J. Martin Fellow.

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*Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.105.090449.

ABBREVIATIONS: mGlu5, metabotropic glutamate 5 receptor; CHPG, (R,S)-2-chloro-5-hydroxyphenylglycine; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; MTEP, 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine; LTP, long-term potentiation; FH, Fawn-Hooded rat; iP, inbred alcohol-prefering rat; DMISO, dimethyl sulfoxide; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; NMDA, N-methyl-D-aspartate; [125I]RTI-55, 2β-carboxethyl-3β-(4-iodophenyl)tropane; GBR12935, 1-(2-diphenylmethoxyethyl)-4-(3-phenylpropyl)piperazine; [125I]SCH23982, (1R)(+)-1-phenyl-3-methyl-7-[(2S,3S,5R)-5-hydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine; SKF77434, (±)-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine; [125I]NCQ298, S(−)-3-iodo-N-(1′-ethyl-2′-pyrrolydiny]methyl-2-hydroxy-5,6-dimethoxybenzamide; DAT, dopamine transporter; NCQ634, S-N-[1′-ethyl-2′-pyrrolydiny]methyl-2-hydroxy-5,6-dimethoxybenzamide.
hydroxyphenylglycine (CHPG) (Doherty et al., 1997) and antagonists, in particular 2-methyl-6-(phenylethynyl)-pyridine (MPEP) (Gasparini et al., 1999) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (MTEP) (Anderson et al., 2002; Cosford et al., 2003). Thus, mGlur5 receptors appear to have a role in modulating pain (Walker et al., 2001a,b; Varty et al., 2005), stress and anxiety-like responses (Schulz et al., 2001; Tatarczynska et al., 2001; Brodkin et al., 2002a,b; Spooren et al., 2002; Busse et al., 2004; Klokodinska et al., 2004), and depressive-like responses (Tatarczynska et al., 2001; Pilc et al., 2002). Furthermore, mGlur5 receptors also have a role in certain forms of long-term potentiation (LTP) and spatial memory formation (La et al., 1997; Balschun and Wetzel, 2002; Naie and Manahan-Vaughan, 2004).

mGlur5 receptors also appear to modulate the reinforcing aspects of a number of drugs of abuse. Mine homozygous null for the mGlur5 receptor do not self-administer cocaine (Chi-amulera et al., 2001), nor do rats or mice administered MPEP self-administer cocaine or nicotine (Paterson et al., 2003; Tessari et al., 2004; Kenny et al., 2005; Lee et al., 2005). Morphine and cocaine-induced place preference was inhibited by MPEP (McGeehan and Olive, 2003; Aoki et al., 2004). Although MPEP did not substitute for ethanol in a discriminative stimulus test (Besheer and Hodge, 2005), MPEP did prevent reinstatement of ethanol seeking behavior by olfactory cues (Bäckstrom et al., 2004). Thus, glutamatergic neurotransmission mediated via the mGlur5 receptor is strongly implicated in the reinforcing properties of a number of drugs of abuse, although the anatomical loci of this role are unclear. Although these studies were performed using MPEP, the more recently developed mGlur5 antagonist MTEP shows greater selectivity for the mGlur5 receptor and greater bioavailability (Anderson et al., 2002; Cosford et al., 2003).

The aims of the present study therefore were to examine whether MTEP could regulate ethanol self-administration and consumption and, if so, whether this related to altered affect. Given the ability of MPEP to modulate anxiety- and depressive-like phenotypes, we first examined the effect of MTEP on ethanol consumption by alcohol-prefering iP rats (Liang et al., 2003), which display an anxiety-like phenotype, and alcohol-prefering FH/WjD rats, which represent a useful animal model of comorbid depressive-like and alcohol-seeking behavior (Rezvani et al., 2002). Thus, this strain of rat consumes large quantities of ethanol under voluntary conditions (Rezvani et al., 1991; Cowen et al., 1999) and demonstrates elevated serum corticosterone levels and relatively high immobility in the forced swim test (Overstreet and Rezvani, 1996; Overstreet et al., 1999). We further examined the ability of MTEP, at a dose effective in decreasing ethanol consumption, to affect anxiety-like and depressive-like behavior. Finally, we examined the effect of chronic MTEP treatment on markers of the glutamatergic and dopaminergic systems, which may play a role in the mGlur5-mediated modulation of drug-seeking behavior.

Methods and Materials

All experiments were performed in accordance with the Prevention of Cruelty to Animals Act, 1986 under the guidelines of the National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia.

Animals. Male FH rats were bred from stock parents obtained from Dr. Amir Rezvani (while at the University of the North Carolina School of Medicine, Chapel Hill, NC). Inbred alcohol-prefering (iP) rats were obtained from the breeding colony at the Howard Florey Institute, University of Melbourne. Parental stock had previously been obtained from Professor T. K. Li (while at Indiana University, Indianapolis, IN).

Operant Ethanol Self-Administration. The effect of MTEP in an acute behavioral paradigm, operant self-administration of orally available ethanol was examined using alcohol-prefering FH rats (n = 11; 357 ± 20 g at 4 months) and iP rats (n = 12; 478 ± 6 g at 4 months). Operant chambers supplied by MED Associates (St. Albans, VT) were employed. Each chamber was housed individually in sound attenuation cubicles, featuring a fan to provide airflow and mask external noise, and the chambers were connected to a computer running Med-PC IV software (MED Associates) to record activity. Within the chambers, a house light provided soft illumination during operant sessions. On either side of the operant chambers, a retractable lever (exerted during operant sessions) was centrally placed below a stimulus light and adjacent to a fluid receptacle. Each receptacle was fed by a solenoid-controlled liquid dispenser with a 20-ml reservoir.

Training of the rats followed a standard sucrose fade protocol as described previously (Cowen et al., 2005). The ethanol and water response levers were alternated from left to right between sessions to avoid place-preference, with a small drop of the current solution (either water or ethanol/sucrose) left in each receptacle to indicate the current orientation of the solutions. Ultimately, rats were responding for 10% ethanol solution under a fixed ratio requirement of 3. For each session, total ethanol and water responses were recorded, and the difference in fluid in the ethanol reservoir between the beginning and end of the session was recorded to ensure correct calibration of the delivery system.

Drug administration commenced once responding for ethanol was stable across sessions for most rats (<10% variation across sessions). For the FH rats, drug administration weeks were structured so that Mondays, Thursdays, and Fridays were no-injection days, vehicle (5% DMSO in 0.9% w/v saline i.p.) was injected Tuesdays (i.p.), and MTEP was administered on Wednesdays (i.p.). FH rats received MTEP (2 mg/kg i.p.) 20 min before the self-administration session on 2 consecutive weeks. For the iP rats, drug administration weeks were structured so that Mondays, Tuesdays, and Fridays were no-injection days, vehicle (5% DMSO in 0.9% w/v saline i.p.) was injected Wednesdays (i.p.), and MTEP was administered on Thursdays (i.p.). Alcohol-prefering iP rats received MTEP 20 min before operant self-administration sessions in the following sequence: 2 mg/kg (week 1), 1 mg/kg (week 2), and 0.5 mg/kg (week 3).

Locomotor Activity. The effect of MTEP on locomotor activity was examined in both the FH and iP rats that had previously self-administered ethanol under an operant paradigm. Rats were habituated to the locomotor cells (TruScan Photobeam Activity Monitors, 26 × 26 × 40 cm) for 1 h/day for 3 to 4 days. Rats were subsequently divided randomly to receive either MTEP (2 mg/kg i.p.) or vehicle (5% DMSO in 0.9% w/v saline i.p.) and were injected 20 min before being put in the cells. The following day, the treatments were reversed so that all rats received both vehicle and MTEP. Locomotor activity, in terms of total distance traveled and total movement time in the horizontal plane, vertical plane entries, and time in vertical planes, was determined automatically using the TruScan software.

Continual Access Ethanol Consumption/Behavioral Analysis. FH rats (8–9 weeks) were randomly divided into two groups (n = 8–12/group) to ultimately receive either drug or vehicle. All rats had unlimited access to both water with free access to standard chow and experienced a 12-h light/dark cycle from 7:00 AM to 7:00 PM. For daily fluid consumption monitoring, rats were individually housed with each cage equipped with two drink bottles, one filled with tap water, and the other with 5% v/v ethanol. Daily consumption rates of...
water and ethanol were measured for a period of 6 weeks before drug treatment began. Drink container positions were changed randomly to prevent the development of place preference. After stable drinking, rats were injected once daily with either MTEP (2 mg/kg i.p.) or vehicle (1 ml/kg i.p.), and consumption of ethanol and water was monitored daily.

Elevated Plus Maze. FH rats were injected once daily with either MTEP (2 mg/kg i.p.) or vehicle (1 ml/kg i.p.) for 10 days, during which time rats were moved into a dim-lit behavioral room to acclimate and experience the same 12-h light/dark cycle.

On the 11th day, rats were injected with either MTEP (2 mg/kg i.p.) or vehicle (1 ml/kg i.p.) and subsequently placed in the center of an elevated plus maze consisting of an X-shape, having two opposite arms that are open to the environment (open arms) and two that have side walls (closed arms) (Lodge and Lawrence, 2003). Within a period of 5 min, the number of entries and time spent in the closed and open arms were recorded. The time spent in the center was calculated as the total time minus the time spent in the closed and open arms. Four paws out of and into the center square defined an arm entry and exit, respectively. Any feces were removed, and the maze was cleaned after each trial.

Porsolt Forced Swim Test. On the 12th day of treatment, rats were tested in the Porsolt swim test as previously described (Hall et al., 1998) but were tested only once due to the chronic drug treatment regime. On the day of behavioral testing, rats were placed into a plastic cylinder from which there was no escape, containing water (22–23°C), at a depth of 15 cm for 10 min. Once this time elapsed, each rat was removed from the water, partially dried with a towel and placed in a plastic cage illuminated with a heat lamp. These trials were captured using a Panasonic CCTV camera with a 3.5- to 8-mm lens attached to a video recorder. Tapes were then scored according to three criteria: time to immobility, time mobile, and time immobile (calculated as total time, i.e., 10 min minus time mobile). Rats were killed the next morning by decapitation, and the brains rapidly removed and frozen over liquid nitrogen. The brains were then stored at −80°C until used for neurochemical analysis (in situ hybridization histochemistry and autoradiography).

**Neurochemical Analysis**

**In Situ Hybridization Histochemistry.** In general, a standard in situ hybridization histochemistry protocol was used (Cowan and Lawrence, 2001). Coronal sections (14 μm) of brain tissue were cut using a cryostat (Leica) and mounted onto poly-l-lysine-coated slides for in situ hybridization histochemistry. Brain sections were collected encompassing the rostral striatum (from Bregma 2.2 mm). The slides were stored at −80°C and thawed to room temperature when required for use. Tissue was fixed (4% paraformaldehyde in phosphate-buffered saline: 0.05 M NaH2PO4, 0.05 M Na2HPO4, and 0.9% w/v NaCl, pH 7.4, 5 min, 4°C), delipidated (100% chloroform, 10 min), and dehydrated through serial ethanol before use. In general, six tissue sections per rat from the region of interest were used to determine specific hybridization (typically 150 sections per experiment). Additional sections were randomly chosen to determine nonspecific signal for each probe. All antisense oligonucleotides were verified against the relevant sequences using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Antisense oligonucleotides complementary to the rat coding sequences were used to examine expression of the metabotropic glutamate receptor mGlur5 (5′-GGA CCA CAC TTC GTC ATC ATC ATC CAT TTT TAA TTC ACC TTC GCA CAC-3′), the GluR2 subunit of the AMPA receptor (5′-TTC ACT ATG TGT TCT CTC TTA TCC ATT CTC CTC AGT GTG-3′), and the NR1 subunit of the NMDA receptor (5′-TTC TCT CTC CTC CTC CTC CTC ACT GTC ATC CTC GCA TCT GAA TTC CGC AAA GGT ACT-3′). Oligonucleotides were diluted to a working stock of 0.3 pmol/μl per oligonucleotide, and aliquots (2 μl) were then labeled with [32P]dATP (2000 Ci/mmol; PerkinElmer Life and Analytical Sciences, Boston, MA) in the presence of terminal deoxynucleotidyl transferase (Roche Diagnostics, Mannheim, Germany). Unincorporated nucleotides were separated from the labeled probes using Sephadex G-25 medium spin columns. The labeling reactions yielded an activity of 180,000 to 500,000 cpm/μl. Note, to ensure valid comparison and obviate inter-assay variability, all tissue sections to be compared by subsequent densitometry and statistical analysis were processed simultaneously under the same procedure and conditions, utilizing the same specific activity probes.

The labeled oligonucleotide probes (1 pg/μl, 100 μl) were applied to adjacent sections of rat brain in a hybridization buffer containing 50% formamide, 4× saline sodium citrate, pH 7.0, and 10% dextran sulfate. Control hybridizations were carried out using a 100-fold molar excess of unlabeled (relative to labeled) oligonucleotide. For each oligonucleotide, hybridization signals were abolished when in situ hybridization histochemistry was carried out in the presence of a 100-fold molar excess of the same unlabeled oligonucleotide probe. Sections of Parafilm were placed over the tissue to prevent drying of the sections during hybridization. Slide-mounted sections were allowed to hybridize in sealed humidified chambers at 42°C overnight.

After hybridization, sections were washed in 1× saline sodium citrate for 1 h at 55°C, rinsed, and then dehydrated in serial ethanol and allowed to air-dry. When dry, slides were exposed to X-ray film (Kodak XAR-5; Eastman Kodak, Rochester, NY) with a set of standard [125I]microscales. Films were developed automatically using an Agfa CP1000 X-ray automatic developer and analyzed using the SCION imaging system (Scion Corporation, Frederick, MD) for densitometry. Data are expressed as disintegrations per minute per millimeter squared.

**Autoradiography.** In general, for each brain region analyzed per ligand, four slide-mounted sections from the region of interest from each rat (typically 100 sections) were used to determine specific binding, with an additional 10 sections chosen randomly to determine nonspecific binding. Tissue levels of the dopamine transporter were determined using a previously published protocol (McGregor et al., 2003). Sections were preincubated in buffer (0.1 M NaH2PO4/0.1 M sucrose, pH 7.4) for 30 min at room temperature. Sections were then incubated in buffer containing 50 pM [125I]RTI-55 (2200 Ci/ mmol; PerkinElmer Life and Analytical Sciences) and 100 nM fluoxetine (60 min, room temperature). GBR12935 (10 μM) was used to determine nonspecific binding. Sections were then washed in ice-cold buffer (1 × 1 min, 2 × 20 min), rinsed in ice-cold dH2O, and desiccated overnight. Slides were then exposed to Kodak X-omat AR film in the presence of standard [14C]microscales (American Radiolabeled Chemicals, St. Louis, MO) for 7 h.

Tissue levels of the dopamine D1 receptor were determined using a standard protocol (Djouma and Lawrence, 2002). Sections were incubated in buffer (50 nM Tris-HCl, 120 mM NaCl, 5 mM KCl, 2 mM CaCl2, and 1 mM MgCl2, 30 min, room temperature) with 50 nM ketanserin (to occlude binding to 5-hydroxytryptamine2A receptors) and 50 pM [125I]SCH23982 (2200 Ci/mmol; PerkinElmer Life and Analytical Sciences). SKF77434 (10 μM) was used to determine nonspecific binding. Sections were washed in ice-cold buffer (2 × 5 min) and rinsed in ice-cold dH2O. After overnight desiccation, slides were exposed to film for 24 h.

Tissue levels of the dopamine D2-like receptor were determined using a standard protocol (Djouma and Lawrence, 2002).

[125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method. [125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method. [125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method. [125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method. [125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method. [125I]NCQ298 was freshly prepared from the des-iodo derivative NCQ634 using Na125I (2200 Ci/mmol; GE Healthcare, Ltd., Little Chalfont, Buckinghamshire, UK) via the chloramine-T iodination method.
using the SCION imaging system for densitometry by comparing the optical densities resulting from the radioactive ligands with that of corresponding standard microscales under constant illumination. Data are expressed as disintegrations per minute per millimeter squared.

**Statistics.** A significance level of $p = 0.05$ was used throughout. Comparisons were by analysis of variance or by repeated measures analysis of variance where appropriate. For the neurochemical analysis, a Bonferroni correction for multiple comparisons was used for a given ligand or in situ hybridization probe.

**Results**

We first sought to establish whether MTEP could impact upon appetitive responding for ethanol in different models of alcohol-prefering rats, namely FH and iP rats. Under a fixed ratio requirement of 3 schedule, MTEP (2 mg/kg i.p.) caused a significant reduction in responding for ethanol by FH rats compared with prior no-injection and vehicle treatment days [$F(2,22) = 15.442, p < 0.001; \text{Fig. 1A}$], with no significant effect on water responding [$F(2,22) = 0.0479, p = 0.953; \text{Fig. 1C}$]. Time course analysis indicated that MTEP caused a significant reduction in responding for ethanol in the first 10 min of the operant session compared with prior vehicle treatment and no injection days (treatment $\times$ time interaction, $F(14,154) = 7.022, p < 0.001; \text{Fig. 1B}$). MTEP also caused a dose-dependent reduction in responding for ethanol by alcohol-prefering iP rats [treatment, $F(2,20) = 22.100, p < 0.001$; dose, $F(2,22) = 16.455, p < 0.001, p < 0.001; \text{Fig. 2A}$], which was significant at 1 and 2 mg/kg. Time course analysis indicated that MTEP caused a significant reduction in responding for ethanol in the first 10 to 15 min of the operant session, compared with prior vehicle treatment and no injection days [treatment $\times$ time interaction, 1 mg/kg, $F(14,154) = 8.052, p < 0.001, \text{Fig. 2B}$; 2 mg/kg, $F(14,154) = 8.196, p < 0.001, \text{Fig. 2C}$]. However, although the reduction in responding for ethanol at 1 mg/kg MTEP was associated with a significant increase in water responding [dose, $F(2,22) = 16.455, p < 0.001$; treatment $\times$ dose interaction, $F(4,40) = 6.079, p < 0.001; \text{Fig. 2D}$], the higher dose of 2 mg/kg MTEP caused a significant reduction in water responses by iP rats [Fig. 2D], suggestive of a sedative effect.

As a consequence, we assessed the effect of 2 mg/kg MTEP on locomotor activity in FH and iP rats using automated measurement of activity. MTEP (2 mg/kg i.p.) did not decrease locomotor activity in FH rats relative to vehicle-treated animals but rather increased locomotor activity to prehabitation levels [distance moved, $F(22,220) = 2.448, p < 0.001; \text{Fig. 3A}$]. However, in iP rats, consistent with the reduction in water responding noted at this dose, 2 mg/kg MTEP caused a significant reduction in distance moved and movement time in the first 15 min after the rats were placed in the locomotor cells, relative to the same rats treated with vehicle [distance moved, $F(11,121) = 8.964, p < 0.001; \text{Fig. 3B}$]. Critically, this is the time period of maximal operant responding (refer to Fig. 1D). Therefore, MTEP reduces operant responding for a 10% ethanol solution in both FH and iP rats; however, the window of efficacy may be narrower in the iP rats because of apparent sedation at 2 mg/kg. Accordingly, we chose to concentrate the remaining studies on FH rats and next determined the impact of MTEP treatment on ethanol consumption during a continual access two-bottle free-choice paradigm.

**Fig. 1.** Effect of MTEP (2 mg/kg i.p.) on operant responding for orally available ethanol (A and B) and water (C) by Fawn-Hooded rats. Data are expressed as the mean ± S.E.M. A and C, session totals. B, time course of the effect of MTEP. A, treatment with MTEP caused a significant reduction in total session responding for ethanol by Fawn-Hooded (A) rats, compared with prior no-injection (Nol) and vehicle (5% DMSO) treatment days (*, $p < 0.05$). B, * time course analysis indicated a significant effect of MTEP (closed circles) on operant responding for ethanol in the first 10 min of the operant session. C, treatment with MTEP had no effect on responding for water by Fawn-Hooded rats.

Before treatment with MTEP (2 mg/kg/day) or vehicle control, there was no significant difference between rats randomly assigned to receive either drug or vehicle in terms of body weight ($t_{18} = 0.505, p = 0.619$; data not shown), total fluid intake ($t_{18} = 0.474, p = 0.641; \text{Fig. 3D}$), or ethanol intake (grams per kilogram per day; $t_{18} = 1.845, p = 0.082; \text{Fig. 4A}$). However, there was a significant difference in preference for ethanol ($t_{18} = 2.731, p = 0.014; \text{Fig. 4B}$) as the water intake of the group to be treated with MTEP had no effect on responding for water by Fawn-Hooded rats.
significance compared with vehicle-treated rats [factor treatment, \(F(1,18) = 0.0223\)]. In contrast, treatment with MTEP caused a dose-dependent reduction in total session responding for ethanol by iP rats (*, significantly different from prior no-injection (NoI) and vehicle (5% DMSO) treatment (Veh) days at 1 (1 MTEP) and 2 (2 MTEP) mg/kg, \(p < 0.05\)]. B and C, time course analysis indicated a significant effect of MTEP (closed circles) in the first 10 to 15 min of the operant session at both 1 (B) and 2 (C) mg/kg compared with prior vehicle treatment (open triangles) and no-injection days (data not shown). D, treatment with MTEP caused a significant increase in responding for water at 1 mg/kg but a significant decrease at 2 mg/kg (*, \(p < 0.05\)).

Fig. 2. Effect of MTEP on operant responding for orally available ethanol (A–C) and water (D) by alcohol-preferring iP rats. Data are expressed as the mean ± S.E.M. A and D, session totals. B and C, time course of the effect of MTEP. For simplicity, an average of the three no-injection (NoI) days is shown (A and D). A, treatment with MTEP caused a dose-dependent reduction in total session responding for ethanol by iP rats (*, significantly different from prior no-injection (NoI) and vehicle (5% DMSO) treatment (Veh) days at 1 (1 MTEP) and 2 (2 MTEP) mg/kg, \(p < 0.05\)]. B and C, time course analysis indicated a significant effect of MTEP (closed circles) in the first 10 to 15 min of the operant session at both 1 (B) and 2 (C) mg/kg compared with prior vehicle treatment (open triangles) and no-injection days (data not shown). D, treatment with MTEP caused a significant increase in responding for water at 1 mg/kg but a significant decrease at 2 mg/kg (*, \(p < 0.05\)).

Fig. 3. Locomotor activity (distance moved/5-min time bin) of FH (A) and iP (B) rats 20 min after receiving MTEP (2 mg/kg i.p., closed circles) or vehicle (5% DMSO, open triangles). Data are expressed as the mean ± S.E.M. MTEP raised locomotor activity of Fawn-Hooded rats to prehabitation levels; in contrast, MTEP caused a significant reduction in locomotor activity by iP rats. *, significantly different from vehicle-treated (\(p < 0.05\)).
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0.0017; Fig. 5, A and D) and the piriform cortex (−19%, $t_{47} = 3.647, p = 0.0005$) and similarly a significant decrease in the expression of the GluR2 subunit of the AMPA receptor within the cingulate cortex (−14%, $t_{62} = 4.362, p < 0.0001$; Fig. 5B), compared with vehicle-treated rats. Treatment with MTEP also led to a significant decrease in mGlu5 receptor expression within the olfactory tubercle (−25%, $t_{61} = 2.737, p = 0.0081$; Fig. 5, C and F). It is noteworthy that these changes in glutamate receptor expression occurred predominantly in olfactory-associated nuclei and also within the cingulate cortex, which provides excitatory input to the basal ganglia (Wang and Pickel, 2000). Therefore, we next analyzed markers of the dopaminergic system and found that 12 days of treatment with MTEP led to a significant increase in DAT binding (+9%, $t_{74} = 2.691, p = 0.0088$; Fig. 6, A and D) and dopamine D2-like binding (+10%, $t_{62} = 2.685, p = 0.0098$; Fig. 6, C and F), compared with vehicle-treated rats. Critically, these alterations were only observed within the olfactory tubercle; there were no other significant effects of MTEP.

### Table 1

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<th>Measure/Treatment</th>
<th>Vehicle</th>
<th>MTEP</th>
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<td>Open arms Entries</td>
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<td>5.3 ± 0.8</td>
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<td>Duration (s)</td>
<td>77 ± 19</td>
<td>75 ± 12</td>
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<tr>
<td>Closed arms Entries</td>
<td>7.0 ± 1.2</td>
<td>7.6 ± 0.6</td>
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<tr>
<td>Duration (s)</td>
<td>126 ± 22</td>
<td>142 ± 13</td>
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### Table 2

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<th>Measure/Treatment</th>
<th>Vehicle</th>
<th>MTEP</th>
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<tr>
<td>Latency to immobility (s)</td>
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<td>34 ± 8</td>
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<tr>
<td>Duration immobile (s)</td>
<td>501 ± 10</td>
<td>496 ± 11</td>
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Fig. 4. Ethanol intake (A), ethanol preference (percent total fluid intake; B), water intake (C), and total fluid intake (D) by Fawn-Hooded rats before (days −4 to −1) and during (days 1–4) treatment with MTEP (2 mg/kg/day; closed circles) or vehicle (5% DMSO; open triangles). Data are expressed as the mean ± S.E.M. MTEP caused a significant reduction in ethanol consumption and total fluid intake compared with pretreatment levels (*, $p < 0.05$).
treatment on binding to the dopamine transporter or D₂-like dopamine receptors, nor was there any effect of MTEP treatment on dopamine D₁ receptor binding (Fig. 6, B and E).

**Discussion**

In the present study, MTEP caused a significant decrease in responding for ethanol by alcohol-preferring FH and iP rats. However, although MTEP increased locomotor activity by FH rats to prehabituation levels, MTEP caused a significant reduction in locomotor activity by iP rats, within the same time frame as the decrease in responding for ethanol by these rats and suggestive of sedation. Repeated dosing with MTEP also caused a significant decrease in continual-access ethanol consumption by FH rats. Repeated treatment with MTEP, at the same dose that caused a significant reduction in ethanol consumption, had no effect on measures of depressive-like and anxiety-like behavior in FH rats. However, repeated treatment with MTEP did cause a significant decrease in the expression of the NR1 subunit of the NMDA receptor and the expression of the GluR2 subunit of the AMPA receptor in the cingulate cortex and a significant decrease in NR1 expression in the piriform cortex. Repeated treatment with MTEP also caused a significant decrease in mGlu5 expression and a significant increase in DAT and dopamine D₂-like receptor binding within the olfactory tubercle. It is noteworthy that the olfactory tubercle and the cingulate and piriform cortices are all involved in the processing of olfactory information (Slotnick and Schoonover, 1992; Weismann et al., 2001).

MPEP, one of the first compounds discovered to have some selectivity for the mGlu5 receptor, has recently been shown to decrease ethanol self-administration (Schroeder et al., 2005) and cue-induced reinstatement of ethanol-seeking behavior (Bäckstrom et al., 2004) in a dose-dependent manner. MPEP has also been shown to decrease cocaine self-administration in mice (Chiamulera et al., 2001) and rats (Tessari et al., 2004; Kenny et al., 2005; Lee et al., 2005), cocaine-induced place preference (McGehee and Olive, 2003), nicotine self-administration (Paterson et al., 2003), and reinstatement of nicotine self-administration (Tessari et al., 2004). However, MPEP has been shown to have a number of off-target effects, notably antagonism of NMDA receptors (OLeary et al., 2000), inhibition of the noradrenaline transporter (Heidbreder et al., 2003), and positive allosteric modulation of the mGlu4 receptor (Mathiesen et al., 2003). Therefore, we have used the more recently discovered MTEP (Brodkin et al., 2002a; Anderson et al., 2003; Cosford et al., 2003), which has greater selectivity for the mGlu5 receptor. This is the first study of which we are aware to demonstrate a dose-dependent reduction of ethanol self-administration by
MTEP; furthermore, we have also demonstrated a small but significant decrease in ethanol consumption by MTEP in FH rats in a two-bottle preference test over several days. Although food intake was not measured during the two-bottle test, this effect of MTEP does not seem to be a general effect on consummatory behavior, because the body weight of both vehicle- and MTEP-treated rats increased over this period, with no significant difference between the groups.

Although MTEP was associated with an increase in locomotor activity by the FH rats, MTEP caused a decrease in locomotor activity in the iP rats, within the same time frame as the decrease in ethanol consumption by these rats. The data would seem to suggest that only a small window exists between therapeutic dose and doses that cause side effects in certain subpopulations. Decreases in locomotor activity by MPEP have been reported previously (Spooren et al., 2000a; Herzig and Schmidt, 2004; Tessari et al., 2004). Spooren et al. (2000a) suggested that MPEP may affect a premotor component of activity such as planning. Another possibility is that the decreases in locomotor activity in iP rats may be secondary to an anxiolytic-like effect of MTEP, because both MPEP (Spooren et al., 2000b; Schulz et al., 2001; Tatarczynska et al., 2001) and MTEP (Busse et al., 2004; Klodzinska et al., 2004) produce anxiolytic-like effects and alcohol-prefering P rats have an anxiety-like phenotype (Stewart et al., 1993). Although there are no other reports of MTEP-induced sedation to date (cf. Klodzinska et al., 2004), no other study has examined locomotor activity so soon after MTEP administration. The reports of MPEP-enhanced PCP-induced locomotor activity in rats (Henry et al., 2002; Kinney et al., 2003) may suggest a comparative hyperglutamatergic state in FH rats that facilitates their responsiveness to MTEP. In support of this hypothesis, FH rats have elevated levels of AMPA receptor binding within the cingulate cortex compared with alcohol-nonpreferring counterparts (Chen et al., 1999). It is noteworthy that a striatal hyperglutamatergic state has been proposed to underlie the enhanced ethanol consumption of mice homozygous null for the clock gene Per2 (Spanagel et al., 2005). Presumably therefore, either a hyperglutamatergic state does not underlie the high levels of ethanol consumption of the iP rats or the mGlu5 receptor is involved in the mediation of alertness in iP rats in a differential manner to Fawn-Hooded rats. The anatomical locus or loci of such an effect remains unclear at present. We note that differential neurochemical profiles between alcohol-preferring strains of
rats have been observed frequently (see Cowen and Lawrence, 1999).

In the present study, repeated treatment with MTEP altered neither anxiety-like behavior as measured by the elevated plus maze nor depressive-like behavior as measured with the forced swim test. To date, there are no reports of MTEP altering depressive-like behavior; therefore, the significant amelioration of this behavior by MPEP (Tatarczynska et al., 2001; Picc et al., 2002) could conceivably reflect an off-target effect. However, acute and chronic MPEP (Spooren et al., 2000b; Tatarczynska et al., 2001; Brodkin et al., 2002b; Picc et al., 2002; Wieronska et al., 2004) and MTEP (Cosford et al., 2003; Busse et al., 2004; Klodzinska et al., 2004; Varty et al., 2005) have well documented anxiolytic-like effects in a range of experimental models. Therefore, the dose of MTEP used in the present study (2 mg/kg i.p.), although effective in causing a decrease in ethanol consumption and self-administration of the FH rat strain, seems to be subthreshold for decreasing anxiety-like responses in this strain of rats. Although one recent report has indicated tolerance to the effect of MTEP on punished responding (Busse et al., 2004), the dose used was somewhat higher than in the present study; in contrast, another study using a similar dose (Klodzinska et al., 2004) indicated no development of tolerance.

From a mechanistic viewpoint, the regulation of ionotropic glutamate receptor subunits and components of the dopaminergic system by chronic MTEP treatment are consistent with interactions among mGlu5, AMPA, and NMDA receptors and also among mGlu5 and the dopamine D2 receptor and dopamine transporter. Thus, agonists acting at mGlu5 receptors can potentiate AMPA- and NMDA-mediated responses (Ugolini et al., 1999; Mannaioni et al., 2001; Pisani et al., 2001; Kotecha et al., 2003), and activation of the mGlu5 receptor was shown to be necessary for the induction of LTP of NMDA/AMPA-mediated excitatory postsynaptic potentials in the lateral amygdala (Fendt and Schmid, 2002). Conversely, NMDA-dependent LTP in the hippocampus was inhibited in mGlu5-deficient mice (Lu et al., 1997; Jia et al., 1998). Since the MTEP-induced down-regulation of AMPA and NMDA receptor subunits occurred specifically within the cingulate and piriform cortices while mGlu5-like immunoreactivity occurs throughout the cortex (Romano et al., 1995), the data may indicate that activation of these cortical subregions is of particular importance in drug-seeking behavior. This would concur with recent reports that the cingulate cortex is involved in cue- and stress-induced reinstatement of cocaine-seeking behavior (Ciccioppo et al., 2001; McLaughlin and See, 2003; McFarland et al., 2004). However, the role of these subregions in the processing of olfactory information may also be significant, particularly in light of the ability of mGlu5 receptor antagonists to attenuate an olfactory cue-induced reinstatement of ethanol seeking behavior under operant conditions (Bäckstrom et al., 2004). In addition, metabotropic glutamate receptors within the piriform cortex are linked to habituation of odor-evoked behaviors (Best et al., 2005). Moreover, in our operant paradigm, the location of the active (ethanol) lever was indicated to the rats by a single drop of 10% v/v ethanol in the corresponding receptacle; an amount too small to act as a priming dose but sufficient to evoke sensory stimulation and enable the rat to orientate within the operant chamber.

Functional interactions between mGlu5 and dopamine D2 receptors and the dopamine transporter have been observed within the striatum, of which the olfactory tubercle is the most ventral component. Thus, infusion of the mGlu5 agonist CHPG into the nucleus accumbens activated the ventral striatopallidal pathway and facilitated the release of GABA within the ventral pallidum (Diaz-Cabiale et al., 2002). This effect was antagonized by MPEP and confusion of the dopamine D2 receptor agonist quinpirole (Diaz-Cabiale et al., 2002). Conversely, infusion of the dopamine D2 receptor antagonist eticlopride into the dorsal striatum caused an increase in preproenkephalin mRNA (which is generally coexpressed in dopamine D2-expressing neurons); this effect was antagonized by MPEP (Parelkar and Wang, 2003). Fuxe et al. (2003) have suggested that heteromeric dopamine D2/mGlu5/adenosine A2A receptor complexes may occur in vivo. Activation of the individual receptor subtypes within this complex may regulate the other receptors present. Finally, activation of the mGlu5 receptor by CHPG has been shown to cause a significant decrease in dopamine transporter capacity and efficiency in a rat striatal synaptosomal preparation (Page et al., 2001). Our data suggest that repeated antagonism of mGlu5 receptors by MTEP may in contrast cause an increase in levels of the dopamine transporter. Why the alterations observed in the present study should occur specifically within the olfactory tubercle remain unclear; however, olfactory stimuli associated with drug availability have particular effectiveness in reinstating drug-seeking behavior (c.f. Bäckstrom et al., 2004).

In summary, our data indicate that MTEP produces significant and long-lasting changes in ethanol self-administration and consumption at doses that are subthreshold for anxiolytic-like and antidepressive-like actions, at least in FH rats. However, the sedation induced in iP rats indicates that as a therapeutic agent, there may be some drawbacks to the use of MTEP, or it may be useful (for example) for a certain subgroup of alcoholics. The interaction of MTEP with markers of glutamatergic and dopaminergic signaling involved in the processing of olfactory information provides further evidence for a role of mGlu receptors in processing olfactory stimuli that drive subsequent behaviors.

Acknowledgments

The supply of MTEP from Merck is gratefully acknowledged.

References


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