Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 528 (2005) 110–118
www.elsevier.com/locate/ejphar

Suppression of alcohol self-administration and cue-induced reinstatement of alcohol seeking by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG

Pia Bäckström, Petri Hyytiä *

Department of Mental Health and Alcohol Research, National Public Health Institute, P.O. Box 33, FI-00251 Helsinki, Finland

Received 14 July 2005; received in revised form 20 October 2005; accepted 26 October 2005

Abstract

Glutamatergic neurotransmission has been suggested to modulate cue-induced drug-seeking behavior. Here we examined the effects of metabotropic glutamate receptor agonists on alcohol self-administration and cue-induced reinstatement. Rats were trained to self-administer 10% w/v ethanol under an FR1 schedule of reinforcement during 30-min sessions. In the reinstatement experiments, ethanol and a non-rewarding quinine solution (available on alternating days) were paired with olfactory stimuli (S^+/S^-) as well as light (CS^+) or tone (CS^-) stimuli. Following extinction training, reinstatement of responding was induced by the ethanol-associated stimuli (S^+/CS^+). The mGlu2/3 receptor agonist LY379268 (0, 1, 3 and 5 mg/kg i.p.) and the mGlu8 receptor agonist (*S*)-3,4-DCPG (0, 5, 10 and 15 mg/kg i.p.) attenuated alcohol self-administration and reinstatement at doses that decreased also spontaneous locomotor activity. The results suggest that metabotropic glutamate receptors may have a role in the modulation of alcohol seeking and self-administration. However, further studies with ligands with fewer motor-suppressant side effects are needed.

Keywords: Metabotropic glutamate receptor; Glutamate; Reinstatement; Self-administration; Conditioning; Alcohol

1. Introduction

Discrete or contextual stimuli that have been repeatedly paired with the availability and pharmacological effects of alcohol can induce craving in abstinent users. For example, exposure to ethanol odor or the presentation of an alcoholic beverage increases subjective reports of craving and the desire to drink (Cooney et al., 1997; Greeley et al., 1993; Schneider et al., 2001). Although a priming dose of alcohol increases the probability of further alcohol consumption (de Wit and Chutuape, 1993), a correlation between craving and subsequent drug use has not been established (Tiffany and Carter, 1998). However, the activation of certain brain areas by alcohol-associated stimuli has been shown to predict subsequent relapse in abstinent alcoholics (Grüsser et al., 2004). Similar to humans, alcohol-seeking behavior in laboratory animals is influenced by alcohol cues, as extinguished alcohol seeking has been demonstrated to be reinstated reliably by alcohol-associated visual or olfactory stimuli (Ciccocioppo et al., 2001; Katner et al., 1999; Nie and Janak, 2003).

Evidence is accumulating for the role of glutamate transmission in processing of drug-associated stimuli. Presentation of drug-associated stimuli increases accumbal dopamine and glutamate levels in laboratory animals (Hotsenpiller et al., 2001; Ito et al., 2000; Katner and Weiss, 1999; Weiss et al., 2000). These alterations in transmitter levels are probably coupled to drug-seeking behavior because stimulus-induced drug seeking is attenuated by intra-accumbal injections of dopamine or glutamate receptor antagonists (Di Ciano and Everitt, 2001; Samson and Chappell, 2004). Also when administered systemically, D1 and D2 type dopamine antagonists as well as NMDA and AMPA/kainate glutamate receptor antagonists are effective in suppressing drug seeking (Alleweireldt et al., 2002; Bäckström and Hyytiä, 2004; Crombag et al., 2002; Liu and Weiss, 2002).

* Corresponding author. Tel.: +358 9 4744 8120; fax: +358 9 4744 8133.
E-mail address: petri.hyytia@ktl.fi (P. Hyytiä).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.10.051

Glutamate receptor antagonists decrease also the expression of conditioned place preference (Bespalov, 1996; Kotlinska and Biala, 2000; Popik et al., 2003), behavioral sensitization and conditioned locomotor activity (Hotsenpiller et al., 2001; Jackson et al., 1998; Mead and Stephens, 1998), suggesting that antagonizing the actions of stimulus-induced glutamate release at postsynaptic receptors attenuates the expression of conditioned drug-related behaviors. Alternatively, it could be hypothesized that reduction of stimulus-evoked glutamate release through presynaptic metabotropic glutamate receptors could also be possible.

Metabotropic glutamate (mGlu) receptors are divided into three groups, mGluI–mGluIII, based on signal transduction pathways and sequence homology (Schoepp, 2001). Group I is comprised of mGlu1 and mGlu5 receptors, group II of mGlu2 and mGlu3 receptors and group III of mGlu4 and mGlu6-8 receptors (Schoepp, 2001). mGlu1/5 receptors are predominantly postsynaptic, whereas mGlu2/3 receptor agonists have been shown to reduce glutamate release via a presynaptic mechanism (Schoepp, 2001). Similar to group II agonists, group III agonists can negatively modulate glutamate transmission (Pothecary et al., 2002; Thomas et al., 2001).

The present study aimed at clarifying whether cue-induced reinstatement of alcohol-seeking behavior and alcohol self-administration in rats could be attenuated by pretreatment with group II/III metabotropic glutamate receptor agonists. LY379268 is a selective group II, mGlu2/3 receptor agonist that is centrally active following systemic administration (Monn et al., 1999). It has previously been shown to attenuate cocaine and heroin seeking triggered by discriminative stimuli and contextual cues, respectively, and cocaine self-administration (Baptista et al., 2004; Bossert et al., 2004). (*S*)-3,4-DCPG was chosen because it has been described as a potent group III, mGlu8 receptor agonist that reduces excitatory transmission during excessive glutamate release, presumably through a presynaptic action (Pothecary et al., 2002; Thomas et al., 2001), and reaches the brain at relevant concentrations after systemic administration (Linden et al., 2003).

2. Materials and methods

2.1. Animals

Fifty-six male Long–Evans rats (HsdBlu:LE, Harlan Sprague Dawley, Indianapolis, IN) weighing 160–200 g upon arrival were used. Rats were housed in pairs in Eurostandard Type IV cages (transparent polycarbonate, dimensions 595 × 380 × 200 mm) in a temperature and humidity controlled room under a 12-h light–dark cycle (lights on at 4 p.m.). Water and pellet food (RM1, SDS, Witham, UK) were available ad libitum in the home cage except during initial training (see below). All behavioral testing was carried out during the dark phase of the light–dark cycle between 0800 and 1200 h, 5 days a week, unless otherwise stated. All experimental procedures using animals were carried out according to the European Community guidelines for the use of experimental animals and were approved by the Institutional Animal Care and Use Committee at the National Public Health Institute.

2.2. Apparatus

All experimental sessions took place in operant chambers (Lafayette Instrument, Lafayette, IN) enclosed in ventilated sound-attenuating cubicles. The front panel of each chamber was equipped with two response levers and two drinking cups between the levers. A blue stimulus light was mounted above the right response lever. Auditory stimuli (2.9 kHz, 65 dB) were delivered from a loudspeaker positioned on top of the self-administration chamber. Responses at the appropriate lever activated a syringe pump that delivered a 0.1-ml drop of fluid to one of the two drinking cups. MED-PC behavioral software (MED Associates Inc., Georgia, VT) was used for controlling the operant chambers and collecting data.

2.3. Drugs

LY379268 [(−)-2-oxa-4-aminobicyclo hexane-4,6-dicarboxylic acid] was a generous gift from Eli Lilly and Co. and (*S*)-3,4-DCPG [(*S*)-3,4-dicarboxyphenylglycine] was obtained from Tocris Cookson Ltd. (Bristol, UK). LY379268 and (*S*)-3,4-DCPG were dissolved in saline and administered intraperitoneally (i.p.) in a volume of 1 ml/kg 30 and 15 min before behavioral testing, respectively.

2.4. Oral ethanol self-administration training

Rats were trained to orally self-administer ethanol by using a modification of a training protocol described previously by Samson (1986). Briefly, rats were deprived of water for 12 h prior to training sessions for 3 consecutive days and were trained to respond for a 0.1-ml drop of 0.2% (w/v) saccharin solution on both levers under a fixed ratio 1 (FR1) schedule of reinforcement. After this initial training, water deprivation was terminated, and animals had free access to food and water in their home cages throughout the subsequent training and testing. Non-deprived rats were given two additional saccharin sessions to confirm that they had acquired responding for saccharin before ethanol self-administration training started. Then, during the next three sessions, responses at the right lever resulted in the delivery of 0.1 ml of 5% (w/v) ethanol +0.2% saccharin solution. Responses at the left lever were recorded but had no programmed consequences. Thereafter, the concentration of ethanol was increased first to 8% and then to 10% w/v and the concentration of saccharin was decreased until saccharin was eliminated completely from the drinking solution.

2.5. Experiment 1: effects of mGlu receptor agonists on ethanol self-administration

The final schedule of reinforcement for the 10% w/v ethanol concentration was similar to the training schedule except that a stimulus light was added. Thus, during the 30-min sessions

responses on the active lever resulted in the delivery of 0.1 ml of ethanol and, in addition, in the illumination of the stimulus light for 3 s. The left lever remained inactive.

When rats had reached stable ethanol self-administration under these conditions, the effects of the mGlu2/3 receptor agonist LY379268 (0, 1, 3 and 5 mg/kg i.p., $n=10$) and the mGlu8 receptor agonist (*S*)-3,4-DCPG (0, 5, 10 and 15 mg/kg i.p., $n=8$) on ethanol self-administration were examined in a Latin-square, within-subjects design. The agonists were dissolved in saline and administered 30 min (LY379268) or 15 min [(*S*)-3,4-DCPG] before start of the self-administration session. The LY379268 doses were chosen based on previous findings, indicating that doses of 1–10 mg/kg decrease phencyclidine-induced locomotor activity and behavioral sensitization (Cartmell et al., 1999; Clark et al., 2002), as well as cocaine and heroin seeking in rats (Baptista et al., 2004; Bossert et al., 2004). (*S*)-3,4-DCPG has been shown to increase c-Fos expression at the higher end of the 3–100 mg/kg dose range in mice (Linden et al., 2003).

2.6. Experiment 2: effects of mGlu receptor agonists on cue-induced reinstatement of ethanol seeking

2.6.1. Conditioning and extinction procedure

Rats participating in this experiment were also trained using the saccharin fading procedure. The conditioning phase began when rats started self-administering 10% ethanol. During conditioning, olfactory discriminative stimuli (S^D) predicting either 10% ethanol or 80 μM quinine hydrochloride solution availability were presented during 30-min sessions. Ethanol availability (S^+) was signaled by anise odor (*trans*-anethole, Sigma-Aldrich Chemie GmbH, Steinheim, Germany), whereas quinine availability (i.e. non-reward) was signaled by citrus odor [S^-, (*R*)-(+)-limonene, Sigma-Aldrich Chemie GmbH, Steinheim, Germany]. The olfactory stimuli were generated by placing a small piece of absorbent paper containing a drop of either anethole or limonene next to the self-administration chamber inside the sound-attenuation cubicle immediately before the start of the session. In addition to these discriminative stimuli, each ethanol delivery was accompanied by a 3-s light stimulus (CS^+), while a 3-s auditory stimulus (CS^-) was presented with quinine delivery. During the first week of the 7-week conditioning phase, rats were given ethanol sessions only. Thereafter, ethanol and quinine sessions were given in a random order until rats received a total of 18 ethanol and 17 quinine sessions.

Extinction training followed after the conditioning phase. During 30-min extinction sessions, responding had no programmed consequences, the olfactory stimuli signaling ethanol or quinine availability were withheld, and the liquid delivery lines remained empty. Extinction sessions continued until no further trend for either increased or decreased responding was seen in the group mean for at least five consecutive sessions.

2.6.2. Reinstatement of ethanol-seeking behavior

Reinstatement sessions began on day 1 post-extinction. During the first session, rats were presented with the S^D predictive of ethanol non-availability (i.e. quinine availability, citrus odor), and responses on the previously active lever resulted in a 3-s presentation of the auditory stimulus (CS^-) and activation of the syringe pump motor, but not in the delivery of any drinking solution. During subsequent sessions, rats were presented with the ethanol-associated S^D (anise odor) and active lever responses turned on the syringe pump motor and the ethanol-associated CS^+, the 3-s light. In addition, the first two lever responses produced 0.1 ml of ethanol solution to the drinking cup. This provided rats with two additional stimuli, namely the taste and smell of ethanol that had been present during the conditioning, but not the extinction phase. Reinstatement sessions were conducted twice a week (on Tuesdays and Fridays) with the rats remaining in their home cages on intervening days.

2.6.3. Effects of LY379268 and (S)-3,4-DCPG on ethanol-seeking behavior

Only rats that had a history of stable ethanol self-administration during the conditioning phase (≥ 20 ethanol responses per session) and showed reliable reinstatement of responding (≥ 7 active lever responses per session) were included in the experiment. The effects of the mGlu2/3 receptor agonist LY379268 ($n=12$) and the mGlu8 receptor agonist (*S*)-3,4-DCPG ($n=9$) on ethanol-seeking behavior were examined in a Latin-square, within-subjects design. The doses and the pretreatment times were as in Experiment 1. On the reinstatement session preceding the first drug pretreatment session, rats were administered a saline injection (1 ml/kg i.p.) 30 or 15 min before testing to habituate them to the injection procedure.

2.7. Experiment 3: effects of mGlu receptor agonists on spontaneous locomotor activity

To clarify whether the changes in reinstatement responding observed after pretreatment with mGlu receptor agonists were caused by effects on motor performance, spontaneous locomotor activity was measured after agonist pretreatment.

Locomotor activity was measured in transparent Eurostandard Type III cages (transparent polycarbonate, dimensions $43\times27\times16$ cm) that were placed inside photocell frames (Cage Rack Activity System, San Diego Instruments, CA, USA). The frames were equipped with seven pairs of photocells (5 cm off the cage floor) for measuring horizontal activity and eight pairs of photocells (12 cm off the floor) for measuring vertical activity. The number of photocell interruptions was recorded by a computer at 5-min intervals for 30 min. During the first three daily sessions, rats were habituated to the test cages and, on the third day, also administered a habituating saline injection (1 ml/kg i.p.). Thereafter, sessions were carried out twice a week. The effects of the mGlu2/3 receptor agonist LY379268 (0 and 5 mg/kg i.p., $n=8$) and the mGlu8 receptor agonist (*S*)-3,4-DCPG (0, 10 and 15 mg/kg i.p., $n=8$) on locomotor activity

were examined in a within-subjects design. The pretreatment times were as in Experiment 1. For technical reasons, there were two groups consisting of eight rats each in the (*S*)-3,4-DCPG experiment. One group was administered the vehicle and the 10 mg/kg dose, the other group the vehicle and the 15 mg/kg dose.

2.8. Statistical analysis

Data from self-administration and reinstatement sessions were expressed as the mean total number of responses during the 30-min session. Data from self-administration and reinstatement sessions were analyzed with a within-subjects one-way analysis of variance (ANOVA) with repeated measures either on dose (mGlu receptor agonist treatments in Experiments 1 and 2) or reinstatement condition (S^D/CS^D-induced reinstatement in Experiment 2). Following a significant main effect, each condition was compared with the vehicle injection (mGlu agonist treatments in Experiments 1 and 2) or the extinction baseline (S^D/CS^D-induced reinstatement in Experiment 2) using a post hoc means comparison with Bonferroni correction. Data from the locomotor activity experiment were analyzed with paired *t*-tests between the drug dose and its vehicle. In all statistical analyses, criterion for significance was set at the 0.05 level.

3. Results

3.1. Experiment 1: effects of mGlu receptor agonists on ethanol self-administration

Rats were allowed 28 sessions of self-administration with the 10% w/v ethanol solution before the agonist pretreatments. During the last three sessions, the average number of responses (±S.E.M.) was 26.7±1.9 on the active lever and 4.2±1.5 on the inactive lever for the subjects participating in this experiment. This corresponded to an average ethanol intake of 0.59±0.04 g/kg.

Both the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG suppressed ethanol self-administration (Fig. 1) [active lever, $F(3,27)=3.12$, $P=0.042$ and $F(3,21)=8.38$, $P=0.001$, respectively]. Inactive lever responding was not significantly affected by the agonist pretreatments. The cumulative response patterns during self-administration sessions indicate highest responding during the first 5 min of the session in the vehicle-treated rats. Rats treated with LY379268 maintained this response pattern and the effects

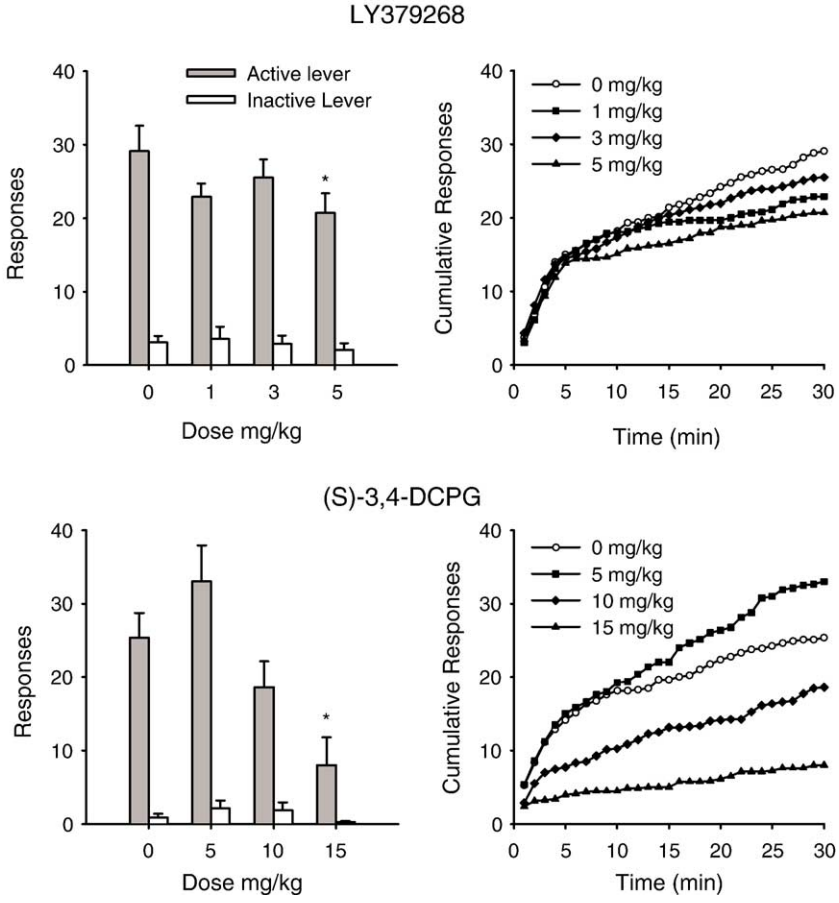

Fig. 1. Effects of pretreatment with LY379268 ($n=10$) and (*S*)-3,4-DCPG ($n=8$) on ethanol self-administration. The left panel shows the mean (±S.E.M.) number of responses on the active and inactive lever during the 30-min self-administration session. *$P<0.05$, significantly different from the vehicle. In the right panel, the mean cumulative number of responses on the active lever is shown in 1-min intervals.

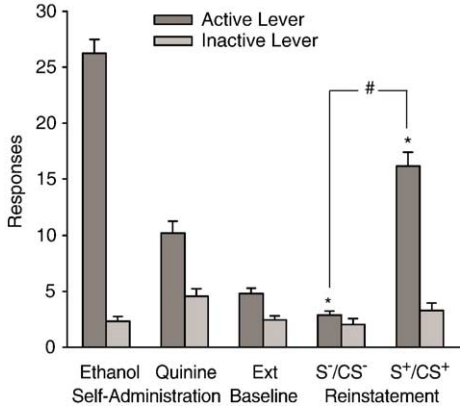

Fig. 2. Mean (±S.E.M.) number of lever-press responses on the active and inactive lever during 30-min conditioning, extinction and reinstatement sessions ($n=24$). For the conditioning and extinction sessions, the average number of responses over the last three sessions is shown. *$P<0.05$, significantly different from the extinction baseline. #$P<0.05$, significantly different from the S^-/CS^- condition. "S^-/CS^- reinstatement", reinstatement session with contingent presentations of stimuli previously associated with non-reward (quinine); "S^+/CS^+ reinstatement", reinstatement session with a priming dose of ethanol and stimuli previously associated with ethanol reward.

of the agonist became visible later in the session. With (*S*)-3,4-DCPG, however, a clear decrease in responding for ethanol was evident from the beginning of the session.

3.2. Experiment 2: effects of mGlu receptor agonists on cue-induced reinstatement of ethanol seeking

3.2.1. Conditioning and extinction phase

Following acquisition of ethanol self-administration, rats were subjected to ethanol and quinine conditioning sessions. During the conditioning phase, rats developed stable responding for ethanol with a mean (±S.E.M.) number of 26.2±1.2 responses on the active lever across the last three sessions (Fig. 2). The number of inactive lever responses during this period was 2.3±0.4. Ethanol responses corresponded to an average ethanol intake of 0.56±0.03 g/kg during the 30-min sessions. At the same time, responding for quinine decreased gradually to a level of 10.2±1.1 and 4.5±0.7 responses on the active and inactive lever during the last three conditioning sessions. Inspection of the drinking cups after the sessions revealed that rats drank only part of the quinine solution they earned. Responding for ethanol and quinine differed significantly during these sessions ($P<0.001$).

On the first day of extinction training, the number of responses on the previously active and inactive levers was 13.5±1.3 and 5.4±0.8, respectively. During subsequent sessions, responding decreased further and reached stable levels within 15 sessions. Over the last 3 days of extinction training, the mean number of responses was 4.8±0.5 for the

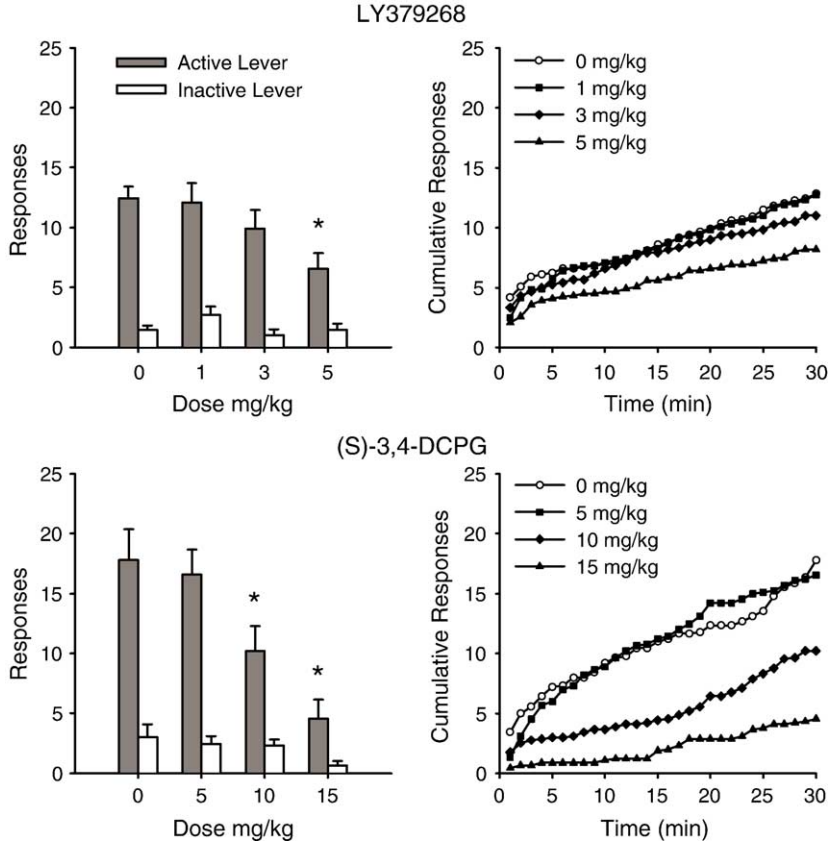

Fig. 3. Effects of pretreatment with LY379268 ($n=12$) and (*S*)-3,4-DCPG ($n=9$) on reinstatement responding induced by ethanol-associated stimuli together with a priming dose of ethanol (S^+/CS^+ condition). The left panel shows the mean (±S.E.M.) number of responses on the active and inactive lever during the 30-min reinstatement session. *$P<0.05$, significantly different from the vehicle. In the right panel, the mean cumulative number of responses on the active lever is shown in 1-min intervals.

previously active lever and 2.5±0.3 for the previously inactive lever.

3.2.2. Reinstatement of ethanol-seeking behavior

The mean number of active lever responses emitted during the quinine reinstatement session was 2.9±0.4 (Fig. 2). During the ethanol reinstatement session, the mean number of responses on the active lever was 16.2±1.2. On average, the 0.2-ml ethanol priming resulted in an ethanol dose of 0.04±0.0006 g/kg. All rats consumed this ethanol dose during the session. ANOVA revealed a significant main effect of reinstatement condition [$F(3,93)=65.55$, $P<0.0001$]. Further analyses showed that the ethanol-associated olfactory stimulus (anise odor, S^+) and ethanol-associated light stimulus (CS^+) together with the small ethanol priming dose increased responding over the extinction baseline, whereas the stimuli signaling quinine availability (non-reward) decreased responding. The number of inactive lever responses did not differ significantly across reinstatement conditions.

3.2.3. Effects of LY379268 and (S)-3,4-DCPG on ethanol-seeking behavior

Responding during reinstatement sessions remained stable as confirmed by paired *t* tests conducted separately for each agonist between the 0 mg/kg dose and the baseline vehicle reinstatement session preceding drug treatment sessions (P's>0.05).

Fig. 3 shows that both the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG attenuated reinstatement induced by the ethanol-associated stimuli and ethanol priming [$F(3,33)=3.54$, $P=0.025$ and $F(3,24)=22.78$, $P<0.0001$, respectively]. The post hoc test indicated significant attenuation at the 5 mg/kg dose for LY379268 and at the 10 and 15 mg/kg doses for (*S*)-3,4-DCPG. The number of inactive lever responses was not significantly decreased by the pretreatments.

Fig. 3 shows also the cumulative response patterns during reinstatement sessions. In the vehicle pretreated rats, the highest levels of responding were seen during the first 5 min of the session. Thereafter, responding leveled off, but continued at a low rate throughout the 30-min session. Both agonists, especially (*S*)-3,4-DCPG, decreased responding from the beginning of the session.

3.3. Experiment 3: effects of mGlu receptor agonists on spontaneous locomotor activity

Fig. 4 shows that LY379268 and (*S*)-3,4-DCPG decreased ambulatory locomotor activity at all doses tested (P's<0.05). In addition, (*S*)-3,4-DCPG, but not LY379268 decreased vertical activity (P's<0.05).

4. Discussion

In the present study, we examined the effects of two mGlu receptor agonists, the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG, on alcohol self-administration and cue-induced reinstatement of alcohol seeking. Alcohol self-administration reflects mainly the unconditioned, primary reinforcing effects of alcohol, whereas the cue-induced reinstatement model measures the potency of alcohol-associated environmental cues to reinstate extinguished behavior and thus represents the conditioned effects of alcohol.

LY379268 and (*S*)-3,4-DCPG decreased responding during reinstatement tests at the 5 mg/kg and the 10 and 15 mg/kg doses, respectively. Inspection of cumulative response records revealed that (*S*)-3,4-DCPG markedly suppressed responding from the beginning of the session, whereas the effects of LY379268 emerged more slowly. The same effect could be seen in the cumulative response patterns of alcohol self-administration. Self-administration was attenuated by the 5 mg/kg dose of LY379268 and, unlike in the reinstatement experiment, only by the highest dose of (*S*)-3,4-DCPG.

To clarify whether the reductions in operant responding could be ascribed to impairment of motor function, a control experiment measuring spontaneous locomotor activity after agonist pretreatment was conducted. All agonist doses that significantly attenuated operant responding decreased also ambulatory locomotor activity. Vertical activity was decreased by both doses of (*S*)-3,4-DCPG tested, but not by LY379268. Previously, LY379268 has been reported to decrease basal

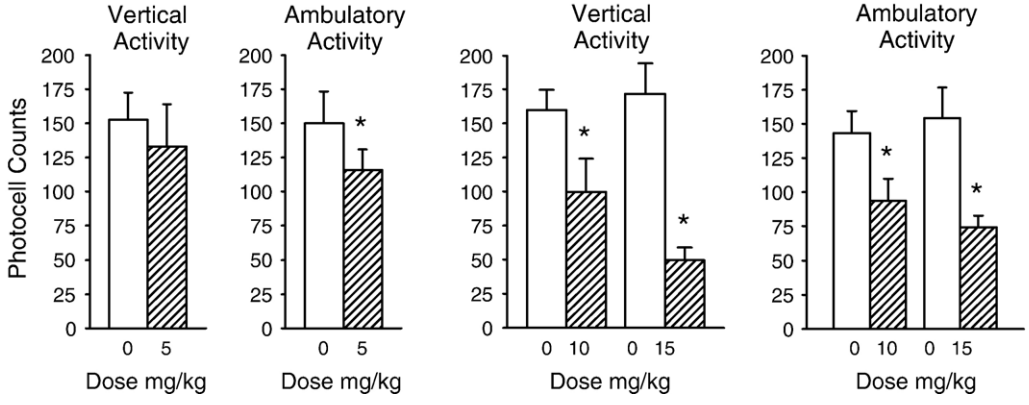

Fig. 4. Effects of pretreatment with LY379268 ($n=8$) and (*S*)-3,4-DCPG ($n=8$) on spontaneous vertical and horizontal (ambulatory) locomotor activity. *$P<0.05$, significantly different from the vehicle.

ambulatory activity at 1 and 3 mg/kg (Cartmell et al., 1999). However, it is possible that attenuation of basal activity with these doses does not indicate gross impairment of motor ability, because rotarod performance was not affected until at the 10 mg/kg dose (Cartmell et al., 2000) and responding for a primary reinforcer, sweetened milk, was not affected by the 3 mg/kg dose (Baptista et al., 2004). Therefore, we cannot exclude the possibility that LY379268 could also alter the reinforcing properties of alcohol and the ability of alcohol-paired stimuli to reinstate alcohol seeking at the present doses. In contrast, the effects of (*S*)-3,4-DCPG on locomotor activity were more pronounced and this agonist tended to suppress responding from the onset of the session. Intracerebroventricular administration of (*S*)-3,4-DCPG has previously been found to impair performance dose-dependently on the rotarod in mice (Moldrich et al., 2001), but the effects of systemic administration on motor functions in rats are not known. However, our observations from the present experiments suggest that motor suppressant effects by (*S*)-3,4-DCPG may have influenced responding in the operant tasks.

In order to assess the behavioral selectivity of LY379268, the effects of this agonist both on the conditioned and unconditioned effects of drugs of abuse have been examined separately. In addition, LY379268 effects on behaviors maintained by drug reinforcers compared with conventional reinforcers have been reported. Thus, reinstatement of cocaine seeking by cocaine-associated cues was attenuated by a lower LY379268 dose than cocaine self-administration and the 3 mg/kg dose that suppressed cocaine self-administration did not affect consumption of sweetened milk (Baptista et al., 2004). Similarly, heroin seeking induced by a heroin-paired context was decreased at a dose of 3 mg/kg, but a 6 mg/kg dose was needed to reduce responding for oral sucrose (Bossert et al., 2004). Our present results that LY379268 attenuated reinstatement of alcohol seeking by alcohol-paired environmental stimuli and alcohol consumption are compatible with previous findings. However, compared with the effects of LY379268 on cocaine or heroin seeking, a higher LY379268 dose was needed to alter alcohol seeking, and the previously seen greater potency of LY379268 to antagonize conditioned than unconditioned drug effects was not observed (Baptista et al., 2004; Bossert et al., 2004). Therefore, the effects of LY379268 on alcohol-related behaviors resembled those on behaviors maintained by conventional reinforcers. Further studies are needed to see whether these results describe the effects of LY379268 on alcohol-maintained behavior or whether they reflect the specific features of the reinstatement model. In our model, reinstatement of extinguished alcohol seeking was repeatedly induced by an alcohol-associated olfactory stimulus together with a response-contingent light stimulus previously paired with alcohol delivery. In addition, we showed previously that reinstatement responding is enhanced by a small priming dose of ethanol compared to the olfactory and visual stimuli alone (Bäckström and Hyytiä, 2004). Although the ethanol priming dose is pharmacologically insignificant, it may provide orosensory activation that typically initiates neural processes related to ingestive behavior, and therefore renders our model less sensitive to the effects of LY379268 than models where drug seeking is reinstated by drug-associated stimuli that are not directly related to sensory activation produced by ethanol.

In contrast to LY379268, very little is known about the effects of (*S*)-3,4-DCPG on drug-related behaviors. The racemic form (*R*,*S*)-3,4-DCPG decreased amphetamine-induced hyperactivity in mice at doses that did not affect spontaneous locomotor activity (Ossowska et al., 2004). However, as the *R*-isomer has been reported to have antagonistic effects at AMPA receptors (Thomas et al., 2001), it is unclear whether the effects were due to actions at mGlu or AMPA receptors, although a role for mGlu8 receptor agonism was proposed (Ossowska et al., 2004). To our knowledge, the effects of (*S*)-3,4-DCPG on drug-seeking behavior and drug-self-administration have not been previously investigated.

Evidence for the involvement of glutamate transmission in mediating the effects of drug-associated cues on behavior comes mainly from cocaine studies. Repeated treatment with cocaine in a distinct environment was reported to decrease basal levels of extracellular glutamate in the rat nucleus accumbens (Bell et al., 2000; Hotsenpiller et al., 2001; McFarland et al., 2003). Subsequent re-exposure to cocaine or cocaine-paired stimuli elicited a more pronounced increase in accumbal glutamate release in these animals compared to saline pre-exposed controls or animals treated with cocaine in their home cage (Bell et al., 2000; Hotsenpiller et al., 2001). In accordance with these results, administration of ionotropic glutamate receptor agonists into the accumbens induced drug seeking (Cornish et al., 1999; Cornish and Kalivas, 2000) and, conversely, ionotropic glutamate receptor antagonists attenuated cocaine- and alcohol-seeking behavior induced by drug-associated stimuli or priming doses of the drug (Bäckström and Hyytiä, 2003, 2004; Cornish and Kalivas, 2000; Di Ciano and Everitt, 2001; Park et al., 2002). The augmented glutamate release evoked by cocaine-paired environmental cues could partly be a consequence of a functional down-regulation of the presynaptic mGlu2/3 receptors by repeated cocaine administration (Xi et al., 2002). Ethanol-induced changes in the capacity of mGlu2/3 receptors to regulate glutamate release have not been described, but it is possible that the relatively small ethanol intake by non-dependent rats in ethanol administration models is not sufficient to cause major alterations in the efficacy of mGlu2/3 receptor signaling. Therefore, mGlu2/3 receptor agonism may not be as potent in decreasing ethanol- as cocaine-seeking behavior. Interestingly, LY379268 pretreatment reduced enhanced dopamine and glutamate overflow in the nucleus accumbens of amphetamine-sensitized rats (Kim et al., 2005). This effect of LY379268 on accumbal dopamine could be another mechanism for the agonist-induced suppression of alcohol seeking, because dopamine release has been shown to be enhanced following exposure to alcohol-associated stimuli (Katner and Weiss, 1999) and cue-induced alcohol-seeking behavior can be reduced by dopamine antagonists (Liu and Weiss, 2002).

In conclusion, the present results confirm and extend findings that attenuation of glutamatergic neurotransmission through agonism at presynaptic metabotropic glutamate

receptors may be effective in suppressing drug consumption and seeking. However, significant alterations in alcohol seeking and self-administration by the mGlu2/3 receptor agonist LY379268 and the mGlu8 receptor agonist (*S*)-3,4-DCPG were achieved only at doses that affected also spontaneous activity. Accordingly, for exploration of the role of these receptors in drug-related behaviors, selective compounds possessing fewer undesirable side effects are needed.

Acknowledgements

The authors would like to thank Dr. Schoepp from Eli Lilly for the generous donation of LY379268. This study was supported by a grant from the Finnish Foundation for Alcohol Studies to P.B.

References

Alleweireldt, A.T., Weber, S.M., Kirschner, K.F., Bullock, B.L., Neisewander, J.L., 2002. Blockade or stimulation of D1 dopamine receptors attenuates cue reinstatement of extinguished cocaine-seeking behavior in rats. Psychopharmacology 159, 284–293.

Baptista, M.A., Martin-Fardon, R., Weiss, F., 2004. Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: comparison between cocaine and a potent conventional reinforcer. J. Neurosci. 24, 4723–4727.

Bell, K., Duffy, P., Kalivas, P.W., 2000. Context-specific enhancement of glutamate transmission by cocaine. Neuropsychopharmacology 23, 335–344.

Bespalov, A., 1996. The expression of both amphetamine-conditioned place preference and pentylenetetrazol-conditioned place aversion is attenuated by the NMDA receptor antagonist (+/−)-CPP. Drug Alcohol Depend. 41, 85–88.

Bossert, J.M., Liu, S.Y., Lu, L., Shaham, Y., 2004. A role of ventral tegmental area glutamate in contextual cue-induced relapse to heroin seeking. J. Neurosci. 24, 10726–10730.

Bäckström, P., Hyytiä, P., 2003. Attenuation of cocaine-seeking behaviour by the AMPA/kainate receptor antagonist CNQX in rats. Psychopharmacology 166, 69–76.

Bäckström, P., Hyytiä, P., 2004. Ionotropic glutamate receptor antagonists modulate cue-induced reinstatement of ethanol-seeking behavior. Alcohol., Clin. Exp. Res. 28, 558–565.

Cartmell, J., Monn, J.A., Schoepp, D.D., 1999. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively attenuate phencyclidine versus d-amphetamine motor behaviors in rats. J. Pharmacol. Exp. Ther. 291, 161–170.

Cartmell, J., Monn, J.A., Schoepp, D.D., 2000. Tolerance to the motor impairment, but not to the reversal of PCP-induced motor activities by oral administration of the mGlu2/3 receptor agonist, LY379268. Naunyn-Schmiedeberg's Arch. Pharmacol. 361, 39–46.

Ciccocioppo, R., Angeletti, S., Weiss, F., 2001. Long-lasting resistance to extinction of response reinstatement induced by ethanol-related stimuli: role of genetic ethanol preference. Alcohol., Clin. Exp. Res. 25, 1414–1419.

Clark, M., Johnson, B.G., Wright, R.A., Monn, J.A., Schoepp, D.D., 2002. Effects of the mGlu2/3 receptor agonist LY379268 on motor activity in phencyclidine-sensitized rats. Pharmacol. Biochem. Behav. 73, 339–346.

Cooney, N.L., Litt, M.D., Morse, P.A., Bauer, L.O., Gaupp, L., 1997. Alcohol cue reactivity, negative-mood reactivity, and relapse in treated alcoholic men. J. Abnorm. Psychology 106, 243–250.

Cornish, J.L., Kalivas, P.W., 2000. Glutamate transmission in the nucleus accumbens mediates relapse in cocaine addiction. J. Neurosci. 20, 1–5.

Cornish, J.L., Duffy, P., Kalivas, P.W., 1999. A role for nucleus accumbens glutamate transmission in the relapse to cocaine-seeking behavior. Neuroscience 93, 1359–1367.

Crombag, H.S., Grimm, J.W., Shaham, Y., 2002. Effect of dopamine receptor antagonists on renewal of cocaine seeking by reexposure to drug-associated contextual cues. Neuropsychopharmacology 27, 1006–1015.

de Wit, H., Chutuape, M.A., 1993. Increased ethanol choice in social drinkers following ethanol preload. Behav. Pharmacol. 4, 29–36.

Di Ciano, P., Everitt, B.J., 2001. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. Neuropsychopharmacology 25, 341–360.

Greeley, J.D., Swift, W., Prescott, J., Heather, N., 1993. Reactivity to alcohol-related cues in heavy and light drinkers. J. Stud. Alcohol 54, 359–368.

Grüsser, S.M., Wrase, J., Klein, S., Hermann, D., Smolka, M.N., Ruf, M., Weber-Fahr, W., Flor, H., Mann, K., Braus, D.F., Heinz, A., 2004. Cue-induced activation of the striatum and medial prefrontal cortex is associated with subsequent relapse in abstinent alcoholics. Psychopharmacology 175, 296–302.

Hotsenpiller, G., Giorgetti, M., Wolf, M.E., 2001. Alterations in behaviour and glutamate transmission following presentation of stimuli previously associated with cocaine exposure. Eur. J. Neurosci. 14, 1843–1855.

Ito, R., Dalley, J.W., Howes, S.R., Robbins, T.W., Everitt, B.J., 2000. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J. Neurosci. 20, 7489–7495.

Jackson, A., Mead, A.N., Rocha, B.A., Stephens, D.N., 1998. AMPA receptors and motivation for drug: effect of the selective antagonist NBQX on behavioural sensitization and on self-administration in mice. Behav. Pharmacol. 9, 457–467.

Katner, S., Weiss, F., 1999. Ethanol-associated olfactory stimuli reinstate ethanol-seeking behavior after extinction and modify extracellular dopamine levels in the nucleus accumbens. Alcohol., Clin. Exp. Res. 23, 1751–1760.

Katner, S.N., Magalong, J.G., Weiss, F., 1999. Reinstatement of alcohol-seeking behavior by drug-associated discriminative stimuli after prolonged extinction in the rat. Neuropsychopharmacology 20, 471–479.

Kim, J.H., Austin, J.D., Tanabe, L., Creekmore, E., Vezina, P., 2005. Activation of group II mGlu receptors blocks the enhanced drug taking induced by previous exposure to amphetamine. Eur. J. Neurosci. 21, 295–300.

Kotlinska, J., Biala, G., 2000. Memantine and ACPC affect conditioned place preference induced by cocaine in rats. Pol. J. Pharmacol. 52, 179–185.

Linden, A.M., Bergeron, M., Baez, M., Schoepp, D.D., 2003. Systemic administration of the potent mGlu8 receptor agonist (*S*)-3,4-DCPG induces c-Fos in stress-related brain regions in wild-type, but not mGlu8 receptor knockout mice. Neuropharmacology 45, 473–483.

Liu, X., Weiss, F., 2002. Reversal of ethanol-seeking behavior by D1 and D2 antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. J. Pharmacol. Exp. Ther. 300, 882–889.

McFarland, K., Lapish, C.C., Kalivas, P.W., 2003. Prefrontal glutamate release into the core of the nucleus accumbens mediates cocaine-induced reinstatement of drug-seeking behavior. J. Neurosci. 23, 3531–3537.

Mead, A.N., Stephens, D.N., 1998. AMPA-receptors are involved in the expression of amphetamine-induced behavioural sensitisation, but not in the expression of amphetamine-induced conditioned activity in mice. Neuropharmacology 37, 1131–1138.

Moldrich, R.X., Beart, P.M., Jane, D.E., Chapman, A.G., Meldrum, B.S., 2001. Anticonvulsant activity of 3,4-dicarboxyphenylglycines in DBA/2 mice. Neuropharmacology 40, 732–735.

Monn, J.A., Valli, M.J., Massey, S.M., Hansen, M.M., Kress, T.J., Wepsiec, J.P., Harkness, A.R., Grutsch Jr., J.L., Wright, R.A., Johnson, B.G., Andis, S.L., Kingston, A., Tomlinson, R., Lewis, R., Griffey, K.R., Tizzano, J.P., Schoepp, D.D., 1999. Synthesis, pharmacological characterization, and molecular modeling of heterobicyclic amino acids related to (+)-2-aminobicyclo[3.1.0] hexane-2,6-dicarboxylic acid (LY354740): identification of two new potent, selective, and systemically active agonists for group II metabotropic glutamate receptors. J. Med. Chem. 42, 1027–1040.

Nie, H., Janak, P.H., 2003. Comparison of reinstatement of ethanol- and sucrose-seeking by conditioned stimuli and priming injections of allopregnanolone after extinction in rats. Psychopharmacology 168, 222–228.

Ossowska, K., Pietraszek, M., Wardas, J., Wolfarth, S., 2004. Potential antipsychotic and extrapyramidal effects of (*R*,*S*)-3,4-dicarboxyphenylglycine [(*R*,*S*)-3,4-DCPG], a mixed AMPA antagonist/mGluR8 agonist. Pol. J. Pharmacol. 56, 295–304.

Park, W.K., Bari, A.A., Jey, A.R., Anderson, S.M., Spealman, R.D., Rowlett, J.K., Pierce, R.C., 2002. Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. J. Neurosci. 22, 2916–2925.

Popik, P., Wrobel, M., Rygula, R., Bisaga, A., Bespalov, A.Y., 2003. Effects of memantine, an NMDA receptor antagonist, on place preference conditioned with drug and nondrug reinforcers in mice. Behav. Pharmacol. 14, 237–244.

Pothecary, C.A., Jane, D.E., Salt, T.E., 2002. Reduction of excitatory transmission in the retino-collicular pathway via selective activation of mGlu8 receptors by DCPG. Neuropharmacology 43, 231–234.

Samson, H.H., 1986. Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. Alcohol., Clin. Exp. Res. 10, 436–442.

Samson, H.H., Chappell, A.M., 2004. Effects of raclopride in the core of the nucleus accumbens on ethanol seeking and consumption: the use of extinction trials to measure seeking. Alcohol., Clin. Exp. Res. 28, 544–549.

Schneider, F., Habel, U., Wagner, M., Franke, P., Salloum, J.B., Shah, N.J., Toni, I., Sulzbach, C., Hönig, K., Maier, W., Gaebel, W., Zilles, K., 2001. Subcortical correlates of craving in recently abstinent alcoholic patients. Am. J. Psychiatry 158, 1075–1083.

Schoepp, D.D., 2001. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J. Pharmacol. Exp. Ther. 299, 12–20.

Thomas, N.K., Wright, R.A., Howson, P.A., Kingston, A.E., Schoepp, D.D., Jane, D.E., 2001. (*S*)-3,4-DCPG, a potent and selective mGlu8a receptor agonist, activates metabotropic glutamate receptors on primary afferent terminals in the neonatal rat spinal cord. Neuropharmacology 40, 311–318.

Tiffany, S.T., Carter, B.L., 1998. Is craving the source of compulsive drug use? J. Psychopharmacol. 12, 23–30.

Weiss, F., Maldonado-Vlaar, C.S., Parsons, J.H., Kerr, T.M., Smith, D.L., Ben-Shahar, O., 2000. Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. Proc. Natl. Acad. Sci. U. S. A. 97, 4321–4326.

Xi, Z.X., Ramamoorthy, S., Baker, D.A., Shen, H., Samuvel, D.J., Kalivas, P.W., 2002. Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. J. Pharmacol. Exp. Ther. 303, 608–615.