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Combined antagonism of glutamate mGlu5 and adenosine A_{2A} receptors interact to regulate alcohol-seeking in rats



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Abstract

Adenosine and glutamate have been implicated as mediators involved in the self-administration of alcohol. In the present study we sought to determine whether adenosine receptors could interact with metabotropic glutamate receptors to regulate operant responding for alcohol and also the integration of the salience of alcohol-paired cues. Alcohol-preferring (iP) rats were trained to self-administer alcohol under operant conditions. The availability of alcohol was paired with an olfactory cue plus a stimulus light. Rats were examined under fixed ratio responding and also following extinction under a cue-induced reinstatement paradigm. Administration of the selective adenosine A_{2A} receptor antagonist, SCH 58261, reduced fixed ratio responding for alcohol in iP rats in a dose-related manner. Furthermore, the combination of a subthreshold dose of SCH 58261 with a subthreshold dose of the mGlu5 receptor antagonist MTEP also reduced alcohol self-administration and increased the latency to the first reinforced response, suggesting a pre-ingestive effect. Moreover, this combination of SCH 58261 and MTEP also prevented the conditioned reinstatement of alcohol-seeking elicited by the re-presentation of cues previously paired with alcohol availability. In contrast, combinations of the selective adenosine A₁ receptor antagonist, DPCPX, with either SCH 58261 or MTEP had no effect on alcohol responding. Collectively, these data suggest a functional interaction between adenosine A_{2A} and mGlu5 receptors in relation to alcohol-seeking and the integration of the drug-related cues.

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Introduction

Alcohol is one of the most widely abused substances in the world, and alcohol abuse causes as much, if not more death and disability as measles, malaria, tobacco and illegal drugs combined (WHO, 2001). In economic terms, alcohol abuse has been estimated to cost US\$167 billion per year; however, 'in human terms, the costs cannot be calculated' (National Institute on Alcohol Abuse and Alcoholism, 2004). For these reasons there has been extensive research into

the pathophysiology underlying alcoholism; however, current therapeutic approaches are far from adequate. Relapse rates remain high under treatment (Anton, 2001; Garbutt et al., 1999; Kranzler and Van Kirk, 2001; Swift, 1999) largely due to the polymodal nature of the disease. For this reason further research is required to identify new therapeutic targets for the improved treatment of alcoholism.

The metabotropic glutamate 5 (mGlu5) receptor is one of a family of eight G-protein coupled glutamate receptors found within the central nervous system (Conn and Pin, 1997; Hermans and Challiss, 2001). Biochemically, mGlu5 receptors have been associated with phosphoinositide hydrolysis and activation of phospholipase C, stimulation of adenylate cyclase and inhibition of voltage-operated calcium channels (Conn

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and Pin, 1997; Hermans and Challiss, 2001). At a behavioural level, mGlu5 receptors appear to have a role in a number of behaviours including anxiety-like and stress responses (Brodkin et al., 2002; Busse et al., 2004; Klodzinska et al., 2004; Spooren et al., 2000), depressive-like behaviour (Li et al., 2006; Pilc et al., 2002; Tatarczynska et al., 2001) and spatial memory formation (Lu et al., 1997; Balschun and Wetzell, 2002). The mGlu5 receptor also appears to have some involvement in the reinforcing properties of a number of drugs of abuse. The mGlu5 receptor antagonist MPEP [2-methyl-6-(phenylethynyl)-pyridine] has been shown to decrease cocaine and nicotine self-administration (Kenny et al., 2005; Paterson et al., 2003; Tessari et al., 2004) as well as alter morphine- and cocaine-induced place preference (Aoki et al., 2004; McGeehan and Olive, 2003).

MPEP has also been shown to decrease self-administration of ethanol in rats and mice (Hodge et al., 2006; Olive et al., 2005; Schroeder et al., 2005) and prevented reinstatement of ethanol-seeking behaviour induced by olfactory cues in rats (Backstrom et al., 2004). We have recently demonstrated that MTEP (3-[2-methyl-1,3-thiazol-4-yl]ethynyl)-pyridine), an mGlu5 receptor antagonist with greater selectivity and bioavailability than MPEP (Anderson et al., 2002; Cosford et al., 2003), caused a decrease in ethanol self-administration in two strains of alcohol-preferring rats: the inbred alcohol-preferring (iP) and the Fawn-Hooded rat (FH/Wjd') (Cowen et al., 2005b). Moreover, MTEP also reduces both consummatory and appetitive responding for ethanol in C57/BL6J mice (Cowen et al., 2007).

Emerging evidence suggests that adenosinergic systems may also be pertinent in alcohol consumption and the behavioural effects of alcohol. Mice lacking the equilibrative nitrobenzylthioinosine-nucleoside transporter (ENT1), largely responsible for adenosine uptake, show elevated levels of alcohol consumption compared to wild-type littermates (Choi et al., 2004). The adenosine A_{2A} receptor belongs to a family of four G-protein coupled adenosine receptors that are widely distributed throughout the body with strong expression in the immune system and basal ganglia (Fredholm et al., 2000; Yaar et al., 2005). Activation of the A_{2A} receptor via G_S or G_{OLF} stimulates adenylyl cyclase, subsequently raising cAMP and intracellular calcium levels (Fredholm et al., 2000; Kull et al., 1999; Ongini and Fredholm, 1996; Yaar et al., 2005). Behaviourally, antagonism of the A_{2A} receptor seems to be associated with relieving anxiety and depressive-like behaviour, and promoting wakefulness (El Yacoubi et al., 2001a, 2003; Ledent et al., 1997). The

A_{2A} receptor is a target for the most widely used psychoactive drug and non-selective adenosine receptor antagonist, caffeine. This receptor also appears to be involved in the reinforcing properties of at least some drugs of abuse. The A_{2A} receptor antagonist DMPX reversed reward impairments caused by cocaine withdrawal (Baldo et al., 1999) and, when injected directly into the nucleus accumbens (NAc), blocked heroin priming-induced reinstatement of lever-pressing for heroin (Yao et al., 2006). Adenosine A_{2A} receptor knockout mice appear less sensitive to the acute intoxicating effects of ethanol (Naassila et al., 2002), and exhibit blunted ethanol withdrawal effects, as do wild-type mice following treatment with adenosine A_{2A} receptor antagonists (El Yacoubi et al., 2001b). Compared to wild types, mice lacking the A_{2A} receptor self-administer less drug and make fewer nose-pokes in a cocaine self-administration paradigm, and appear resistant to locomotor sensitization caused by chronic cocaine administration (Soria et al., 2006). Furthermore, acute A_{2A} receptor antagonism attenuates operant self-administration of ethanol in rats (Arolfo et al., 2004).

The striatal complex, an integral component in the mesocorticolimbic pathway which is intimately involved in natural and drug-induced reward, is rich in both mGlu5 and A_{2A} receptors. This localization of receptors has been shown via immunohistochemical methods (A_{2A} receptor – see Rosin et al., 1998; mGlu5 receptor – see Shigemoto et al., 1993), and autoradiographic methods (mGlu5 receptor – see Patel et al., 2003; A_{2A} receptor – see Sihver et al., 2004). Co-immunoprecipitation of receptor complexes from both transfected cell cultures and rat striatal extracts suggests the existence of mGlu5– A_{2A} receptor heterodimers (Ferre et al., 2002). Further potential for cross-talk exists between the receptors' signal transduction pathways (Agnati et al., 2003), and there is some functional evidence of a relationship between mGlu5 and A_{2A} receptors. Submaximal concentrations of mGlu5 and A_{2A} receptor agonists enhanced glutamate release in the rat striatum *in vitro*, displaying a greater-than-additive effect (Rodrigues et al., 2005). Nishi et al. (2003) found co-activation of mGlu5 and A_{2A} receptors in mouse striatal slices synergistically increased DARPP-32 phosphorylation. Functional interdependence of mGlu5 and A_{2A} receptors has also been demonstrated *in vivo*, suggesting a clear rationale for combinatorial approaches to therapeutics for basal ganglia-related disorders (Kachroo et al., 2005).

The adenosine A_1 receptor has also been suggested to play a role in the effects of drugs of abuse. Functional studies indicate the existence of striatal

A₁ receptors which modulate dopamine release (Jin et al., 1993). A reduction in ethanol withdrawal signs following treatment with an A₁ agonist has been noted in mice (Kaplan et al., 1999). A₁ receptor agonists have also been found to potentiate, and antagonists attenuate, ethanol-induced motor uncoordination in mice (Meng et al., 1997). More recently, heterodimeric complexes comprising adenosine A₁ and A_{2A} receptors have been identified that can regulate glutamate release from corticostriatal terminals (Ciruela et al., 2006a,b). Moreover, these complexes are sensitive to regulation by chronic caffeine (Ciruela et al., 2006a), suggesting a possible relevance in drug-induced plasticity.

Therefore, given the ability of both mGlu5 and adenosine A_{2A} receptor antagonists to independently regulate alcohol self-administration, and the well-established interactions between these receptors within pertinent brain nuclei, the present study was designed to examine whether interactions between mGlu5 and A_{2A} receptors are biologically relevant to alcohol-seeking and self-administration behaviour in alcohol-preferring iP rats. To determine whether such an interaction was specific, we also examined the possibility of mGlu5 and A₁ receptor interactions in this process.

In-bred alcohol-preferring (iP) rats (developed in Indiana) exhibit many traits desirable in a rodent model of alcohol use. Thus, P rats develop tolerance to alcohol (Waller et al., 1983); are vulnerable to the alcohol deprivation effect (McKinzie et al., 1998), which is prolonged following multiple deprivation cycles (Rodd-Henricks et al., 2000) and are less sensitive to the hypnotic effects of ethanol than non-preferring (NP) rats (reviewed in McBride and Li, 1998). In operant paradigms iP rats self-administer more ethanol at any concentration tested than water, whilst NP rats self-administer more water than ethanol at concentrations over 10% (Murphy et al., 2002). Consequently, this rat line represents a suitable animal model for the described studies.

Methods

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. Inbred alcohol-preferring (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, USA). Animals were pair-housed with ad libitum access to standard rat chow and water, and a 12 h light/dark cycle, lights on 07:00 hours. A sucrose-fade training procedure was employed as previously described (Cowen et al., 2005a; Samson et al., 1988) to initiate ethanol self-administration.

Operant alcohol self-administration

Alcohol-preferring iP rats ($n = 30$) were trained to self-administer ethanol (10% v/v) under operant conditions using a fixed ratio of 3 (FR3) during 20-min sessions as previously described (Cowen et al., 2005a,b; Liang et al., 2006). Operant chambers supplied by Med Associates (St Albans, VT, USA) were employed. Each chamber was housed individually in sound-attenuation cubicles, featuring a fan to provide airflow and mask external noise. 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 (protruding 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. Availability of ethanol was conditioned by the presence of an olfactory cue (CS+; 2 drops of vanilla essence, placed on the bedding of the operant chamber directly under the active lever), plus a 1-s light stimulus (CS+) occurred when FR3 was obtained. Ultimately, rats were responding for 10% ethanol solution under a fixed ratio requirement of 3 (FR3) with the sides the alcohol and water were presented on randomized to minimize any side preference. For each session, total ethanol and water responses were recorded, and the difference of fluid in the ethanol reservoir between the beginning and end of the session was also recorded to ensure correct calibration of the delivery system.

Following acquisition of lever-pressing behaviour and stable sessional alcohol self-administration (10% ethanol v/v), rats were administered the A_{2A} receptor antagonist SCH 58261 (Sigma, St Louis, MO, USA; 1 mg/kg, 2 mg/kg and co-administered as detailed) or the A₁ receptor antagonist DPCPX (RBI Sigma, 0.5 mg/kg, 0.25 mg/kg i.p. and co-administered as detailed) suspended in methyl cellulose 30 min prior to the beginning of operant sessions. The mGlu5 antagonist MTEP (Ascent Scientific, N. Somerset, UK; 0.25 mg/kg i.p.) was dissolved in 1% dimethyl sulphoxide (DMSO) and administered 20 min prior

to the operant session. Administration of MTEP as a combination with either SCH 58261 or DPCPX involved two injections both at 0.5 ml/kg at the time-points mentioned above; vehicle controls were delivered in the same way. SCH 58261 and DPCPX as a combination was suspended in methyl cellulose and administered at 1 ml/kg 30 min prior to the operant session. Drug administration weeks were structured so that Mondays and Fridays were no-injection days, vehicle was injected either Tuesday or Wednesday (i.p.) followed by drug the following day (i.p.).

Cue-induced reinstatement of alcohol-seeking

Following normal operant training as detailed above, a cohort of iP rats were then subjected to extinction training, during which time there were no cues placed in the operant chamber and there was no programmed response subsequent to task completion (Lawrence et al., 2006). Extinction sessions continued until responding on the 'active' lever was similar to that on the water lever, and stable between trials. Reinstatement was then triggered by replacing CS+ (i.e. the olfactory cue) under the 'active' lever and also reprogramming the software such that the stimulus light was activated (for 1 s) after every FR3 response, although there was no delivery of ethanol into the receptacle. Prior to the reinstatement session, rats were treated with either vehicle or a combination of SCH 58261 (0.5 mg/kg i.p. 30 min prior) and MTEP (0.25 mg/kg i.p. 20 min prior).

Locomotor activity

The effect of SCH 58261 (0.5 mg/kg i.p. 30 min prior) combined with MTEP (0.25 mg/kg i.p. 20 min prior) on locomotor activity was examined in the previously mentioned cohort of iP rats ($n=10$). Habituation of the rats to the locomotor cells (TruScan photobeam activity monitors, Colbourn Instruments, Allentown, PA, USA, 26 × 26 × 40 cm) was performed for 30 min/d for 3 d. Subsequently, rats were treated over 2 d with drug or vehicle via a Latin squares design and 20 min later placed in the test cells for 30 min. Locomotor activity, in terms of total distance travelled in the horizontal plane and entries into the vertical plane (rearing), was determined automatically using the TruScan software, as previously described (Liang et al., 2006; McPherson and Lawrence, 2006).

Statistics

Statistical analysis using one and two-way ANOVAs was performed with SigmaStat (version 3; SPSS Inc.,

Chicago, IL, USA). The data are presented as mean ± S.E.M. A significance level of $p=0.05$ was used. In general, session totals and time-courses were analysed using a repeated-measures two-way ANOVA with Student–Newman–Keuls post-hoc tests. For every dose of drug, there is a corresponding vehicle injection, thus the factors for the session totals were treatment vs. drug/vehicle; for the time-course analysis the factors were drug/vehicle vs. time-point. The effect of 0.5 mg/kg i.p. SCH 58261 was also examined using a paired t test. Vehicle injections prior to extinction sessions were not significantly different in ethanol or water lever presses (as examined via a paired t test), and were therefore pooled. The resultant data were analysed using a one-way ANOVA with a Student–Newman–Keuls post-hoc test.

Results

Effect of SCH 58261 on operant responding for ethanol

We have previously published a dose–response curve for MTEP to reduce alcohol self-administration in iP rats (Cowen et al., 2005b), and accordingly our first experiment defined a dose–response for the selective A_{2A} receptor antagonist SCH 58261. A cohort of 10 iP rats responding stably and preferentially for 10% v/v ethanol (36 ± 3 responses representing 0.54 ± 0.04 g/kg per 20 min session) compared with water (3 ± 1 responses) were treated with either vehicle or SCH 58261 (Figure 1). A significant main effect of treatment occurred [treatment: $F(3,27)=3.217$, $p=0.038$; drug vs. vehicle: $F(1,27)=8.764$, $p=0.016$] indicating that SCH 58261 at 2 mg/kg (-47% , $p<0.001$) caused a significant reduction in ethanol responding (Figure 1a). Note that at 0.5 mg/kg, SCH 58261 had no significant effect on ethanol-reinforced responding in a separate cohort of rats [vehicle treated: 49 ± 17 reinforced responses vs. SCH 58261: 51 ± 17 ($t_9=0.32$, $p=0.757$)]. When considering the time-course of effects, SCH 58261 at 2 mg/kg caused a significant reduction in responding compared with vehicle [$F(1,45)=16.456$, $p=0.003$]; post-hoc analysis indicated a significant effect at 5 min ($p<0.009$) and 20 min ($p<0.013$, Figure 1b). Water responding remained unchanged (Figure 1a).

Effect of subthreshold doses of SCH 58261 and MTEP on operant responding for ethanol

Next we verified that low doses of both SCH 58261 and MTEP were independently subthreshold and

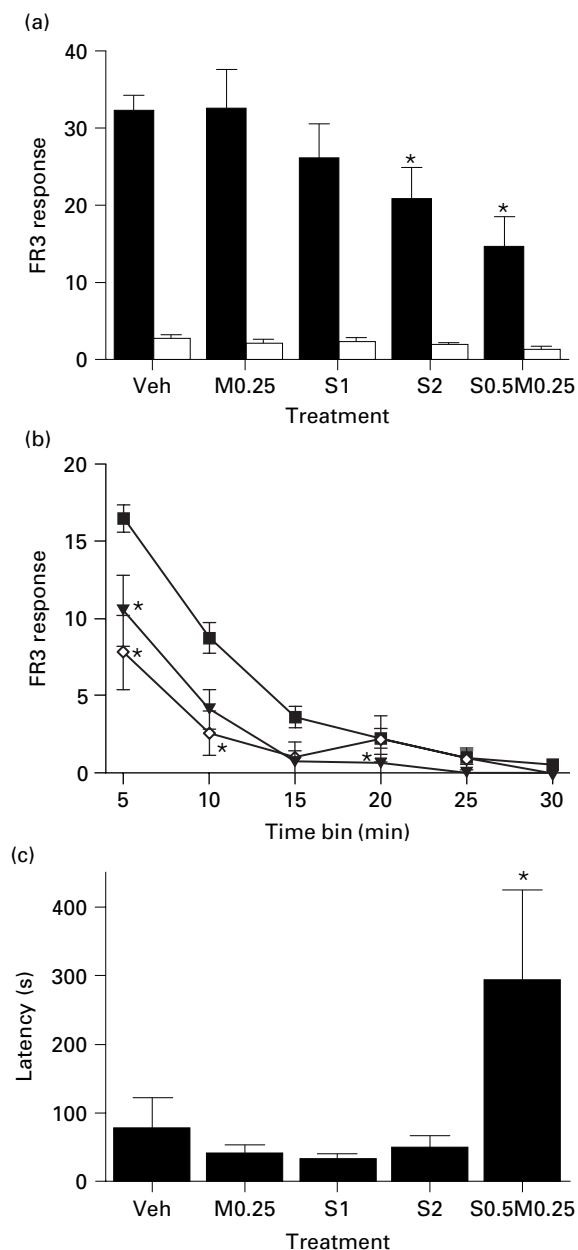


Figure 1. The effects of SCH 58261 and MTEP on operant ethanol self-administration in iP rats ($n=10$). Black bars represent ethanol responses; white bars represent water responses; * denotes significantly different to vehicle. (a) Ethanol self-administration was not significantly altered by SCH 58261 at 1.0 mg/kg i.p., nor MTEP at 0.25 mg/kg i.p. SCH 58261 at 2.0 mg/kg i.p. significantly reduced responding for ethanol ($p<0.001$) without altering responding for water. When co-administered, SCH 58261 and MTEP significantly reduced responding for ethanol without affecting responding for water. Veh, Vehicle; M0.25, MTEP (0.25 mg/kg i.p.); S1 and S2, SCH 58261 at 1.0 and 2.0 mg/kg; S0.5M0.25, SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg). (b) The ethanol-lever time-course shows SCH 58261 at 2.0 mg/kg i.p.

ineffective at reducing operant responding for alcohol (Figure 1a). Notably, the combination of SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg) reduced operant responding for alcohol by -53% ($p=0.001$). Moreover, post-hoc analysis indicated that the effect mediated by a combination of SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg) was significantly greater than subthreshold doses of either MTEP ($q=6.017$, $p<0.001$) or SCH 58261 ($q=3.866$, $p=0.021$) alone. In addition, the combination of SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg) also altered the pattern of responding compared with vehicle [$F(1,45)=15.710$, $p=0.003$]; post-hoc analysis indicated responding was significantly reduced compared to vehicle at 5 and 10 min ($p<0.001$, Figure 1b). Water responding remained unchanged by either drug or in combination (Figure 1a). Finally, analysis of the latency from the session start until the first reinforced response indicated an interaction between factors [drug vs. vehicle \times treatment: $F(3,27)=4.076$, $p=0.016$] with the SCH 58261–MTEP combination increasing latency by almost 5 min ($p<0.001$, Figure 1c).

Effect of MTEP/DPCPX on operant responding for ethanol

To investigate if this functional interaction between mGlu5 and A_{2A} receptor antagonists was specific, the experiment was repeated with a second cohort of iP rats to examine the effects of the selective A₁ receptor antagonist DPCPX (0.25 mg/kg and 0.5 mg/kg i.p.) administered alone or in combination with subthreshold doses of MTEP (0.25 mg/kg i.p.) or SCH 58261 (0.5 mg/kg i.p.). A significant drug effect occurred for ethanol responses [$F(1,24)=6.833$, $p=0.031$] with DPCPX at 0.5 mg/kg lowering responding significantly compared with vehicle (-43% , $p=0.018$; Figure 2). Responding for water and latency to the first reinforced response remained unchanged. A combination of a subthreshold dose of DPCPX (0.25 mg/kg) with either MTEP (0.25 mg/kg) or SCH 58261 (0.5 mg/kg) had no effects on operant responding for alcohol (Figure 2).

significantly reduced responding in the 5-min ($p=0.009$) and 20-min ($p=0.013$) bins. SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg i.p.) significantly attenuated responding for the first 10 min ($p<0.001$). ■, Vehicle; ▼, SCH 58261 (2 mg/kg); ◇, SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg). (c) SCH 58261 at 0.5 mg/kg and MTEP at 0.25 mg/kg i.p. significantly increased the latency from the session start until the first reinforced ethanol reward ($p<0.001$).

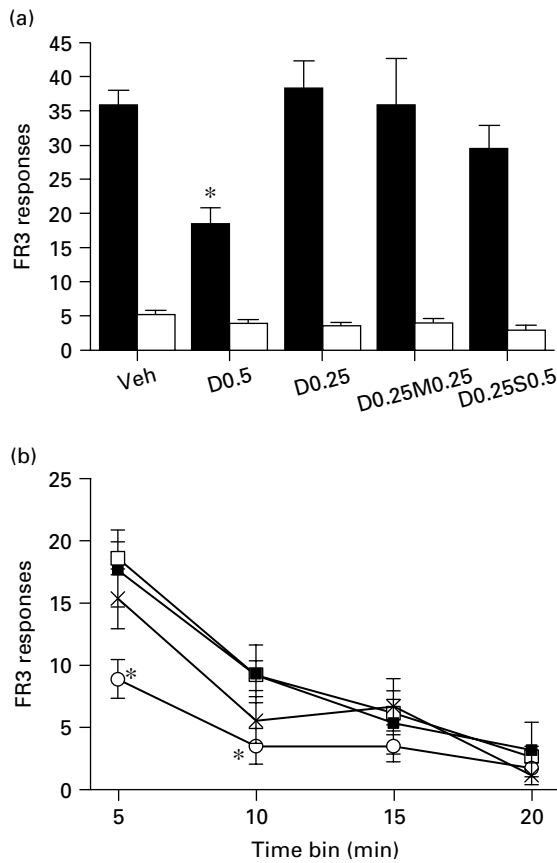


Figure 2. The effects of DPCPX on operant ethanol self-administration in iP rats ($n = 10$). Black bars represent ethanol responses; white bars represent water responses; * denotes significantly different to vehicle. (a) DPCPX at 0.5 mg/kg i.p. significantly reduced responding for ethanol in iP rats ($p < 0.018$) without affecting their responding for water. No interaction was observed between DPCPX and MTEP nor DPCPX and SCH 58261 when co-administered at subthreshold doses. Veh, Vehicle; D0.5, DPCPX (0.5 mg/kg); D0.25, DPCPX (0.25 mg/kg); D0.25M0.25, DPCPX (0.25 mg/kg) and MTEP (0.25 mg/kg); D0.25S0.5, DPCPX (0.25 mg/kg) and SCH 58261 (0.5 mg/kg). (b) The session time-course was significantly altered by DPCPX (0.5 mg/kg) with fewer responses made within the first 10 min ($p < 0.001$). □, Vehicle; ○, DPCPX (0.5 mg/kg); ■, DPCPX (0.25 mg/kg) and MTEP (0.25 mg/kg); ×, DPCPX (0.25 mg/kg) and SCH 58261 (0.5 mg/kg).

Effect of SCH 58261 and MTEP on cue-induced reinstatement of alcohol-seeking

As the reduction in alcohol self-administration was specific to the combination of SCH 58261 and MTEP, we next examined drug-seeking behaviour using a cue-induced reinstatement paradigm to further define the nature of this A_{2A} -mGlu5 receptor interaction. Prior to extinction, alcohol-preferring iP rats

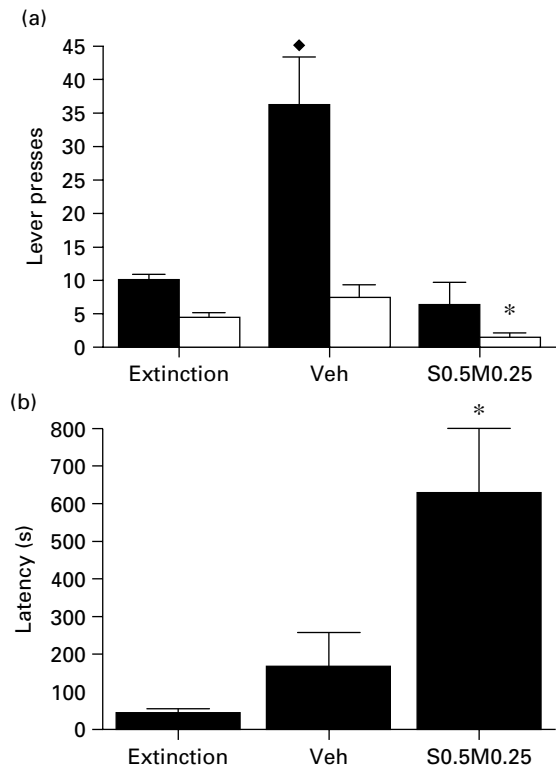


Figure 3. The effect of co-administered SCH 58261 and MTEP on conditioned reinstatement in iP rats ($n = 10$). Black bars represent ethanol-paired or actual ethanol responses; white bars represent water-paired or actual water responses; * denotes significant compared to vehicle; ♦, significant compared to extinction and SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg). (a) The combination of SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg i.p.) effectively blocked conditioned reinstatement of alcohol-seeking ($p = 0.762$) and significantly decreased ethanol-paired lever pressing compared to vehicle ($p < 0.001$). SCH 58261 and MTEP co-administration also significantly reduced water responding compared to vehicle ($p = 0.002$), suggesting that drug-seeking may generalize to the inactive lever during this paradigm. Veh, Vehicle; S0.5M0.25, SCH 58261 (0.5 mg/kg) and MTEP (0.25 mg/kg i.p.). (b) SCH 58261 and MTEP co-administration significantly increased the latency to the first ethanol-paired lever press ($p = 0.004$).

responded stably and preferentially for 10% ethanol (v/v; 52.7 ± 7.3 responses per session) compared with water-responding rats (2.6 ± 0.4 responses per session), corresponding to an average ethanol intake of 0.86 ± 0.12 g/kg per session. Over 10 sessions, ethanol responding was extinguished (no availability of ethanol or water and no presentation of ethanol-associated cues; average ethanol lever presses: 10.4 ± 1.6 ; average water lever presses: 4.5 ± 0.8 ; Figure 3a). At this point, re-presentation of the cues previously associated

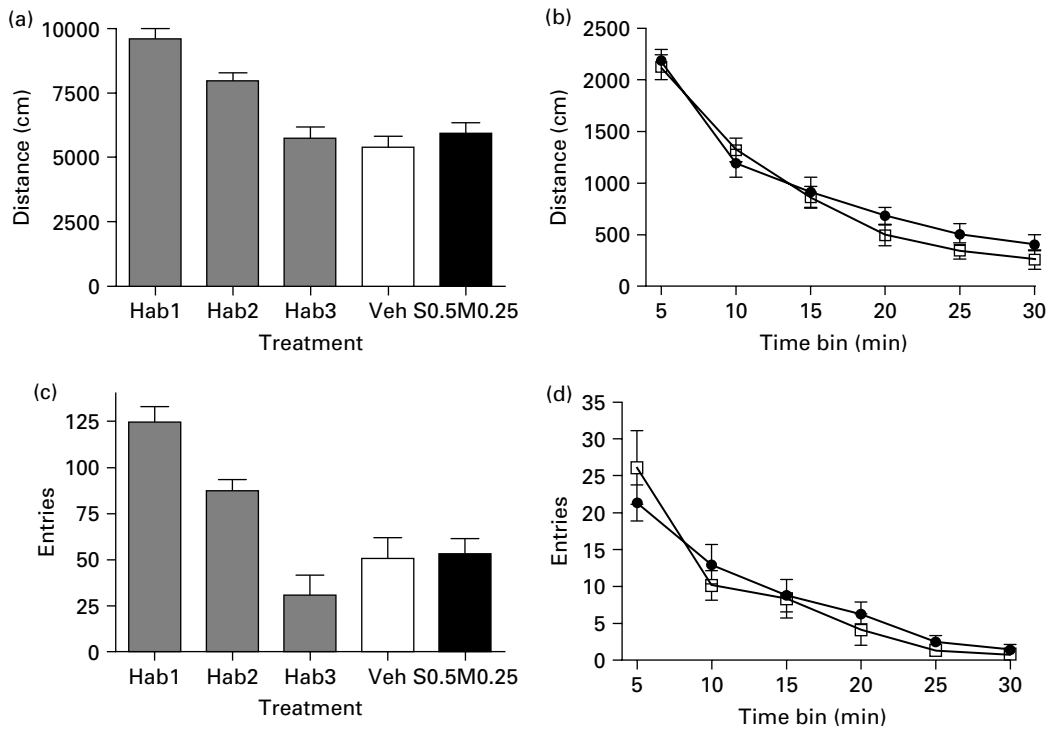


Figure 4. Locomotor effects of SCH 58261 co-administered with MTEP in iP rats ($n=10$). The co-administration of SCH 58261 (0.5 mg/kg i.p. 30 min prior) and MTEP (0.25 mg/kg i.p. 20 min prior) produced no significant locomotor effects on iP rats. Hab, Habituation session. (a) Total distance moved; (b) distance moved time-course; (c) total vertical plane entries (rearing); (d) vertical plane time course. Veh/□, Vehicle; S0.5M0.25/●, SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg).

with ethanol availability caused a robust and significant increase in responding on the 'active' (olfactory cue) lever in vehicle-treated rats that was completely prevented when a combination of SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg i.p.) were co-administered [$F(3,33)=13.555$, $p<0.001$; treatment effect, -83% compared to vehicle, $p<0.001$; Figure 3a]. In addition, the latency to the first lever press was significantly increased by treatment [Figure 3b: $F(3,33)=6.616$, $p<0.001$] and a significant increase in active-lever latency occurred after drug treatment compared to vehicle (a three-fold increase, $p=0.020$).

Locomotor response to SCH 58261 and MTEP

Previously we have found that high doses of MTEP can cause sedation in iP rats which may have explained the observed reductions in responding (Cowen et al., 2005b). We therefore next examined the locomotor effects of co-administration of SCH 58261 and MTEP in iP rats ($n=10$). Locomotor activity was measured over five consecutive days. Rats were habituated to the test chambers then divided into

two groups according to a Latin-squares design for vehicle and drug administration. To ensure a valid comparison, pre-treatment times were the same as those employed for the operant studies. Rats treated with either vehicle or a combination of SCH 58261 (0.5 mg/kg i.p.) and MTEP (0.25 mg/kg i.p.) exhibited no difference in movement distance [$F(1,45)=0.448$, $p=0.52$; Figure 4a,b] or rearing as measured by elevated vertical plane entries [$F(1,45)=0.025$, $p=0.878$; Figure 4c,d], nor in any other parameter examined (not shown).

Discussion

Here we report that an interaction between glutamate mGlu5 and adenosine A_{2A} receptors regulates operant self-administration of alcohol and conditioned reinstatement of alcohol-seeking in iP rats. A combination of individually subthreshold doses of MTEP and SCH 58261 produced a marked reduction in alcohol self-administration compared to vehicle or either drug in isolation. This combination treatment also completely blocked conditioned reinstatement of

alcohol-seeking driven by the presentation of cues previously associated with the availability of alcohol. In contrast, at least in the paradigms employed, we found no support for interactions between adenosine A_1 and A_{2A} receptors, or A_1 and mGlu5 receptors, suggesting this reduction in alcohol self-administration and alcohol-seeking behaviour was specific to an interaction between A_{2A} and mGlu5 receptors. These data therefore provide a clear rationale for the development of therapeutic approaches simultaneously targeting these receptor proteins.

The increase in the latency to the first lever-press following combined treatment with SCH 58261 and MTEP under FR3 conditions (almost a 5-min increase, one sixth of the total session length) indicates the resultant reductions in ethanol-seeking are not mediated by a post-ingestive mechanism, but rather suggests a reduced motivation to seek out and engage in ethanol-seeking behaviour. This was also evident in the conditioned reinstatement paradigm where cues previously paired with availability of ethanol were re-presented after a period of extinction. Given that the rats were not sedated and therefore capable of completing the instrumental task, these data provide strong evidence that co-administration of SCH 58261 and MTEP can dampen the salience of alcohol-related cues and thereby reduce alcohol-seeking.

Immunohistochemical localization of receptors indicates dense staining of A_{2A} receptors throughout the striatum (Ongini and Fredholm, 1996; Rosin et al., 1998) and similarly strong staining of the mGlu5 receptor in the same area (Shigemoto et al., 1993). A_{2A} receptors have been localized to striatopallidal GABA-containing neurons expressing enkephalin and dopamine D_2 receptors (Ongini and Fredholm, 1996) whilst mGlu5 receptors are expressed more widely, occurring on striatonigral, striatopallidal and striatal interneurons (Tallaksen-Greene et al., 1998). Metabotropic glutamate receptors including the mGlu5 receptor are commonly found in a homodimeric, disulphide-bridged complex. This complex appears to exhibit a biphasic response in that binding of an agonist to one receptor in the homodimeric complex causes some activation, but binding to both receptors is required to fully activate the receptor (Kniazeff et al., 2004). It is possible that a similar situation exists when considering an mGlu5- A_{2A} heterodimeric receptor complex. Tonic activation of the A_{2A} receptor by endogenous adenosine may cause an mGlu5 receptor agonist (i.e. glutamate), at relatively low concentrations, to fully activate the complex.

Cue-controlled drug-seeking involves the basal ganglia (Vanderschuren et al., 2005), and reinstatement

of drug-seeking appears to be mediated via activation of corticostriatal glutamatergic inputs to the basal ganglia (Kalivas and McFarland, 2003). In concert with this, alcohol-seeking can be regulated by glutamate receptor antagonists (Backstrom et al., 2004; Backstrom and Hyytia, 2004; Sanchis-Segura et al., 2006; Vengeliene et al., 2005) and transgenic mice with excessive glutamate levels within the striatum consume large amounts of alcohol (Spanagel et al., 2005). It would therefore appear that under the conditions of a cue-induced reinstatement, prior co-administration of SCH 58261 and MTEP may prevent the cue-elicited release of glutamate, attenuate the ability of glutamate to bind to receptors on medium spiny neurons and/or pharmacologically interfere with the downstream signalling caused by glutamate release. The interaction produced by combined antagonism of adenosine A_{2A} and glutamate mGlu5 receptors in this regard is consistent with the existence of heterodimeric complexes within the basal ganglia (Ferre et al., 2002).

While future studies will undoubtedly examine these issues, a possible mechanism could involve a synergistic interaction between mGlu5 and A_{2A} receptors to reduce the signalling efficacy of corticostriatal afferent synapses within the basal ganglia. Thus, in the context of reinstatement, ERK signalling within the basal ganglia has been defined as a molecular substrate for drug-paired contextual cue memories (Miller and Marshall, 2005). In rats, stimulation of corticostriatal afferents activate glutamatergic synapses within the basal ganglia resulting in phosphorylation of ERK and the Glu1 receptor subunit; this can be prevented by pharmacological blockade of adenosine A_{2A} receptors (Quiroz et al., 2006). Whether heterodimeric A_{2A} -mGlu5 receptor complexes can synergize to regulate plasticity of the corticostriatal glutamate synapse awaits confirmation. Nevertheless, our data provide clear functional evidence of a biologically relevant interaction between these two receptor proteins to regulate cue-induced alcohol-seeking.

Our findings are in general agreement with other behavioural reports of a 'synergistic' interaction between mGlu5 and A_{2A} receptors, although to date the majority of studies have been focused on Parkinsonian syndromes. Akinetic symptoms of bilaterally 6-hydroxy-dopamine lesioned rats were improved with combinations of 'ineffective', or subthreshold, doses of mGlu5 and A_{2A} receptor antagonists (Cocurello et al., 2004). Kachroo et al. (2005) found locomotor stimulation evoked in reserpinized mice via co-administration of A_{2A} and mGlu5 receptor antagonists was 'synergistic' in nature. This effect could not be replicated in mice devoid of the A_{2A} receptor. Notably,

we did not observe any significant change in locomotor activity between antagonist and vehicle-treated groups, probably due to the low doses of individual drugs.

While MTEP has been shown to acutely reduce operant responding for food in rats (Varty et al., 2005), this effect was noted with a dose of MTEP four times higher than used in the present study. Moreover, doses of >3 mg/kg (compared to 0.25 mg/kg in the present study) were required to reduce night-time feeding in rats (Bradbury et al., 2005). Similarly, high doses of A_{2A} receptor antagonists are required to reduce feeding in rats (Nagel et al., 2003), while consumption of sucrose (Naassila et al., 2002) and saccharin (Short et al., 2006) are normal in mice lacking adenosine A_{2A} receptors. Under FR3 conditions, there was no effect of co-administration of SCH 58261 and MTEP on water responding; however, during cue-induced reinstatement a small reduction of water lever-pressing was evident. This probably reflects a generalized reduction in non-specific drug-seeking behaviour rather than an anti-dipsogenic effect. However, this could be confirmed in future experiments by associating the second lever with the delivery of negative reinforcer such a quinine solution (cf. Backstrom and Hyytia, 2004), rather than the more neutral water used in the present study.

Adenosine A₁ receptors have been shown to be involved in some of the effects of ethanol within the brain. Rapid tolerance to ethanol measured via rotarod performance in Swiss mice has been prevented by acute A₁ receptor antagonist administration (Batista et al., 2005), which also attenuated handling-stimulated withdrawal hyperlocomotion in CD1 mice (Kaplan et al., 1999). To examine the possibility that A₁ receptors may potentially interact with either A_{2A} or mGlu5 receptors, experiments were conducted with DPCPX, a selective A₁ antagonist. Ethanol self-administration in iP rats was attenuated by DPCPX (0.5 mg/kg i.p.), although no effect on latency to the first reinforced reward was observed in contrast to the effect of SCH 58261. On the surface, it may appear difficult to explain why receptors of the same family with opposing effects on intracellular cAMP (Fredholm et al., 2000) produce similar effects in an ethanol self-administration, operant paradigm. Interestingly however, adenosine A₁ receptors are capable of exerting modulatory influence over both adenosine and dopamine receptor signalling within the striatum. Recent studies have suggested that activation of adenosine A₁ receptors decreases dopamine D₂ receptor activity and thereby disinhibits adenosine A_{2A} receptor signalling (Yabuuchi et al., 2006). If this is

the case, then antagonism of adenosine A₁ receptors could ultimately cause the opposite, which would be consistent with our observations. Alternatively, the anatomic loci where adenosine A₁ receptor antagonism results in reduced operant self-administration of alcohol could be extra-striatal (given the widespread distribution of A₁ receptors). Adenosine A₁ and A_{2A} receptor antagonists have similar effects in relation to the discriminative-stimulus properties of psychostimulants in rats (Justinova et al., 2003). Whilst in Long-Evans rats antagonism of adenosine A₁ receptors has no effect on operant responding for alcohol (Arolfo et al., 2004), iP rats are an inbred strain of alcohol-preferring rats that may differ from out-bred rats in many ways. A subthreshold dose of DPCPX (0.25 mg/kg i.p.) coupled with a subthreshold dose of the mGlu5 receptor antagonist MTEP (0.25 mg/kg i.p.), or the A_{2A} receptor antagonist SCH 58261 (0.5 mg/kg i.p.) produced no significant effects on ethanol self-administration, nor was the session time-course or latency to the first reward altered. These data are indicative of specificity, at least in this paradigm, between mGlu5 and A_{2A} receptors in relation to ethanol self-administration and/or ethanol seeking. Further study is required to examine this receptor interaction in more detail. Of particular interest is the specificity of our observation with low doses of SCH 58261 and MTEP in combination. For example, would this drug combination alter self-administration of a highly palatable food reinforcer (e.g. sucrose), or another drug reinforcer (e.g. cocaine)? In addition, it remains to be established whether this combination of drugs would also be effective in reducing drug-priming or stress-induced reinstatement.

In summary, we have demonstrated for the first time a significant interaction between a mGlu5 and A_{2A} receptor antagonist (at individually subthreshold doses) which produces a marked reduction in ethanol self-administration, and effectively blocks cue-induced reinstatement of ethanol seeking. These effects are specific and do not involve sedation. Due to the low doses of individual drugs used, off-target effects are minimized. This may prove an excellent locus for novel pharmacotherapeutic strategies to address the problem of alcoholism, and possibly other forms of drug abuse.

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Statement of Interest

None.

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