European Journal of Pharmacology 627 (2010) 156–161

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Behavioural Pharmacology

Oral administration of the NAALADase inhibitor GPI-5693 attenuates cocaine-induced reinstatement of drug-seeking behavior in rats

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ARTICLE INFO

Article history:
Received 27 July 2009
Received in revised form 26 October 2009
Accepted 27 October 2009
Available online 31 October 2009

Keywords:
NAAG
NAALADase
GPI-5693
Cocaine
Self-administration
Reinstatement
Relapse

ABSTRACT

We have recently reported that the endogenous mGlu2/3 agonist *N*-acetylaspartylglutamate (NAAG) and the *N*-acetylated-α-linked-acidic dipeptidase (NAALADase, a NAAG degradation enzyme) inhibitor 2-PMPA significantly inhibit cocaine self-administration and cocaine-induced reinstatement of drug-seeking behavior by attenuating cocaine-enhanced extracellular dopamine and glutamate in the nucleus accumbens. However, the poor oral bioavailability of NAAG and 2-PMPA limits their practical use in humans. In the present study, we investigated the effects of the orally active NAALADase inhibitor GPI-5693 and its enantiomers on cocaine-taking and cocaine-seeking behaviours. We found that oral administration of GPI-5693 (15, 30, 60 mg/kg, p.o.) did not significantly alter intravenous cocaine self-administration under fixed-ratio (FR2) reinforcement, but significantly inhibited cocaine-induced reinstatement of the extinguished drug-seeking behavior. This inhibition was blocked by pretreatment with LY341495, a selective mGlu2/3 receptor antagonist. Pretreatment with the same doses (15, 30, 60 mg/kg, p.o.) of GPI-16476 or GPI-16477, two enantiomers of GPI-5693, also inhibited cocaine-induced reinstatement similar to GPI-5693. In contrast, GPI-5693 altered neither oral sucrose self-administration nor sucrose-triggered reinstatement of sucrose-seeking behavior. These data suggest that orally effective NAAG peptidase inhibitors deserve further study as potential agents for the treatment of cocaine addiction.

Published by Elsevier B.V.

1. Introduction

Cocaine addiction is characterized by high rates of relapse to drug use after abstinence. Despite extensive research, there is no effective medication available for the treatment of cocaine addiction (Shalev et al., 2002; Stewart, 2008). It is well known that cocaine priming significantly increases extracellular dopamine and glutamate in the nucleus accumbens in rats during reinstatement of drug-seeking behavior (Anderson and Pierce, 2005; Kalivas, 2004). Also, local perfusion of the group II metabotropic glutamate receptor (mGlu2/3) agonists, (2R,4S)-APDC or DCG-IV, into the nucleus accumbens inhibits dopamine and glutamate release (Hu et al., 1999; Xi et al., 2002a,b). Based on this, we proposed that mGlu2/3 receptor agonists may be effective in attenuating cocaine-induced relapse by inhibiting cocaine-enhanced dopamine and glutamate in the nucleus accumbens (Xi et al., 2002a,b). LY379268 is a well-characterized systemically active mGlu2/3 agonist (Imre, 2007; Gasparini and Spooren, 2007). When administered systemically or locally into the nucleus accumbens or central amygdala, LY379268 significantly inhibits intravenous cocaine self-administration, cocaine-induced reinstatement, and incubation of cocaine craving in rats and non-human primates (Adewale et al., 2006; Baptista et al., 2004; Lu et al., 2007; Peters and Kalivas, 2006). In accordance with these findings, we have recently demonstrated that intranasal administration of the endogenous mGlu2/3 agonist, *N*-acetylaspartylglutamate (NAAG), or intraperitoneal administration of the selective NAALADase (a NAAG degradation enzyme) inhibitor, 2-PMPA, significantly attenuates intravenous cocaine self-administration (Xi et al., 2010), cocaine-enhanced electrical brain-stimulation reward (Xi et al., 2010) and cocaine-induced reinstatement of drug-seeking behavior (Xi et al., 2009). However, the poor oral bioavailability of both NAAG and 2-PMPA limits their practical use in humans (Majer et al., 2003, 2006; Tsukamoto et al., 2005).

In the present study, we investigated the effects of the orally active NAALADase inhibitor GPI-5693 and its enantiomers GPI-16476 and GPI-16477 (Majer et al., 2003, 2006; Tsukamoto et al., 2005) on intravenous cocaine self-administration and cocaine-induced reinstatement of drug-seeking behavior. To determine whether a NAAG-mGlu2/3 receptor mechanism underlies such behavioural effects, we further observed the effects of LY341495, a selective mGlu2/3 receptor antagonist, administered 30 min prior to GPI-5693, on cocaine-induced reinstatement of drug-seeking behavior. Finally, to determine whether GPI-5693 also

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0014-2999/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ejphar.2009.10.062

alters natural (non-drug) reward or reward-seeking, we examined the effects of GPI-5693 on oral sucrose self-administration and sucrose-triggered reinstatement of reward-seeking behavior.

2. Methods

2.1. Animals

Experimentally naïve male Long–Evans rats (Charles River Laboratories, Raleigh, NC, USA) weighing 250 to 300 g were used. Rats were housed individually in a climate-controlled room on a reversed light–dark cycle (lights on at 7:00 PM, lights off at 7:00 AM) with free access to food and water. The animal facility was fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* of the U.S. National Academy of Sciences, and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the U.S. National Institutes of Health.

2.2. Intravenous cocaine self-administration

2.2.1. Surgery

All animals were prepared for experimentation by surgical catheterization of the right external jugular vein. The venous catheters were constructed of microrenathane (Braintree Scientific Inc., Braintree, MA, USA), and catheterization was performed under sodium pentobarbital anaesthesia (60 mg/kg i.p.) with aseptic surgical technique. After exiting the jugular, the catheter passed subcutaneously to the top of the skull, where it exited into a connector (a modified 24 gauge cannula; Plastics One, Roanoke, VA, USA) mounted to the skull with jeweler's screws and dental acrylic. During experimental sessions, the catheter was connected to the injection pump via tubing encased in a protective metal spring from the head-mounted connector to the top of the experimental chamber. To help prevent clogging, the catheters were flushed daily with a gentamicin–heparin–saline solution (30 IU/ml heparin; ICN Biochemicals, Cleveland, OH, USA).

2.2.2. Apparatus

The i.v. self-administration experiments were conducted in operant response test chambers ($32 \times 25 \times 33$ cm) from MED Associates Inc. (Georgia, VT, USA). Each test chamber had 2 levers located 6.5 cm above the floor, 1 active and 1 inactive. Depression of the active lever activated the infusion pump; and depression of the inactive lever was counted but had no consequence. A cue-light and a speaker were located 12 cm above the active lever. The house light was turned on at the start of each 3 h test session. When the animal performed a lever-press that resulted in a drug infusion, it was exposed to 2 drug-paired environmental cues: a cue-light and a cue-sound (tone) that lasted for the duration of the infusion. Scheduling of experimental events and data collection was accomplished using MED Associates software.

2.2.3. General procedure

After recovery from surgery, each rat was placed into a test chamber and allowed to lever-press for i.v. cocaine (1 mg/kg/injection) delivered in 0.08 ml over 4.6 s, on an FR1 reinforcement schedule. During the 4.6 s injection time, additional responses on the active lever were recorded but did not lead to additional infusions. Each session lasted 3 h. The FR1 reinforcement schedule was used for 3–5 days until stable cocaine self-administration was established. The initial cocaine dose of 1 mg/kg/infusion was chosen based on our previous experience that this dose produces the most rapid and facile acquisition of cocaine self-administration behavior. Then, animals were transitioned from FR1 to FR2 reinforcement for continued cocaine (0.5 mg/kg/infusion) self-administration until the following criteria for stable cocaine-maintained responding were met: less than 10% variability in mean inter-response interval and less than 10% variability in mean number of presses on the active lever for at least 3 consecutive days. To avoid cocaine overdose during the self-administration period, each animal was limited to a maximum of 50 cocaine injections per session. After stable cocaine-maintained responding was achieved, each rat randomly received 1 of 3 doses of GPI-5693 (15, 30, 60 mg/kg) or vehicle (0.5% Tween-80), by gavage, 30 min prior to the test session. Animals then received an additional 5–7 days of cocaine self-administration alone until baseline response rates were re-established prior to testing the next dose of GPI-5693. The order of testing for the various doses of drug or vehicle was counterbalanced according to a Latin square design.

2.3. Cocaine-induced reinstatement of drug-seeking behavior

Additional groups of rats were used to study the effects of GPI-5693 and its enantiomers on cocaine-induced reinstatement. The general procedures for cocaine self-administration prior to behavioural extinction were as described above. After stable cocaine self-administration was established, animals were exposed to extinction conditions, during which cocaine was replaced by saline, and the cocaine-associated cue-light and tone were turned off. Active lever pressing led only to saline infusion. Daily 3 h extinction sessions for each rat continued until that animal lever-pressed less than 10 times per 3 h session for at least 3 consecutive days. After the animals met this established extinction criterion, they were divided into 10 dose groups (6–10 rats per group) for reinstatement testing. We chose between-subjects design, because lever responses appeared to decrease over repeated reinstatement tests, making it difficult to re-stabilize 'basal' reinstatement responding before testing the next drug dose.

On the reinstatement test day, each group of animals received either vehicle (0.5 M HEPES, 1 ml.) or one dose of GPI-5693 (15, 30, 60 mg/kg), GPI-16476 (15, 30, 60 mg/kg) or GPI-16477 (15, 30, 60 mg/kg), by oral gavage. Three additional groups of rats were used to further determine whether mGlu2/3 receptors are involved in the pharmacological action of GPI-5693, by pretreating animals with LY341495, a selective mGlu2/3 antagonist, 30 min prior to GPI-5693 (30 mg/kg) or vehicle administration. Thirty minutes after GPI compound administration, all rats were given a priming injection of cocaine (10 mg/kg, i.p.) immediately before initiation of reinstatement testing. During the reinstatement test, the conditions were identical to those in extinction sessions. Active-lever presses (reinstatement) were recorded, although these did not lead to either cocaine infusions or presentation of the conditioned cue-light and tone. Reinstatement test sessions lasted 3 h.

2.4. Sucrose self-administration

To determine whether GPI-5693 selectively inhibits non-drug reward or reward-seeking behavior, we further observed the effects of GPI-5693 on oral sucrose self-administration and sucrose-triggered reinstatement of sucrose-seeking behavior in rats. The procedures for sucrose self-administration were identical to the procedures for cocaine self-administration except for the following: 1) no surgery was performed on the animals in the sucrose experiment; 2) active-lever presses led to delivery of 0.1 ml of 5% sucrose solution into a liquid food tray on the operant chamber wall. After stable sucrose self-administration was achieved, animals were divided into three dose groups (vehicle, 30, 60 mg/kg GPI-5693, i.p., 30 min prior to sucrose self-administration), and the effects of GPI-5693 on sucrose self-administration under FR2 reinforcement conditions were assessed.

2.5. Sucrose-triggered reinstatement of sucrose-seeking behavior

The procedures for oral sucrose self-administration were the same as described above. The procedures for extinction and reinstatement testing were identical to the procedures used in the cocaine-triggered