Effects of a Positive Allosteric Modulator of Group II Metabotropic Glutamate Receptors, LY487379, on Cognitive Flexibility and Impulsive-Like Responding in Rats

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ABSTRACT

Orthosteric group II metabotropic glutamate receptor (mGluR) agonists are regarded as novel, effective medications for all major symptom domains of schizophrenia, including cognitive disturbances. mGluR2s also can be affected in a more subtle way by positive allosteric modulators (PAMs) characterized by a unique degree of subtype selectivity and neuronal frequency-dependent activity. Because currently available treatments for schizophrenia do not improve cognitive dysfunction, the main aim of the present study was to examine the effects of a mGluR2 PAM, N-(4-(2-methoxyphenoxy)-phenyl)-(2,2,2-trifluoroethylsulfonyl)-pyrid-3-ylmethylamine (LY487379), on rat cognitive flexibility and impulsive-like responding, assessed in an attentional set-shifting task (ASST) and a differential reinforcement of low-rate 72 s (DRL72) schedule of food reinforcement. In addition, in vivo microdialysis was used to assess the drug’s impact on cortical levels of dopamine, norepinephrine, serotonin, and glutamate. Rats treated with LY487379 (30 mg/kg) required significantly fewer trials to criteria during the extradimensional shift phase of the ASST. Under a DRL72 schedule, LY487379 (30 mg/kg) decreased the response rate and increased the number of reinforcers obtained. These effects were accompanied by the shift of the frequency distribution of responses toward longer inter-response time durations. LY487379 significantly enhanced extracellular norepinephrine and serotonin levels in the medial prefrontal cortex. In summary, the present study demonstrates that a mGluR2 PAM, LY487379, promotes cognitive flexibility and facilitates behavioral inhibition. These procognitive effects may contribute to the therapeutic efficacy of agents stimulating mGluR2 in schizophrenia.

Introduction

Group II metabotropic glutamate receptors (mGluRs), comprising mGluR2 and mGluR3, provide a promising pharmacotherapeutic target for psychiatric disorders associated with aberrant glutamatergic transmission (Witkin and Eiler, 2006; Patil et al., 2007). Preclinical studies support this view because mGluR2/3 agonists reversed various behavioral, neurochemical, and electrophysiological effects induced by N-methyl-D-aspartate receptor (NMDAR) blockade (Marek et al., 2010). Certain behavioral effects of NMDAR antagonists such as psychomotor activation and cognitive impairment are attributed to the blockade of NMDARs located on γ-aminobutyric acid interneurons, which in turn leads to disinhibition of corticollimbic glutamate circuits (Lisman et al., 2008). Therefore, mGluR2/3 activation may inhibit this secondary effect of NMDAR blockade by reducing the enhanced glutamate release in the medial prefrontal cortex (mPFC) (Moghaddam and Adams, 1998). Electrophysiological studies (Homayoun et al., 2005) and neuroimaging studies (Gozzi et
al., 2008) have supported the effectiveness of mGluR2/3 agonists in blocking the aberrant response elicited by NMDAR antagonists in cortical regions. The role of mGluR2/3 is not, however, restricted to conditions of NMDAR blockade, because activation of mGluR2/3 also counteracts excitatory signaling in the mPFC induced by hallucinogenic drugs such as the 5-HT$_{3A}$ agonist 2,5-dimethoxy-4-iodoamphetamine (Ben-neyworth et al., 2007).

Consistent with these data, a number of studies have shown that mGluR2/3 activation ameliorates various behavioral symptoms arising from NMDAR blockade, including hyperlocomotion and stereotypies (Cartmell et al., 2000; but see Ossowska et al., 2000 for negative data regarding prepulse inhibition experiments). A phase 2a study confirmed expectations that mGluR2/3 agonists possess clinically significant antipsychotic efficacy (Patil et al., 2007). Although these results generated substantial excitement, one of the most intriguing unanswered questions concerns the effects of mGluR2/3 agonists on cognition, because the cognitive domain was not directly assessed in this phase II study. There have been studies published to date indicating that mGluR2/3 stimulation reverses cognitive impairments produced by phencyclidine (PCP)-like NMDAR antagonists in laboratory animals and humans (Moghaddam and Adams, 1998). However, administration of mGluR2/3 agonists to adult animals impairs working memory (Aultman and Moghaddam, 2001; Higgins et al., 2004) and enhances impulsive-like responding (Moore et al., 1999). These findings are consistent with the hypothesis that the utility of orthosteric mGluR2/3 agonists may be limited to conditions associated with enhanced cortical excitability as evoked by NMDAR blockade. Indeed, activation of mGluR2/3 under normal conditions presumably leads to an inhibition of the slow asynchronous phase of glutamate release that is necessary for active maintenance of information (Aultman and Moghaddam, 2001).

Positive allosteric modulators (PAMs) of mGluR2 provide an opportunity to overcome these undesirable side effects of orthosteric agonists (Johnson et al., 2004). In addition to being selective for the mGluR2 subtype, PAMs are characterized by the unique ability to modulate glutamate release in a state-dependent manner, i.e., to reduce excessive glutamate release while having little effect on glutamate levels under basal conditions (Johnson et al., 2004). These state-dependent effects of mGluR2 PAM therefore may sharpen the contribution of glutamate to various physiological processes, including cognitive performance, even under normal, undisturbed conditions. To test this hypothesis, the present study used N-[(4-(2-methoxyphenoxy)-phenyl)-N-(2,2,2-trifluoroethylsulfonyl)-pyrid-3-ylmethylamine) (LY487379) as a representative subtype-selective PAM at mGluR2 showing no activity at mGluR3 and no intrinsic agonist or antagonist activity at mGluR2 (Schaffhauser et al., 2003). Similar to mGluR2/3 PAMs, this compound is effective in preclinical models of anxiety and psychoses (Johnson et al., 2005). For example, it was shown to inhibit PCP-induced hyperlocomotion, reverse amphetamine-induced deficits in prepulse inhibition (Galici et al., 2005), and attenuate long-term developmental deficits produced by PCP in the social discrimination task (Harich et al., 2007). Taken together with the data on other mGluR2 PAMs such as biphenyl-indanone A (Galici et al., 2006), these studies suggest that the selective targeting of mGluR2 via positive allosteric modulation may be as effective as direct stimulation by the less selective mGluR2/3 agonists in animal models of antipsychotic drug action.

To characterize the ability of mGluR2 stimulation to facilitate cognitive performance, effects of LY487379 were evaluated in two models: the attentional set-shifting task (ASST) and the differential reinforcement of low-rate 72 s (DRL72) schedule. Although differing in various aspects, these two models represent cognitive domains that are impaired in schizophrenia and depend on integrity of higher-order, executive functions associated with prefrontal cortical processing.

In the ASST, rats have to select a bowl containing food reward, based on the ability to discriminate the odors and the media covering the bait (Birrell and Brown, 2000). The ASST requires rats to initially learn a rule and form an attentional set within the same stimulus dimensions. In the extradimensional (ED) shift, the crucial part of the test, subjects have to switch their attention to a new, previously irrelevant stimulus dimension (e.g., from the odors to the media covering the bait). The ED component is impaired by lesions of the mPFC (Birrell and Brown, 2000) and blockade of NMDARs (Niki-foruk et al., 2010). The utility of the ASST in measuring functions associated with prefrontal cortical processing in rodents is taken from the fact that this test has been developed as an equivalent of the Wisconsin Card Sorting Test (Roberts et al., 1988), and deficits in Wisconsin Card Sorting Test performance are one of the most widely studied aspects of cognitive dysfunction encountered in schizophrenic patients (Elliott et al., 1998).

In DRL72, food-deprived animals learn to wait at least 72 s between responses to obtain food, and this task is often used to probe impulsive behaviors related to inhibiting prepotent responses (Evenden, 1999). Impaired performance on DRL tasks was reported in rats with prefrontal dopamine lesions (Sokolowski and Salamone, 1994) and after administration of NMDAR antagonists (Stephens and Cole, 1996).

The mGluR2/3 agonists (-)-1R,4S,5S,6S-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) and (-)-1R,4S,5S,6S-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid (LY404039) have been reported to increase extracellular dopamine and serotonin levels in the cortex of freely moving rats (Imre, 2007; Rork-Keun et al., 2007). Taking into account the involvement of monoaminergic neurotransmission in both cognitive flexibility and impulsiveness, the purpose of the microdialysis studies described in this article was to investigate whether systemic administration of LY487379 could affect the levels of dopamine, norepinephrine, serotonin, and glutamate in the mPFC of freely moving rats.

**Materials and Methods**

**Ethics**

All experiments were conducted in accordance with the recommendations and policies of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996 edition) and all applicable European, German, and Polish laws and were approved by the respective Ethic Committee and Animal Welfare Offices. DRL72 and microdialysis experiments were conducted in facilities with full Association for Assessment and Accreditation of Laboratory Animal Care accreditation.

**Animals**

In general, rats were housed in temperature-controlled (21 ± 1°C) and humidity-controlled (40–50%) colonies under 12/12-h light/dark
cycle (lights on at 6:00 AM). Behavioral testing was carried out during the light phase of the light/dark cycle.

**Attentional Set-Shifting Task.** Male Sprague-Dawley rats (Charles River, Sulzfeld, Germany), weighing 250 to 280 g at the time of arrival, were allowed to acclimatize for at least 7 days before the start of the experimental procedure and were initially group-housed (five rats/cage). For 1 week before testing the rats were individually housed under conditions of mild food restriction (15 g of food pellets per day) and ad libitum access to water.

**Differential Reinforcement of Low Rates of Responding**
72 s. Drug and experimentally naive male Wistar rats (12 weeks old at the beginning of behavioral training) were purchased from Janvier (Le Genest St. Isle, France). Animals were housed individually in Macrolon type 3 cages (Tecniplast, Buguggiate, Italy), with two enrichment items per cage (wooden and plastic objects of various shapes). They had ad libitum access to water and restricted access to food consumption (14–16 g/day given after behavioral testing to limit the body weight gain to 5–6 g/week).

**Microdialysis In Vivo.** Male Sprague-Dawley rats (350–380 g) were purchased from Janvier. Animals had food and water available ad libitum. After surgery, rats were housed singly.

**Experimental Procedures**

**Attentional Set-Shifting Task.** Testing was conducted in a modified wired rat housing cage (length × width × height: 42 × 32 × 22 cm) with a white plywood wall dividing half of the length of the cage into two sections. During behavioral testing, one digging ceramic pot (internal diameter of 10.5 cm and the depth of 4 cm) was placed in each section. Each pot was defined by a pair of cues along with two stimulus dimensions. To mark each pot with a distinct odor, 5 μl of a flavoring essence (Dr. Oetker, Lebocz, Poland) was applied on a piece of blotting paper fixed to the external rim before use. A different pot was used for each combination of the digging medium and odor, and only one odor was ever applied to a given pot. The bait (one-third of a Honey Nut Cheerio; Nestle, Glendale, CA) was placed on the bottom of the “positive” pot and buried in the digging medium.

The procedure was adopted from Birrell and Brown (2000) with slight modifications (Nikiforuk et al., 2010) and entailed 3 days for each rat.

On day 1 (habituation), rats were habituated to the testing area and trained to dig in the pots filled with sawdust to retrieve the food reward. Rats were transported from the housing facility to the testing room where they were presented with one unscented pot (filled with several pieces of cereal) in their home cages. After the rats had eaten a Cheerio from the home cage pot, they were placed in the apparatus and were given three trials to retrieve reward from both sawdust-filled baited pots. With each exposure, the bait was covered with an increasing amount of sawdust.

On day 2 (training), the rats were trained on a series of simple discriminations (SDs), to a criterion of six consecutive correct trials. For these trials, the rats had to learn to associate the food reward with an odor cue (e.g., arrack versus orange, both pots filled with sawdust) and/or digging medium (e.g., plastic balls versus pebbles, no odor). All rats were trained by using the same pairs of stimuli. The positive and negative cues for each rat were presented randomly and equally. These training stimuli were not used again in later testing trials.

On day 3 (testing), 30 min after administration of LY487379 or its vehicle, in a single test session the rats performed a series of increasingly difficult discriminations. The first four trials at the beginning of each discrimination stage were a discovery period (not included in six trials to criteria), in which the rat was allowed to dig in both pots regardless of where it first began to dig. In the subsequent trials, an incorrect choice terminated the trial. Digging was defined as any distinct displacement of the digging media with either paw or the nose; the rat could investigate the digging pot by sniffing or touching without displacing material. Testing was continued at each stage until the rat reached the criterion of six consecutive correct trials, after which testing proceeded to the next stage.

In the SD, involving only one stimulus dimension, the pots differed along one of two dimensions (e.g., an odor or a digging medium). For the compound discrimination (CD), the second (irrelevant) dimension was introduced, but the correct and incorrect items of the relevant dimension remained constant. For the reversal of this discrimination (Rev 1), the items and relevant dimension were unchanged, but the previously correct item became the negative one. For the ED shift, a new pair of items was again introduced, but this time a relevant dimension was also changed. Finally, the last stage was the reversal (Rev 3) of the ED discrimination problem. The order of discriminations was always the same (i.e., SD, CD, Rev 1, ID, Rev 2, ED, and Rev 3). For half the animals, the discriminations began with an odor as a relevant dimension. For the other half, they began with a digging medium as the salient cue.

The items were always presented in pairs and varied so that only one animal within each treatment group received the same combination of odor and medium. The following pairs of items were used: pair 1, lemon versus almond, cotton wool versus crumpled tissue; pair 2, spicy versus vanilla, metallic filler versus shredded paper; and pair 3, rum versus cream, clay pellets versus silk. The assignment of each item in a pair as being positive or negative at a given stage and the left–right positioning of the pots in the test apparatus on each trial were randomized.

**TABLE 1**
An example of the order of discriminations used in the ASST of rats that were shifted from digging media to odors as the relevant dimension.
The correct exemplar is shown in bold and was paired with either of two exemplars from the irrelevant dimension across trials within each discrimination problem, (i.e., at the CD phase, the shredded paper was paired with either spicy or vanilla odor, etc.). In the IDS and EDS, there were novel pairs of exemplars for each dimension. The combination of exemplars into positive and negative stimuli and their spatial presentation in the cage was a pseudorandom series determined in advance.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Relevant Dimension</th>
<th>Correct Exemplar</th>
<th>Discrimination between:</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD</td>
<td>Medium</td>
<td>Shredded paper</td>
<td>Metallic filler</td>
</tr>
<tr>
<td>CD</td>
<td>Medium</td>
<td>Shredded paper</td>
<td>Metallic filler</td>
</tr>
<tr>
<td>Rev 1</td>
<td>Medium</td>
<td>Metallic filler</td>
<td>Vanilla</td>
</tr>
<tr>
<td>ID</td>
<td>Medium</td>
<td>Clay pellets</td>
<td>Silk</td>
</tr>
<tr>
<td>Rev 2</td>
<td>Medium</td>
<td>Silk</td>
<td>Cream</td>
</tr>
<tr>
<td>ED</td>
<td>Odor</td>
<td>Lemon</td>
<td>Almond</td>
</tr>
<tr>
<td>Rev 3</td>
<td>Odor</td>
<td>Almond</td>
<td>Crumpled tissue</td>
</tr>
</tbody>
</table>
Differential Reinforcement of Low Rates of Responding

72 s. Operant experiments were conducted in 12 identical standard operant conditioning chambers (31 × 27 × 33 cm, length × width × height; MED Associates, St. Albans, VT) enclosed in sound- and light-attenuating cubicles and connected to a computer through an interface and controlled by MED-PC software (MED Associates). Each chamber was equipped with a white house light centered 19 cm above two response levers (model ENV-112BMI; MED Associates; positioned 7 cm above the floor), sound generator (model ENV-222AM; MED Associates), and a food dispenser that delivered 45-mg food pellets (Formula A/I, Noyes Precision Pellets; Research Diets, Inc., New Brunswick, NJ) on one side.

The DRL procedure was adapted from original reports (O’Donnell and Seiden, 1983). After a single magazine training session (60 food pellets delivered under a variable 60-s schedule), 10 rats were shaped to lever-press for food during 60-min daily sessions. Training sessions continued until the subjects received 100 pellets per session. Then, animals were shifted to a fixed-interval 12-s schedule, followed by fixed interval 30 s, DRL 3 s, DRL 12 s, DRL 18 s, DRL 24 s, DRL 36 s, and finally DRL 72. Under the DRL72 schedule, rats had to wait at least 72 s between successive lever presses to obtain a food reward. Training on the final DRL72 schedule was continued for 2 to 3 consecutive weeks. Drug tests were given when the response rate did not change by more than 10% for 2 consecutive days. Rats received saline injections before the intervening training sessions. Daily training and test sessions lasted 70 min for each animal.

Microdialysis In Vivo. Individual male Sprague-Dawley rats (290–320 g body weight) anesthetized with pentobarbital (50 mg/kg i.p.; Narcoren, Rhone-Merieux, Lyon, France) were mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA), and microdialysis guide cannulas (CMA/12; Axel Semrau GmbH, Sprockhövel, Germany) were implanted into the mediod prefrontal cortex (anterior-posterior = 2.5; medial-lateral = 0.6; tip of the 3-mm long active membrane −3.2 mm below the brain surface, according to Paxinos and Watson, 1982). The guide cannulas were secured with dental cement (Technovit powder, Technovit polymerization starter fluid; Kulzer GmbH, Dormagen, Germany) and four anchor screws into the skull. The rats were allowed to recover from surgery for 5 to 7 days. The day before the experiment, each animal was transferred into a system allowing for free movement (CMA/120; Axel Semrau GmbH) consisting of a plastic bowl, wire attachment, counter balance arm, and swivel assembly connecting in/outlet of the probe with the perfusion pump. Next, a CMA/12 microdialysis probe (3-mm arm, and swivel assembly connecting in/outlet of the probe with a system allowing for free movement (CMA/120; Axel Semrau GmbH) onto a high-performance liquid chromatography column (Waters Xbridge C18 3.5-μm 10/2.1-mm Guard Cartridge and Waters Xbridge C18 3.5 μm 100/2.1 mm). The mobile phase consisted of 50 mM NaH2PO4, 1 mM Na-EDTA, 0.15 mM sodium octyl sulfate, and 6% isopropanol, pH 5.0 with phosphoric acid. Flow rate was set to 0.55 ml/min (Rheos flux pump; Axel Semrau GmbH), and the sample run time was less than 15 min. Serotonin was measured via an electrochemical detector (LC4C; BAS Bioanalytical Systems, West Lafayette, IN). Oxidation currents were measured at a working potential of ±700 mV. The system was calibrated by a standard solution (serotonin) containing 0.1 pmol/10-μl injection. Serotonin was identified by its retention time and peak height with an external standard method using the chromatography software described above.

Assay of Microdialysate Glutamate Levels. Eight microliters of ortho-phthalaldehydehyde (Calbiochem, San Diego, CA)/ N-isobutyl-tryr-L-cysteine (Fluka, Buchs, Switzerland) solution were added to 20 μl of microdialysate or standard volume. After three times mixing and a reaction time of 3 min, 14 μl was injected (CTC PAL autosampler; Axel Semrau GmbH) onto a high-performance liquid chromatography column (Waters Xbridge C18 3.5-μm 10/2.1-mm Guard Cartridge and Waters Xbridge C18 3.5 μm 100/2.1 mm). The mobile phase consisted of 50 mM NaH2PO4, 1 mM Na-EDTA, and 20% methanol, pH 6.5 with phosphoric acid. Flow rate was set to 0.3 ml/min (Rheos flux pump; Axel Semrau GmbH). Glutamate was measured with a fluorescence detector (CMA/280; Axel Semrau GmbH). The system was calibrated by standard solutions of glutamate containing 10 pmol/10-μl injection. Glutamate was identified by its retention time and peak height with an external standard method using the chromatography software described above.

Drugs

LY487379, synthesized internally at Abbott, was suspended in 0.5% hydroxy-propyl-methylcellulose in sterile water. The suspensions were prepared fresh daily and injected intraperitoneally in a volume of 1 ml/kg body weight. Drug doses were chosen based on our own data indicating reversal of PCB-induced hyperlocomotion in rats (A.L. Relo, A. Bespalov, and M. Mezler, unpublished work) and literature data (Galici et al., 2005). In the DRL72 study, drug doses were tested in a pseudo-random order derived from Latin square design with at least 72 h between consecutive tests.

Data Presentation and Statistical Analysis

Attentional Set-Shifting Task. The number of trials required to achieve the criterion of six consecutive correct responses and the number of errors made were recorded for each rat and each discrimination problem. However, because the analyses of these measures yielded the same results, only the number of trials to criterion parameter is reported. Data were calculated by using two-way mixed-design ANOVA with drug treatment as between-subject factor and discrimination phase as within-subject factor, followed by the Newman-Keul’s post hoc test. The α value was set at the P < 0.05 level. The data fulfilled criteria of normal distribution. Statistical analyses were performed with Statistica 7.0 for Windows (StatSoft, Tulsa, OK).

Differential Reinforcement of Low Rates of Responding.

For DRL, the main dependent variables were the total numbers of lever presses and reinforcements obtained. In addition, the data were expressed as percentage relative to the baseline values collected during the preceding training session. For inter-response time
(IRT) analysis, dependent variables were the peak location and the peak area (Richards et al., 1993). The distribution of IRTs for lever press responses can be depicted in a histogram that indicates the percentage of IRTs that occur in time bins of 6 s. Typically, the pattern of responding generates IRT distributions with several peaks. One peak occurs very early in the distribution (burst responses with IRTs <6 s), and another occurs close to the reinforcer criterion (i.e., close to 72 s in duration). The IRT data can be analyzed by using a peak deviation analysis where a negative exponential curve based on a rat responding in a hypothetical random manner is compared with the actual IRT distribution (Richards et al., 1993). The peak area is the proportion of the pause IRTs (IRTs >6 s) above the negative exponential curve. The peak location is the median IRT duration above the same curve.

After rank transformation, data were analyzed by using a multivariate ANOVA with repeated measures on LY487379 dose (SAS-STAT, version 8.02; SAS Institute, Cary, NC). Whenever applicable for repeated-measures analysis, Mauchly’s test of sphericity was applied, and the degrees of freedom were corrected to more conservative values by using Huynh-Feldt’s epsilon for any terms involving factors in which the sphericity assumption was violated. Dunnett’s test was applied for post hoc comparisons whenever indicated by ANOVA results.

**Microdialysis In Vivo.** Microdialysis time course data (~40 to 120 min) for dopamine, norepinephrine, serotonin, or glutamate were evaluated for significance by using a two-way repeated-measures ANOVA (with treatment as the between-subjects factor and time as the repeated-measures factor) followed by the Bonferroni multiple comparison post hoc test using Prism version 5.0 (GraphPad Software, Inc., San Diego, CA).

**Results**

**Attentional Set-Shifting Task**

As shown in Fig. 1, rats treated with LY487379 at a dose of 30 mg/kg (but not 10 mg/kg) required significantly fewer trials to criterion during the ED phase of the ASST. There was no significant drug effect during any other discrimination stage. The ANOVA demonstrated significant effect of drug treatment \( F_{2,21} = 9.31; P < 0.01 \), discrimination phase \( F_{6,126} = 40.15; P < 0.001 \), and their interaction \( F_{12,126} = 4.35; P < 0.001 \).

**Differential Reinforcement of Low Rates of Responding**

At the end of the training phase, rats achieved average performance efficiency of approximately 10%, i.e., 1 of 10 responses was reinforced. Administration of LY487379 affected both the reinforcement rate and response rate (Fig. 2). At the highest tested dose of 30 mg/kg, the number of earned reinforcers rose by almost 80%, whereas response rates seemed to be decreased by approximately 40%. An ANOVA confirmed these observations (reinforcers: \( F_{3,27} = 7.1, P < 0.01 \); responses: \( F_{3,27} = 7.0, P < 0.01 \)). Analysis of the IRT distribution indicated that LY487379 produced a rightward shift in the distribution while producing only a minor increase in the IRT spread (Fig. 3; peak location: \( F_{3,27} = 4.6, P < 0.05 \); peak area: \( F_{3,27} = 1.3, \text{n.s.} \)).

**Effects of LY487379 on Dopamine Levels.** In the mPFC of freely moving rats, a single administration of LY487379...
resulted in a modest transient increase in microdialysate dopamine levels (Fig. 4). The statistical analysis of percentage basal dopamine level data of all treatment groups (vehicle, 3, 10, and 30 mg/kg) by two-way ANOVA with repeated measures on time indicated a significant interaction ($F_{3,1189} = 3.8; P < 0.01$), a significant effect of time ($F_{9,1189} = 2.2; P < 0.05$) but no significant effects of treatment ($F_{3,1189} = 1.7, \text{n.s.}$). Bonferroni’s test revealed no statistical significant effect of treatment at any time point postdosing across all treatment groups.

**Effects of LY487379 on Norepinephrine Levels.** LY487379 induced an increase in microdialysate norepinephrine levels; the dose-effect resembled a bell-shape relationship. A two-way ANOVA with repeated measures indicated a significant interaction ($F_{18,180} = 2.8; P < 0.001$) and a significant effect of time ($F_{9,189} = 2.2; P < 0.05$) but no significant effects of treatment ($F_{3,189} = 1.7, \text{n.s.}$). Bonferroni’s test revealed no statistical significant effect of treatment at any time point postdosing across all treatment groups.

**Effects of LY487379 on Serotonin Levels.** LY487379 increased, dose-dependently, extracellular serotonin levels in the medial prefrontal cortex (Fig. 6). Data analysis by two-way ANOVA with repeated measures indicated a significant interaction ($F_{18,180} = 2.9; P < 0.01$), a significant effect of time ($F_{9,180} = 10.3; P < 0.01$), and a significant effect of treatment ($F_{2,180} = 7.1; P < 0.01$). Bonferroni’s test revealed significant effects ($P < 0.05$), 20 min postdosing of 10 mg/kg LY487370 and 20, 60 to 120 min postdosing of 30 mg/kg LY487370 (Fig. 6).

**Effects of LY487379 on Glutamate Levels.** A single administration of LY487379 resulted in a modest transient decrease in microdialysate glutamate levels (Fig. 7). However, a two-way ANOVA with repeated measures indicated no significant interaction ($F_{9,171} = 1.0; \text{n.s.}$) and a significant effect of time ($F_{9,171} = 2.8; P < 0.01$), but no significant effect of treatment ($F_{3,171} = 1.4; \text{n.s.}$).

**Discussion**

The present study demonstrates that the mGluR2 PAM LY487379 promoted cognitive flexibility, because the compound significantly and specifically improved rats’ performance at the ED stage of the attentional set-shifting task. Furthermore, the results of the present microdialysis study suggested that LY487379 enhanced norepinephrine and sero-
rotonin neurotransmission in the medial prefrontal cortex. LY487379 also reduced impulsive-like behavior generated by the DRL72 schedule of reinforcement. Similar to what is observed after acute administration of various antidepressant drugs (Sokolowski and Seiden, 1999), this latter effect is expressed as an increase in reinforcer rate with a parallel decrease in the response rate. It should be emphasized that, at the dose levels tested, LY487379 does not affect spontaneous locomotor activity in habituated or nonhabituated rats or response latencies in various operant tasks (e.g., delayed nonmatching to position or delayed lever alternation; A. Bеспалов, A. L. Relo, M. van Gaalen, and M. Mezler, unpublished work).

Previous studies on the effects of mGluR2/3 stimulation on cognitive functioning produced somewhat conflicting results. Although mGluR2/3 agonists reversed certain cognitive deficits induced by NMDAR blockade, in drug-free animals these agents had either no effects or even impaired cognition (Aultman and Moghaddam, 2001; Higgins et al., 2004). Experiments with transgenic mice lacking mGlu2 receptors more specifically implicated mGluR2s in cognitive-impairing effects of mGluR2/3 agonists (Higgins et al., 2004). In the present study, the selective mGluR2 PAM LY487379 did not exert any impairing effects. Instead, LY487379 enhanced cognitive flexibility and inhibitory control, suggesting that frequency-dependent modulation of neurotransmission, characteristic of mGluR2 PAMs, may result in a more favorable pharmacological profile than that of mGluR2/3 agonists (Johnson et al., 2005). Nonetheless, additional studies with other mGluR2 PAMs and subtype-selective antagonists would be necessary to confirm the generality and selectivity of this effect.

Although an impairment of cognitive flexibility caused by the blockade of NMDA glutamate receptors has been broadly reported (Nikiforuk et al., 2010), little is known about the effects of mGluR2/3 stimulation on ASST performance. Glutamatergic neurotransmission also is involved in the modulation of impulsive behaviors, because NMDAR blockade disrupts rats’ responding under the DRL72 schedule (Stephens and Cole, 1996). Moore et al. (1999) suggested that the mGluR2/3 agonist \( \text{R},5 \text{S},6 \text{S})-2\text{aminobicyclo[3.1.0]hexane-2,6-dicarboxylic acid (LY354740) reduced inhibitory control in the DRL10 task, although these results were not confirmed when the task conditions were changed to DRL72 (Marek, 2002). This is in contrast to the effects of the mGluR2 PAM in the present study, where LY487379 administration reduced impulsivity of rats, as shown by decreased response rate, increased the number of reinforcers obtained, and a shift in the frequency distribution of responses toward longer IRT durations. It could be speculated that the effects of LY487379 on DRL responding resemble those produced by antidepressants, although until now there had been no data demonstrating the antidepressant-like activity of mGluR2 PAMs and the ineffectiveness of administration of an mGluR2/3 agonist in models detecting antidepressant-like activity (Kłodzińska et al., 1999).

The present microdialysis studies revealed an increase in extracellular norepinephrine levels in the mPFC. It has been widely accepted that norepinephrine transmission is involved in the prefrontal cortical functions related to attentional set shifting and the control of impulsive behavior under conditions of delayed responding. Elevating norepinephrine neurotransmission in the mPFC by administration of the α2-adrenergic receptor antagonist atipamezole (Lapiz and Morilak, 2006) and norepinephrine reuptake blockade by desipramine (Nikiforuk and Popik, 2010) enhanced cognitive performance on ASST in the rat. Likewise, administration of norepinephrine reuptake inhibitors has been shown to improve the efficiency of rats’ responding under the DRL72 schedule (Wong et al., 2000). Therefore, the selective enhancement of mPFC norepinephrine concentration after LY487379 administration might explain its beneficial profile of action in both ASST and DRL72 tasks. In agreement with the present results are the data showing that administration of mGluR2/3 agonist LY379268 also markedly increased the prefrontal norepinephrine level (Lorrain et al., 2005). It should be noted, however, that in the present study the lower dose of LY487379 (i.e., 10 mg/kg), evoking the most robust increase in norepinephrine level, did not affect behavior. This mismatch could be explained by data from Newman et al. (2008) suggesting that an optimal concentration of norepinephrine is required for cognitive improvement, because the norepinephrine reuptake blocker produced beneficial effects on ASST performance only in norepinephrine-lesioned rats.

Increases in extracellular serotonin also probably contribute to at least some of the observed behavioral effects. For example, it is well established that the inhibition of serotonin reuptake affects performance in the DRL task (Sokolowski and Seiden, 1999), whereas serotonin depletion induces opposite effects (Jolly et al., 1999). It is noteworthy that fluoxetine and several other SSRIs were shown to increase inhibitor forces in this task (Sokolowski and Seiden, 1999) but have a relatively modest effect on IRT distribution, and their administration often results in flattened IRT distribution toward a more random pattern of responding. Coadministration of 5-HT2A receptor antagonists facilitates effects of SSRIs on the DRL task performance, producing clear rightward shifts in the IRT distribution (Marek et al., 2005). Thus, the effects of this combination are somewhat similar to what was observed in the present study with the mGluR2 PAM. This parallel may explain robust efficacy of LY487379 in the DRL task because, in addition to the ability to enhance serotonin levels, mGluR2 stimulation is associated with functional 5-HT2A receptor antagonism (Marek et al., 2000).

Serotonin is also involved in the regulation of cognitive flexibility. However, there is strong evidence indicating that this involvement is not the same for different cognitive flexibility domains. For example, serotonin depletion impairs the ability to switch responding between one of the two visual stimuli on a serial discrimination reversal but leaves intact the higher order ability to shift an attentional set (Clarke et al., 2005). It is unclear whether enhanced serotonin levels would have an impact on attentional set shifting. It is worth noting, however, according to a recent study that stimulation of at least one subtype of serotonin receptors may be associated with enhanced performance during the extradimensional set-shifting phase in the task, very similar to the one used in the present study (Burnham et al., 2010).

In contrast to the limited data concerning the impact of mGluR2 activation on norepinephrine and serotonin levels, it has been repeatedly found that mGluR2/3 agonists increase dopamine levels in rat mPFC (Rorick-Keen et al., 2007). Results of the present microdialysis study showed only a modest increase in mPFC dopamine levels after LY487379 administration that did not reach the levels of statistical
significance. This effect was relatively weak compared with drug-induced norepinephrine and serotonin release. Therefore, it seems that the behavioral effects of LY487379 in ASST and DRL72 tasks do not seem to be driven by drug-induced changes in the cortical dopamine levels.

The antipsychotic efficacy of mGluR2 activation in animal models of schizophrenia has been attributed to normalization of enhanced glutamate levels (see Introduction). In fact, agonists of this group of receptors attenuated disruptive effects of NMDAR antagonists on both cognition and the cortical glutamate efflux (Moghaddam and Adams, 1998). Nevertheless, LY379268 had no effect of its own on extracellular glutamate levels (for review, see Imre, 2007). In the present study, the mGluR2 PAM LY487379 produced only a tendency to reduce glutamate levels in mPFC at the doses that affected levels of other neurotransmitters. Thus, it is possible that the effects of LY487379 on executive cognitive functions and inhibitory control cannot be solely attributed to mGluR2-dependent modulation of extracellular glutamate levels, the mechanism that has been proposed as an explanation of the antipsychotic potential of mGluR2/3 agonists.

One should note that the present study was conducted in animals with undisturbed cognitive functioning and therefore one may argue that these results do not readily extrapolate to the situation where cognition is impaired (e.g., in schizophrenia or via pretreatment with drugs like amphetamine or PCP-like psychotomimetics). Indeed, a previous study associated ability of the mGluR2/3 agonist to reverse cognition-imparing effects of PCP with inhibition of PCP-induced cortical glutamate release (Moghaddam and Adams, 1998). Apparently, this mechanism can hardly explain the potential proognitive action of mGluR2 stimulation in intact animals (as in the present study). In fact, suppressed glutamatergic tone under control (undisturbed) conditions presumably is responsible for adverse effects of mGluR2/3 agonists on cognition (Aultman and Moghaddam, 2001). Instead, the present study revealed other nonglutamatergic neurochemical effects of mGluR2 stimulation that may be associated with enhanced cognitive performance. These effects, taken together with the previously reported antipsychotic-like effects of LY487379 (reversal of amphetamine and phencyclidine-induced hyperlocomotion, amphetamine-induced PPI deficits, Galici et al., 2005; attenuation of social novelty discrimination deficits induced by neonatal phencyclidine treatment, Harich et al., 2007), warrant further evaluation of the therapeutic potential of mGluR2/3 agonists.

In conclusion, in the present study selective mGluR2 stimulation enhanced cognitive flexibility and inhibitory behavioral control. Neurochemical changes (enhanced extracellular norepinephrine and serotonin levels in prefrontal cortex) induced by LY487379 are also in favor of cognitive enhancement in the domains critically affected in schizophrenia.

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