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The mGlu2/3 receptor agonist LY379268 blocks the expression of locomotor sensitization by amphetamine

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Abstract

The present experiments assessed the effect of the Group II-specific metabotropic glutamate receptor (mGluR) agonist, LY379268, on the expression of the locomotor sensitization observed following repeated exposure to amphetamine (AMPH). Rats in different groups were administered five injections of AMPH (1 mg/kg ip), one injection every 2–3 days. Two weeks after the last injection, rats were challenged with either AMPH (1 mg/kg ip) or AMPH coinjected with LY379268 (1 mg/kg ip). As expected, AMPH produced levels of locomotion that increased progressively from the first to the fifth injection. This locomotor sensitization was still evident 2 weeks later in rats challenged with AMPH. Rats challenged on this test with AMPH + LY379268, however, showed levels of locomotion similar to those observed following the first AMPH injection. These results indicate that Group II mGluRs can play an important role in the expression of locomotor sensitization by AMPH. The ability of Group II mGluR activation to block the expression of sensitization indicates that it can be targeted as a possible molecular candidate for the development of therapeutic drugs directed at drugs of abuse.

Keywords: Metabotropic glutamate receptor; LY379268; Amphetamine; Sensitization; Locomotor activity

1. Introduction

Repeated intermittent exposure to psychomotor stimulant drugs such as amphetamine (AMPH) has long been known to lead to the development of behavioral sensitization, most often demonstrated as a progressively enhanced locomotor response to the drug (for review, see Kalivas and Stewart, 1991). Exposure to sensitizing regimens of drugs of abuse has also been suggested to enhance the rewarding or incentive motivational properties of these drugs (Robinson and Berridge, 1993) as evidenced by facilitation of their subsequent self-administration (Piazza et al., 1990; Pierre and Vezina, 1997; Lorrain et al., 2000) and enhanced preferences for places associated with these drugs (Lett, 1989; Shippenberg and Heidbreder, 1995).

Brain glutamatergic neurotransmission is known to contribute to the development and expression of behavioral sensitization to psychomotor stimulant drugs (for review, see Wolf, 1998). It has been shown, for example, that in rats preexposed to cocaine, the subsequent microinjection of α-amino-3-hydroxy-5-methyl isoxazole-4-propionic acid (AMPA) into the nucleus accumbens (NAcc) elicited a greater locomotor response than in rats preexposed to saline (Pierce et al., 1996). The injection of the non-*N*-methyl-D-aspartic acid (NMDA) glutamate receptor antagonists, 6,7-dinitroquinoxaline-2,3-dione (DNQX) systemically in mice (Karler et al., 1991) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) into the NAcc of rats (Pierce et al., 1996), has also been reported to prevent the expression of locomotor sensitization, although another antagonist of the AMPA receptor, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(*f*)quinoxaline (NBQX), failed to block the expression of sensitization (Li et al., 1997). These results indicate that changes in glutamatergic neurotransmission in the brain, especially in the NAcc, are important contributors to the expression of psychomotor stimulant-induced sensitization.

The role of metabotropic glutamate receptors (mGluRs) in psychomotor stimulant-induced behavioral sensitiza- tion has also been studied (for review, see Vezina and Kim, 1999). While repeated coinfusion with AMPH into the ventral tegmental area (VTA) of the broad spectrum mGluR

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antagonist, (*RS*)-α-methyl-4-carboxyphenylglycine [(*RS*)-MCPG], has been shown to prevent the development of locomotor sensitization by AMPH (Kim and Vezina, 1998a), this antagonist also elicits a greater locomotor response in AMPH compared to saline preexposed rats when administered intracranially into the NAcc 2 weeks after the last drug preexposure injection (Kim and Vezina, 1998b). It has been suggested that this latter finding may reflect a contribution by Group II mGluRs in the NAcc to the expression of sensitization by psychomotor stimulant drugs (Vezina and Kim, 1999). Consistent with this view, it was recently reported that the Group II mGluR-selective antagonist LY341495 also elicits a greater locomotor response in AMPH compared to saline preexposed rats (Kim and Vezina, 2001). It remains to be directly determined, however, whether the expression of locomotor sensitization by drugs like AMPH is regulated by Group II mGluRs.

LY379268 is a recently developed agonist with highly potent selectivity for Group II mGluRs ($EC_{50} = 2.1–4.6$ nM for Group II but more than 100,000 nM for Group I and some Group III; Schoepp et al., 1999). Its ability to attenuate the acute motor behaviors produced by some doses of AMPH in rats has been reported (Cartmell et al., 1999, 2000). By using this more selective Group II mGluR agonist, the role played by these receptors in the expression of locomotor sensitization by AMPH was further examined in the present experiment.

2. Methods

2.1. Subjects

Male Sprague–Dawley rats weighing 250–275 g on arrival from Harlan Sprague–Dawley (Madison, WI) were housed individually in a 12-h light/dark reverse cycle room with food and water available at all times. They were allowed to acclimate to housing conditions for 6 days before the start of any procedures. All testing was conducted during the animals' dark cycle. Experimental procedures were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Drugs

The selective Group II mGluR agonist LY379268 was graciously provided by Dr. Darryle D. Schoepp (Lilly Research Laboratories, Indianapolis, IN, USA). It was dissolved in 1.2 equivalent of NaOH solution (i.e., 1.2 mol of NaOH solution was used to dissolve 1 mol of LY379268) just prior to use. D-AMPH sulfate (Research Biochemical International, Natick, MA, USA) was dissolved in sterile 0.9% saline either separately or as a mixture with LY379268. All doses refer to the weight of the salt. The dose of LY379268 was chosen based on its ability to block the locomotor activating effects of LY341495 in AMPH preexposed animals and its lack of effect on basal locomotion (Kim and Vezina, 2001).

2.3. Locomotor activity

A bank of 12 activity boxes was used to measure locomotor activity. Each box ($22 \times 43 \times 33$ cm) was constructed of opaque plastic (rear and two sidewalls), a Plexiglas front-hinged door and a tubular stainless-steel ceiling and floor. Two photocells, positioned 3.5 cm above the floor and spaced evenly along the longitudinal axis of each box, estimated horizontal locomotion. Two additional photocells, positioned on the side walls 16.5 cm above the floor and 5 cm from the front and back walls, estimated rearing. Separate interruptions of photocell beams were detected and recorded via an electrical interface by a computer situated in an adjacent room. The activity boxes were kept in a room lighted dimly with red light.

2.4. Design and procedure

The experiment consisted of two phases: a drug preexposure phase and a test for sensitization. During the drug preexposure phase, two different groups of rats were administered AMPH (1 mg/kg ip) on five occasions, one injection every 2–3 days. Immediately after the first and fifth injections, rats were placed in the activity boxes for 1 h. Following the remaining preexposure injections, rats were returned to their home cages. On the test for sensitization, 2 weeks after the last preexposure injection, animals were administered either AMPH (1 mg/kg ip) or a mixture of AMPH and LY379268 (1 mg/kg ip each) and placed immediately in the activity boxes for 2 h. Prior to preexposure injections 1 and 5 and the test drug injection, rats were habituated to the activity boxes for 1 h.

The data were analyzed with two-way between-within ANOVA with test condition (2) as the between factor and either injection (3, preexposure 1 and 5 and test) or time (3, preinjection, or 6, postinjection, 20-min bins) as the within factor. Post-hoc Scheffé comparisons were made according to Kirk (1968).

3. Results

As expected, AMPH produced levels of locomotion that increased progressively from the first to the fifth preexposure injection. This locomotor sensitization was still evident 2 weeks later in rats challenged with AMPH but not in animals tested following coinjection of AMPH and LY379268 (Fig. 1). The two-way between-within ANOVA conducted on the 1-h locomotion counts obtained during the preexposure and test showed significant effects of injections [$F(2,20) = 16.38$, $P < .001$] and post-hoc comparisons revealed significantly lower levels of locomotion in the group challenged with AMPH + LY379268 compared to

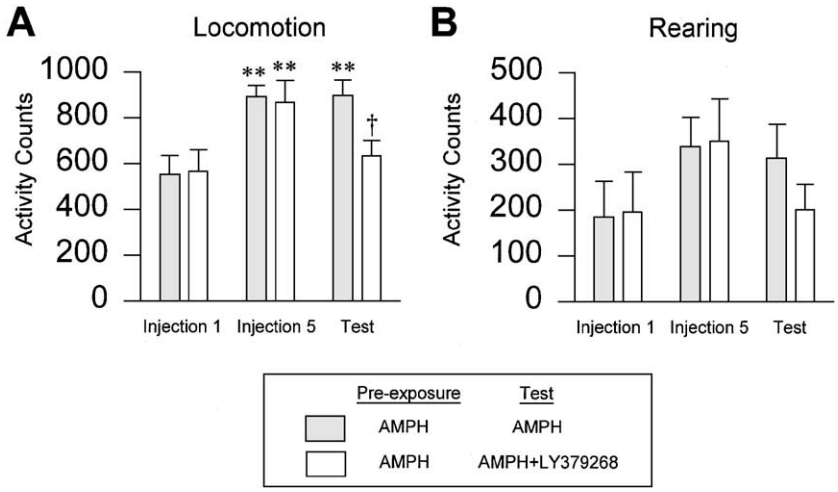

Fig. 1. Locomotion (A) and rearing (B) observed after AMPH preexposure injection (injections 1 and 5) and either AMPH alone or AMPH + LY379268 test injection (test). AMPH-induced locomotor sensitization was observed after the fifth preexposure injection of AMPH. Locomotor activity remained at the same level for injection 5 when tested with AMPH following 2 weeks of withdrawal, but returned to the level for injection 1 when tested with AMPH + LY379268, indicating that the Group II-specific mGluR agonist LY379268 blocks the expression of AMPH-induced locomotor sensitization. The data are shown as group mean (+S.E.M.) 1 h total activity counts observed during preexposure (1-h measurement) and test (first hour of 2-h measurement). Number of rats in each group is six. Symbols indicate significant differences as revealed by post-hoc Scheffé comparisons following two-way between-within ANOVA. $^{**}P < .01$, injection 5 or test compared to injection 1 for each different group. $^{\dagger}P < .05$, AMPH + LY379268 compared to AMPH alone at test.

the group with AMPH alone. The pattern of results observed in the first hour of testing with rearing paralleled those obtained with locomotion but failed to achieve statistical significance. The difference between groups observed with locomotion in the first hour of the test for sensitization was maintained into the second hour of testing. In the case of rearing, the difference between groups became more pronounced and led to a significant overall effect of group (Fig. 2).

When challenged with either AMPH or a mixture of AMPH and LY379268 on the test for sensitization, both groups of rats showed similar increases in locomotion in the first 20-min postinjection compared to levels observed in the 20 min preceding the injection ($P < .001$). However, rats tested with AMPH + LY379268 subsequently showed a more precipitous decrease in activity levels compared to AMPH-tested animals, indicating again that, when it was coinjected with LY379268, AMPH failed to express locomotor sensitization. The two-way between-within ANOVA conducted on the locomotion data (Fig. 2A) revealed significant effects of group [$F(1,10) = 17.70$, $P < .003$] and time [$F(5,50) = 38.89$, $P < .001$]. Post-hoc Scheffé comparisons

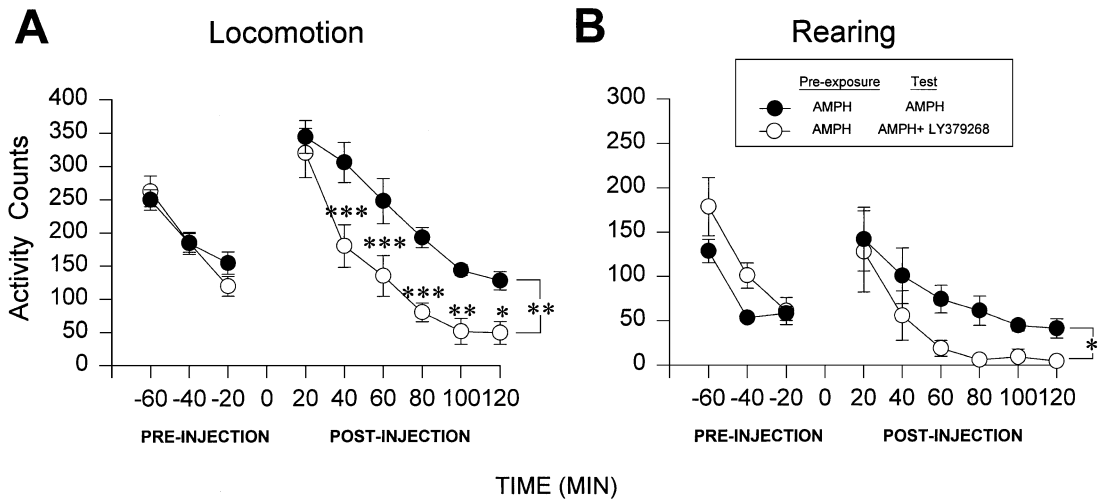

Fig. 2. The Group II-specific mGluR agonist LY379268 blocks the expression of AMPH-induced locomotor sensitization. The data are shown as group mean (±S.E.M.) locomotion (A) and rearing (B) counts obtained during the 1 h preceding (−60 to 0 min) and the 2 h following the sensitization test injection (0–120 min). The test injection was administered at time 0. Number of rats in each group is six. Symbols indicate significant differences as revealed by post-hoc Scheffé comparisons following two-way between-within ANOVA. $^{***}P < .001$, $^{**}P < .01$ and $^{*}P < .05$, AMPH + LY379268 compared to AMPH alone at indicated times. Asterisks to the right of the graphs in A and B indicate significant overall group differences.

showed that the significantly lower levels ($P < .05 - .001$) of locomotion displayed by AMPH + LY379268 animals starting in the second 20-min bin persisted for the remainder of the 2-h test period. The ANOVA conducted on the rearing data (Fig. 2B) also revealed significant effects of group [$F(1,10) = 5.15$, $P < .05$] and time [$F(5,50) = 9.03$, $P < .001$]. Despite the significant overall effect of group, however, post-hoc Scheffé comparisons failed to show significant differences between groups at any time points during the 2-h postinjection time period.

4. Discussion

The present behavioral study found that the Group II-specific mGluR agonist, LY379268, blocked the expression of AMPH-induced locomotor sensitization when coinjected with this drug. This finding indicates that Group II mGluRs can be recruited to regulate the expression of sensitization by psychomotor stimulant drugs and suggest potential therapeutic strategies involving these receptors for use in drug abuse.

Previous studies have shown that the broad spectrum mGluR antagonist, (*RS*)-MCPG, produced hyperlocomotion when microinjected into the NAcc in AMPH compared to saline preexposed rats (Kim and Vezina, 1998b). The lack of this ligand's mGluR subtype selectivity notwithstanding, it was suggested that this finding may reflect a role for Group II mGlu autoreceptors. It was hypothesized, for example, that blocking these autoreceptors in the NAcc could lead to increased extracellular levels of glutamate and DA in this site and subsequently to hyperlocomotion (Vezina and Kim, 1999). On the other hand, activation of mGluRs (including Group II) in the NAcc by 1-amino-cyclopentane-*trans*-1,3-dicarboxylic acid [(1*S*,3*R*)-ACPD] did not produce different locomotor responses in AMPH compared to saline preexposed rats (Kim and Vezina, 1998b), suggesting that this potential plasticity involving Group II mGlu autoreceptors in the NAcc may be under tight control by endogenously released glutamate in AMPH preexposed rats. In these previous experiments, however, it was not tested what effect activation of Group II mGluRs might produce on locomotion in AMPH preexposed rats in the presence of a drug, like AMPH (Reid et al., 1997; Smith et al., 1995), that is known to evoke glutamate release in the NAcc. By using a more specific ligand for Group II mGluRs, LY379268, the present experiment revealed that activation of Group II mGluRs in the brain actually blocks the production of hyperlocomotion by AMPH in AMPH preexposed rats. It is conceivable that this effect of LY379268 resulted from the concurrent activation of Group II mGluR autoreceptors (possibly up-regulated as a result of previous exposure to AMPH) and the consequential reduction of glutamate release that otherwise would be higher in the presence of AMPH alone. This possibility remains to be tested.

Studies have shown that multiple subtypes of mGluRs can serve as presynaptic autoreceptors to reduce glutamatergic neurotransmission. For example, Group II mGluRs are known to serve as autoreceptors in corticostriatal synapses (Lovinger and McCool, 1995), whereas both Groups I and III mGluRs are known to do so in area CA1 of the adult hippocampus (Gereau and Conn, 1995; Manzoni and Bockaert, 1995). In the present experiment, the locomotor reducing effect of LY379268 in AMPH-sensitized rats is most likely due to the activation by this agonist of Group II mGluRs. LY379268 exhibits highly potent specificity for these compared to other subtypes of mGluRs. EC_{50} values for Group II mGluRs range from 3 to 8 nM, while for Group I, these are greater than 100 μM, and for Group III, range from 39 nM to 100 μM (Schoepp et al., 1999).

A number of studies have suggested that glutamate may perhaps contribute as importantly as DA to the expression of sensitization by psychomotor stimulant drugs. For example, previous exposure to these drugs leads to enhanced stimulant-induced DA (Kalivas and Stewart, 1991; Vezina, 1996) and glutamate (Pierce et al., 1996; Reid and Berger, 1996) overflow in the NAcc. Moreover, it has been recently shown that increased glutamate neurotransmission and activation of D1 DA receptors in the NAcc, neither of which is by itself sufficient, together contribute to the expression of locomotor sensitization by AMPH (Kim et al., 2001). These findings well indicate the importance of glutamate–DA interactions in the NAcc in the expression of sensitization. Glutamatergic neurotransmission mediated by mGluRs in the NAcc is also known to interact with DA neurotransmission as evidenced by effects on DA overflow in this site (Arai et al., 1996; Taber and Fibiger, 1995) as well as by locomotor activity (Attarian and Amalric, 1997; Kim and Vezina, 1997; Meeker et al., 1998). Activation of Group II mGluRs by systemic injection of LY379268 has also been shown to influence DA release in the medial prefrontal cortex (Cartmell et al., 2001), suggesting that it may impact the generation of locomotor activity by influencing DA as well as glutamate levels in brain. The sensitization regimen used in the present experiment is known to enhance the overflow of NAcc DA in response to AMPH in AMPH compared to saline preexposed rats (e.g., Lorrain et al., 2000). It is therefore possible that the ability of LY379268 to block the expression of locomotor sensitization by AMPH observed in the present experiment may be due to its ability to block the enhanced release of DA (and glutamate) by AMPH in the NAcc. This question remains to be answered and is currently under investigation in this laboratory.

In conclusion, the present findings support an important role for Group II mGluRs in the expression of psychomotor stimulant drug-induced locomotor sensitization. The possibility that these receptors mediate this effect by recruiting

glutamate–DA interactions in different brain regions remains to be explored.

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