The Regulation of Dopamine Transmission by Metabotropic Glutamate Receptors

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ABSTRACT

Receptor subtype nonselective metabotropic glutamate receptor (mGluR) agonists have been shown to regulate the release of dopamine. The eight mGluR subtypes have been pharmacologically categorized into three groups, and the present study used in vivo microdialysis to examine the capacity of mGluR subgroup-selective drugs to modulate the extracellular levels of dopamine in the nucleus accumbens. By administering the drugs in the dialysis buffer, it was found that the group 3 mGluR agonist L-amino-4-phosphonobutyrate produced a dose-dependent reduction in extracellular dopamine, whereas the group 1 agonist 3,5-dihydroxyphenylglycine was ineffective.

The group 2 agonist (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine produced a reduction that was biphasic with respect to dose. The group 2/3 antagonist α-methyl-4-phosphonophenylglycine elicited a dose-dependent increase in extracellular dopamine that was antagonized by coperfusion with either the L-type calcium channel blocker diltiazem or the group 3 agonist L-amino-4-phosphonobutyrate. These data demonstrate that group 3 and to a lesser extent group 2 mGluR may presynaptically regulate dopamine release or reuptake. Moreover, there exists significant in vivo glutamatergic tone on group 2/3 mGluRs to suppress extracellular dopamine levels.

Glutamate synaptic transmission in the mammalian central nervous system is mediated by both ionotropic and metabotropic receptors (mGluR; Hollmann and Heinemann, 1994). Stimulation of these receptors produces both pre- and postsynaptic changes in ion conductance and intracellular signal transduction. Presynaptic actions include both autoreceptor-like effects to regulate the release of glutamate, as well as heterosynaptic effects to regulate the release of other neurotransmitters. Notably, a number of studies have attempted to demonstrate that the stimulation of glutamate receptors alters the release of dopamine (Nicholls, 1993; Conn and Pin, 1997; Ottersen and Landsend, 1997). To this end, it has been shown that relatively high doses of ionotropic glutamate receptor agonists release dopamine (Imperato et al., 1990a; Mount et al., 1990; Pierce and Kalivas, 1996). More recently, studies have examined the capacity of mGluR agonists to alter dopamine release with apparently conflicting results. Thus, mGluR agonists have been reported to augment and reduce the release of dopamine in the striatum and nucleus accumbens (Ohno and Watanabe, 1995; Taber and Fibiger, 1995; Feenstra et al., 1998; Verma and Moghaddam, 1998). One possible explanation for the divergence between existing studies is that there exist eight subtypes of mGluRs, and most published studies have used agonists and antagonists that do not discriminate between the receptor subtypes.

Several cDNAs encoding mGluRs have been characterized; the receptors are divided into three subgroups based upon receptor pharmacology and coupling to intracellular transduction pathways (Nakanishi, 1992; Watkins and Collingridge, 1994; Conn and Pin, 1997). Group 1 consists of mGluR1 and mGluR5, which are positively coupled to phospholipase C, group 2 consists of mGluR2 and mGluR3 and are negatively coupled to adenylyl cyclase, and group 3 (mGluR4/6/7/8) are also negatively coupled to adenylyl cyclase. Over the last five years, agonists and antagonists that are relatively selective for the subgroups of mGluRs have emerged. In the present report, agonists and antagonists selective for mGluR subgroups were perfused through microdialysis probes located in the nucleus accumbens, and changes in extracellular dopamine were quantified to estimate the capacity of mGluRs to modulate dopamine release.

Materials and Methods

Animal Housing and Surgery. Male Sprague-Dawley rats (Simonsen Laboratories, Gilroy, CA) were individually housed with food

ABBREVIATIONS: ACPD, 1S,3R-1-amino-1,3-cyclopentanedicarboxylate; DHPG, 3,5-dihydroxyphenylglycine; DCG-4, (2S,1'R,2'R,3'R)-2-(2,3-dicarboxycyclopropyl)glycine; L-AP4, L-amino-4-phosphonobutyrate; MPPG, α-methyl-4-phosphonophenylglycine; mGluR, metabotropic glutamate receptor.
and water made available ad libitum. A 12-h light/dark cycle was used with lights on at 6:30 AM. Before surgery, rats were anesthetized with Equithesin (Washington St. University, Pullman, WA; 3.0 mg/kg) and mounted in a stereotaxic apparatus. Bilateral dialysis guide cannulas (14 mm, 20-gauge stainless steel) were implanted 3 mm dorsal to the nucleus accumbens (9.0 mm AP; 1.5 mm ML; −0.5 mm DV; relative to the interaural line according to Pellegrino et al., 1979) and cemented in place by affixing dental acrylic to three stainless steel screws tapped into the skull.

**Drugs.** The following compounds were dissolved in microdialysis buffer for administration through the dialysis probe: diltiazem (Research Biochemicals, Inc., Natick, ME), 1S,3R-1-amino-1,3-cyclopentane dicarboxylic acid (ACPD; Tocris Cookson, Baldinon, MO), 3,5-dihydroxyphenylglycine (DHPG; Tocris Cookson), (2S,1R,2R,3R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-4; Tocris Cookson), l-amino-4-phosphonobutyrate (l-AP4; Tocris Cookson), α-methyl-4-phosphonophenylglycine (MPPG; Tocris Cookson).

**Microdialysis and Drug Administration.** The dialysis probes were constructed as described (Robinson and Wishaw, 1988) with 1.5 to 2.0 mm of active dialysis membrane exposed at the tip. The probes were inserted through the guide cannula into the nucleus accumbens the night before the experiment. The next day, dialysis buffer (2.8 mM KCl, 140 mM NaCl, 1.4 mM CaCl₂, 1.2 mM MgCl₂, 5.0 mM d-glucose, plus 0.2 mM phosphate-buffered saline to give a pH value of 7.4 and a final sodium concentration of 140.7 mM) was advanced through the probe at a rate of 1.9 μl/min via a syringe pump (Harvard Instruments, Boston, MA) for 2 h, after which three to six 20-min baseline samples were collected. Various compounds were inserted through the guide cannula into the nucleus accumbens via perfusion through the dialysis probe. Dose-response curves for the capacity of various mGluR agonists and antagonists to alter extracellular dopamine content were determined by passing increasing concentrations of drug through the dialysis probe after collecting baseline samples. Typically, three different doses were used in each experiment and each dose was passed through the probe for 100 min (i.e., five 20-min dialysis samples). The drugs tested were chosen based on mGluR subgroup selectivity (see Conn and Pin, 1997 for overview of drug selectivity) and included the nonselective agonist ACPD, the group 1 agonist DHPG, the group 2 agonist (2S,1R,2R,3R)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-4), the group 3 agonist l-AP4, and the group 2/3 antagonist MPPG. In some experiments either the L-type calcium channel blocker diltiazem (10 μM) or l-AP4 (10 μM) was perfused through the dialysis probe after collecting baseline samples, and remained in the perfusion buffer for the duration of the experiment. After the first 100 min of perfusion with diltiazem or l-AP4, MPPG (5 and 50 μM) was coperfused through the dialysis probe.

**Quantification of Dopamine.** For the measurement of extracellular dopamine, samples were collected into microfuge tubes containing 20 μl of mobile phase (0.1 M citric acid, 75 mM Na₂HPO₄, 1.5 mM heptane sulfonic acid, 0.1 mM EDTA, 15% methanol, v/v, pH = 4.2) plus 2.0 pmol of dihydroxybenzylamine as an internal standard. After collection, all samples were frozen at −80°C until analyzed. The samples were subsequently thawed and placed in an autosampler (Gilson Medical Supplies, Inc., Middleton, WI) connected to an HPLC system with electrochemical detection. The dopamine was separated using a 10-cm C₁₈ reversed phase column (Bioanalytical Systems, West Lafayette, IN) and oxidized/reduced using coulometric detection (ESA Inc., Bedford, MA). Three electrodes were used: a preinjection port guard cell (+0.4 V) to oxidize the mobile phase, an oxidation analytical electrode (+0.3 V), and a reduction analytical electrode (−0.14 V). Peaks were recorded on a chart recorder and compared to an external standard curve (10–1000 fmol).

**Histology.** After the dialysis experiment, the rats were given an overdose of pentobarbital (>100 mg/kg i.p.) and perfused intracardially with phosphate-buffered saline followed by 10% formalin. The brain was removed and stored in 10% formalin for at least 1 week. The brains were then blocked and coronal sections (100 μm) were taken at the level of the nucleus accumbens with a vibratome. The sections were mounted on gelatin-coated slides and stained with Cresyl violet. Probe and cannula placements were determined according to the atlas of Paxinos and Watson (1986) by an individual unaware of the rats’ neurochemical response.

**Data Analysis.** For statistical analysis of the dose-response curves (Figs. 1 and 2A), the last two samples obtained from each dose of drug were averaged and compared to the average of the last two

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**Fig. 1.** Effect of mGluR agonists on extracellular dopamine in the nucleus accumbens. Five 20-min baseline dialysis samples (B) were collected followed by the perfusion through the dialysis probe of increasing concentrations of various mGluR agonists. Each concentration of drug was perfused for 100 min (5 dialysis samples). For the DCG-4 data, two separate groups were used, one group examining doses from 0.01 to 1.0 nM (N = 6) and another group examining doses from 0.1 to 10.0 nM (N = 5). Right (each drug): average of the last two dialysis samples at each dose. The data were statistically evaluated using a one-way repeated measures ANOVA, except for DCG-4, which was analyzed using a one-way ANOVA. *p < .05 comparing all doses to baseline using a Dunnett’s test.
introduction of diltiazem or L-AP4. The data in A, B, and C were statistically evaluated using a one-way repeated measures ANOVA followed by a least significant difference test. The data in D was evaluated using a two-way ANOVA with repeated measures over drug treatment, followed by a least significant difference test. *p < .05 comparing all treatments to the levels after diltiazem or L-AP4 alone in B and C.

baseline samples using a one-way repeated measures ANOVA. If a significant F score (p < .05) was identified, post hoc comparisons were made using a Dunnett’s test for comparison with basal levels of dopamine. When a pretreatment altered the basal levels of extracellular dopamine (Fig. 2D), the levels were normalized to the percentage of change from the pharmacologically altered baseline and a least significant difference test was performed for each comparison (Milliken and Johnson, 1984).

Results

Effect of mGluR Agonists on Extracellular Dopamine. Figure 1 shows that neither the nonselective agonist ACPD (5–500 μM) nor the group 1 agonist DHPG (1–100 μM) altered the extracellular level of dopamine in the nucleus accumbens. The group 2 agonist DCG-4 produced a biphasic reduction in extracellular dopamine (F(1,4,43) = 2.85, p = .036). Neither the lowest two doses (0.01 or 0.1 μM) nor the highest dose (10.0 μM) of DCG-4 used altered extracellular dopamine content compared with baseline levels. However, the intermediate dose (1.0 μM) elicited a 35% reduction in extracellular dopamine. The group 3 agonist L-AP4 produced a dose-dependent decrease in extracellular dopamine content with 50 and 500 μM, causing a significant reduction (F(3,18) = 6.55, p = .007).

Group 2/3 mGluR Antagonist Increases Extracellular Dopamine. Figure 2 shows the effect of the group 2/3 antagonist MPPG on extracellular dopamine content in the nucleus accumbens. MPPG produced a dose-dependent increase in extracellular dopamine levels, with 50 and 500 μM eliciting a statistically significant increase (F(3,23) = 5.09, p = .013). To determine if this effect of MPPG was calcium-dependent, a group of rats was perfused with the L-type calcium channel blocker diltiazem (10 μM) before coperfusing MPPG (5 and 50 μM). Figure 2B shows that diltiazem alone reduced the basal levels of extracellular dopamine by about 35%, and MPPG did not significantly elevate extracellular dopamine levels in the presence of diltiazem (F(4,20) = 4.85, p = .007). To determine if this effect was mediated by group 3 receptors, the selective group 3 agonist L-AP4 was perfused through the dialysis probe before coperfusing MPPG. Figure 2C shows that L-AP4 (10 μM) significantly reduced the basal levels of dopamine by about 30%. Although the lowest dose of MPPG did not alter extracellular dopamine levels in the presence of L-AP4, the higher dose (50 μM) partly overcame the reduction in levels elicited by L-AP4 (F(3,31) = 12.47, p < .001). Both L-AP4 and diltiazem reduced the basal levels of extracellular dopamine (see Fig. 1); Fig. 2D illustrates the data shown in Fig. 2A-C, converted to percentages of change from the levels of dopamine after pretreatment with these drugs. It can be seen that MPPG (50 μM) alone increased dopamine levels 2.4-fold over baseline and that this elevation was significantly reduced in the presence of either diltiazem or L-AP4 (treatment F(2,16) = 6.181, p = .012; dose F(2,4) = 18.72, p < .001; interaction F(4,32) = 3.44, p = .21).

Histology. Figure 3 illustrates the location of the microdialysis probes in the nucleus accumbens. The majority of dialysis probes were located in the rostromedial quadrants of the nucleus accumbens. Thus, the probes were primarily in the shell, medial core, and rostral pole subcompartments of the nucleus accumbens (Deutch et al., 1993; Zahn and Heimer, 1993). In addition, a portion of the active region of many probes traversed the ventral aspect of the caudate. No neurotoxicity beyond the mechanical destruction produced by...
insertion of the dialysis probe was apparent in the Nissl-stained tissue after any of the drug treatments.

**Discussion**

Selective pharmacological stimulation of subgroups of mGluRs was found to differentially modulate extracellular dopamine in the nucleus accumbens. Groups 2 and 3 mGluR agonists reduced the levels of extracellular dopamine, whereas group 1 agonist was ineffective. The reduction in dopamine was dose-dependent after perfusion of the group 3 mGluR agonist 1-AP4, and the dose-response curve was biphasic after the group 2 agonist DCG4, with a significant reduction occurring at 1.0, but not at 0.1 or 10.0, µM. Moreover, the blockade of groups 2 and 3 mGluRs elicited a dose-dependent increase in extracellular dopamine, suggesting the presence of significant endogenous glutamatergic tone on these receptors.

**Regulation of Extracellular Dopamine Content by mGluRs.** Many studies have concluded that mGluR receptors regulate dopamine release in the nucleus accumbens or striatum. With the exception of a recent report (Moghaddam and Adams, 1998), all of these studies have used the nonselective mGluR agonist ACPD, and the resulting data has been divergent. Using microdialysis to perfuse ACPD into the nucleus accumbens or striatum, most investigators have reported increases in extracellular dopamine (Ohno and Watanabe, 1995; Taber and Fibiger, 1995; Arai et al., 1996; Verma and Moghaddam, 1998). However, Taber and Fibiger (1995) demonstrated that the effect of ACPD on dopamine release in the nucleus accumbens was biphasic with respect to dose and that lower doses reduced extracellular dopamine content. Moreover, a number of studies found that ACPD reduced stimulated dopamine release (Taber and Fibiger, 1995; Feenstra et al., 1998; Verma and Moghaddam, 1998). In the present study, no effect by ACPD was found on extracellular dopamine over a dose range of 5 to 500 µM. However, when subgroup-selective agonists were administered, no agonist elicited an increase, whereas the group 3 agonist produced a dose-dependent reduction in extracellular dopamine content. It is unclear why increases in extracellular dopamine by ACPD were not measured in the present study. One possibility is that the doses used were not high enough to evoke release. Previous studies used 1.0 mM ACPD to increase extracellular dopamine in the nucleus accumbens versus the maximum of 500 µM ACPD in the current study (Ohno and Watanabe, 1995; Taber and Fibiger, 1995). Moreover, all studies showing enhanced release of dopamine by lower doses of ACPD were conducted in the striatum, which may be more sensitive to mGluR stimulation than the nucleus accumbens (Arai et al., 1996; Verma and Moghaddam, 1998).

**Regulation of Extracellular Dopamine Content by Group 3 mGluRs.** The possible regulation of dopamine release in the nucleus accumbens by group 3 mGluRs is supported by anatomical studies showing the expression of moderate to high levels of mRNA-encoding mGluR7 in the ventral mesencephalic region containing the dopamine cells that project to the nucleus accumbens (Ohishi et al., 1995). In addition, a low to moderate density of mRNA-encoding mGluR4 and mGluR7 is present in neurons in the nucleus accumbens, suggesting a postsynaptic as well as a presynaptic action by group 3 mGluR agonist administration (Ohishi et al., 1995; Testa et al., 1995).

The stimulation of group 3 mGluRs reduced extracellular dopamine levels in the nucleus accumbens. This observation implicates a role for group 3 mGluRs in the previous observations that the nonselective mGluR agonist ACPD reduces the capacity of electrical stimulation (Taber and Fibiger, 1995), handling (Feenstra et al., 1998), or K+ (Verma and Moghaddam, 1998) to increase extracellular dopamine content. The reduction in extracellular dopamine by the group 3 agonist 1-AP4 was blocked by the group 2/3 antagonist MPPG. Moreover, MPPG alone increased extracellular dopamine content in a dose-dependent manner. This latter observation indicates the presence of substantial in vivo glutamatergic tone on the group 2/3 mGluRs to presynaptically inhibit dopamine release. The fact that MPPG-induced elevation in extracellular dopamine was inhibited by blocking calcium channels suggests that the release depends upon vesicular exocytosis (Westerink, 1995).

**Regulation of Extracellular Dopamine Content by Group 2 mGluRs.** In addition to group 3 receptors, a reduction in extracellular dopamine was also elicited by the group 2 mGluR agonist DCG-4. However, unlike the reduction in extracellular dopamine produced by the group 3 agonist 1-AP4, the decrease by DCG-4 was biphasic with respect to dose. The biphasic dose-response curve may arise from the fact that DCG-4 has only 10-fold selectivity as an agonist for group 2 mGluRs versus NMDA receptors (Hayashi et al., 1993). Given that NMDA agonists can enhance dopamine release (Imperato et al., 1990b; Ohno and Watanabe, 1995; Pap and Bradberry, 1995), the stimulation of NMDA receptors by higher doses of DCG-4 may mask the reduction in extracellular dopamine produced by selective stimulation of group 2 mGluRs. Alternatively, group 2 mGluRs may not have a presynaptic location on dopamine terminals. The anatomical data to date do not support the expression of high levels of mRNA encoding either mGluR2 or mGluR3 mRNA in dopamine cells in the ventral mesencephalon (Ohishi et al., 1993, 1995; Testa et al., 1995). Moreover, the systemic administration of the group 2 mGluR agonist LY3354740 does not alter extracellular dopamine content in the nucleus accumbens although only a single dose was used (Moghaddam and Adams, 1998). Thus, it is possible that the biphasic effect observed in the present study may arise from multiple actions in the nucleus accumbens on nondopaminergic elements. For example, group 2 mGluRs have an autoregulatory effect on excitatory transmission in the nucleus accumbens (Manzoni et al., 1997) or may modulate spiny neurons having inhibitory feedback onto dopamine perikarya in the ventral mesencephalon (Kalivas et al., 1993).

**Lack of Effect by Group 1 mGluRs on Extracellular Dopamine.** The group 1 agonist DHPG was without effect on extracellular dopamine content in the nucleus accumbens. Consistent with a lack of heterosynaptic group 1 receptors on dopamine terminals, the dopamine-rich cell groups in the ventral mesencephalon do not express large amounts of mRNA for mGluR1 or mGluR5 (Shigemoto et al., 1992; Testa et al., 1994). In contrast, very high levels of mGluR5 mRNA and protein are found in the nucleus accumbens, suggesting a postsynaptic location of receptors (Shigemoto et al., 1993; Romano et al., 1995). Indeed, recent studies have demonstrated the presence of mGluR1a and mGluR5 immunoreac-
tivity and mRNA in striatal and nucleus accumbens spiny neurons (Tallaksen-Greene et al., 1998). A postsynaptic location of group 1 receptors is consistent with findings that DHPG was without effect on evoked excitatory responses in the nucleus accumbens (Manzoni et al., 1997). The presence of postsynaptic group 1 mGluRs in the nucleus accumbens is further supported by the recent finding that the microinjection of DHPG into the nucleus accumbens elicits a dose-dependent elevation in locomotor activity that is not affected by coadministration of dopamine receptor antagonists (Swanson et al., 1998).

**Summary.** The present study uses microdialysis to clarify the role of mGluR subtypes on regulating dopamine release. It was found that group 3, and to a lesser extent group 2, mGluR agonists reduce the level of extracellular dopamine. Moreover, blockade of group 2/3 receptors resulted in an increase in extracellular dopamine, supporting the presence of significant endogenous glutamatergic tone on these receptors.

**References**


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