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Activation of group III metabotropic glutamate receptors inhibits basal and amphetamine-stimulated dopamine release in rat dorsal striatum: an in vivo microdialysis study

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Abstract

Group III metabotropic glutamate (mGlu) receptors are negatively coupled to adenylate cyclase and are distributed pre-synaptically in the striatum. A behavioral study previously conducted in this laboratory shows that activation of this group of mGlu receptors attenuates acute amphetamine-stimulated motor activity. By administering a group III selective agonist or antagonist via the dialysis probe, the present study employed in vivo microdialysis to evaluate the capacity of the group III selective agents to alter extracellular levels of dopamine in the dorsal striatum of normal and amphetamine-treated rats. It was found that the group III agonist L-2-amino-4-phosphonobutyrate (L-AP4) dose-dependently (1, 10 and 100 μM) reduced basal levels of extracellular dopamine. In contrast, the group III antagonist α-methyl-4-phosphonophenylglycine (MPPG) dose-dependently (10, 50 and 250 μM) elevated the basal release of extracellular dopamine. This elevation was antagonized by co-perfusion of L-AP4. Perfusion of 5-μM amphetamine through the dialysis probe increased extracellular dopamine in the dorsal striatum. Co-perfusion of L-AP4 (100 μM) significantly reduced amphetamine-stimulated dopamine levels, whereas co-perfusion of L-AP4 (100 μM) and MPPG (100 μM) did not alter the capacity of amphetamine to elicit dopamine release. The data obtained from this study demonstrate the presence of a tonically active glutamatergic tone on group III mGlu receptors in the dorsal striatum to pre-synaptically regulate basal dopamine release in an inhibitory fashion. Moreover, activation of L-AP4-sensitive group III mGlu receptors can suppress the phasic release of dopamine induced by a dopamine stimulant amphetamine.

Keywords: Glutamate receptor, metabotropic; Excitatory amino acid; Caudoputamen; Nucleus accumbens; Basal ganglia; Drug of abuse

1. Introduction

Group III metabotropic glutamate (mGlu) receptors, which consist of mGlu4/6/7/8 subtypes, are negatively coupled to the adenylate cyclase and cAMP formation (Conn and Pin, 1997). With the recently developed agonists/antagonists that are relatively selective for this group of mGlu receptors, functional studies have emerged over the last few years to define physiological roles of group III mGlu receptors in the regulation of central nervous system functions. Available data demonstrate that group III mGlu receptors are involved in striatal neurotoxicity and extrapyramidal motor modulation. For instance, activation of the group III mGlu receptors with a selective agonist, L-2-amino-4-phosphonobutyrate (L-AP4, Tones et al., 1995), protects striatal neurons from excitotoxicity in vivo and in vitro (Bruno et al., 1996; Gasparini et al., 1999). In behavioral studies, the group III agonists produce anti-convulsive and anti-epileptogenic effects in rats and mice (Abdul-Ghani et al., 1997; Gasparini et al., 1999; Ghauri et al., 1996; Tang et al., 1997). Deficiency of mGlu7 receptors in knockout mice causes spontaneous epileptic seizures (Gasparini et al., 1999). More interestingly, a recent work conducted in this laboratory shows that L-AP4 injected into the dorsal striatum attenuates motor activity induced by a psychostimulant, amphetamine (Mao and Wang, 2000a). By using a group III selective antagonist, α-methyl-4-phosphonophenylglycine (MPPG) (Bedingfield et al., 1996; Salt and Turner, 1996), we were able to reverse the inhibitory action of L-AP4 on amphetamine-stimulated behavior (Mao and Wang, 2000a). Apparently, a glutamater-

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gic tone on group III mGlu receptors exists in the striatum that participates in the modulation of local neuronal activities under normal and stimulated conditions.

Mechanisms underlying the group III regulation of striatal functions may involve both pre- and post-synaptic processes. Since mGlu4 and mGlu7 receptors are expressed in the medium-sized projection neurons of striatum (Bradley et al., 1999; Kosinski et al., 1999; Tanabe et al., 1993; Testa et al., 1994), the group III agonist may achieve its actions by interacting with the post-synaptic receptors. Alternatively, the group III agonist may regulate local transmitter release pre-synaptically. This could involve the regulation of glutamate release via an autoreceptor mechanism, as well as dopamine release via a heteroreceptor mechanism. Using an in vivo microdialysis technique, two studies have recently been attempted to define the influence of the subgroup selective agonists on dopamine release in the rat nucleus accumbens (Bruton et al., 1999; Hu et al., 1999). The data from these studies imply that the regulation of dopamine release is subgroup-specific. The group II (mGlu2/3 subtypes that are also negatively coupled to the adenylate cyclase) or III agonists reduce, whereas the group I (mGlu1/5 subtypes that are positively coupled to phosphoinositide hydrolysis) agonists elevate, the release of dopamine in the nucleus accumbens. However, to our knowledge, no report has been published addressing the capacity of the group III agonist to alter basal and amphetamine-stimulated dopamine release in the dorsal striatum (caudoputamen).

This study was, therefore, designed to investigate the role of group III mGlu receptors in the regulation of basal and stimulated release of dopamine in the rat dorsal striatum. In chronically cannulated rats, the group III agonist, L-AP4, or antagonist, MPPG, was perfused through microdialysis probes located in the dorsal striatum to locally enhance or reduce the glutamatergic tone on group III mGlu receptors, respectively. Alterations in basal and amphetamine-stimulated dopamine levels were quantified to define the ability of group III mGlu receptors to modulate dopaminergic transmission.

2. Materials and methods

2.1. Animals and surgery

Adult male Wistar rats (225–249 g, Charles River, New York, NY) were individually housed in clear plastic cages in a controlled environment at a constant temperature of 23°C and humidity of 50 ± 10% with food and water available ad libitum. The animal room was on a 12/12-h light/dark cycle with lights on at 0700 h. All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee. Rats were anesthetized with 4% chloral hydrate (240 mg/kg, i.p.) and placed in a David Kopf stereotaxic holder. A unilateral microdialysis guide cannula (11 mm, 21-gauge stainless steel, Plastics One, Roanoke, VA) was implanted at the coordinates of 1–2 mm anterior to bregma, 2.5 mm right or left to midline and 4 mm below surface of skull (Paxinos and Watson, 1986). A plastic stylet (11 mm, Plastics One, Roanoke, VA) was screwed into the guide cannula after solidification of the dental acrylic to two stainless steel screws that were tapped into the skull. Rats were given a post-operative analgesic (Toradol, 3 mg/kg, s.c.) and were allowed at least 5 days for recovery from surgery before the microdialysis experiments were carried out.

2.2. Microdialysis probe construction

The concentric microdialysis probes with selectively permeable membrane were constructed according to the methods of Johnston and Justice (1983) and Vezzani et al (1985). Briefly, the ends of two 90-cm long fused silica (0.105 mm o.d. and 0.039 mm i.d., Polymicro Technologies, Phoenix, AZ) were inserted into a 4.0-mm long 25-gauge dialysis tubing (0.2 mm o.d., Plastics One, Roanoke, VA). The distance between the ends of the two fused silica tubing was 2.0 mm, which constitutes the actively dialyzing surface of the porous membrane exposed at the tip. A 7-mm selectively permeable membrane (6000 MW cutoff, Spectrum Medical Industries, Houston, TX) covered the probe tip, encompassing both fused silica lines. A 0.3–0.5-mm distance separated the distal membrane tip from the end of the long fused silica tubing. The top and bottom portions of the membrane in contact with brain tissue that was not intended for perfusion were sealed with polyamide resin (Alltech Associate, Deerfield, IL). One fused silica line was connected to a BAS MD-1001 baby bee syringe drive and BAS MD-1020 bee hive controller (Bioanalytical Systems, West Lafayette, IN) and served as the inlet tube. The second fused silica line was placed into a 0.5-ml microcentrifuge tube that collected dialysate. Each microdialysis probe was flushed with double-deionized water at a perfusion rate of 0.30 μl/min for at least 12 h before each experiment to assure probe fidelity.

2.3. In vivo microdialysis perfusion

On the day of the experiment, the stylet was removed and a dialysis probe was inserted through the guide cannula with 2 mm protruding the opening of the guide cannula in unanesthetized rats. Artificial cerebrospinal fluid (ACSF, in mM: NaCl 149.4, $CaCl_2$ 1.1, KCl 3.2, $MgCl_2$ 1.2, and glucose 6.1, adjusted to pH of 7.4 with 1 N NaOH, filtered by a 0.2-μm vacuum filtration, and de-

gassed with helium for at least 30 min before use) was advanced through the probe at a rate of 2 μl/min via a BAS syringe drive for 3–5 h to allow basal transmitter levels to stabilize. After this washout period, dialysate samples were collected into microcentrifuge tubes containing 5-μl 0.1 M $HClO_4$ at 15-min intervals (a final volume of 35 μl per each collected sample) throughout the experiment. The first four samples were collected for baseline measurements. After collecting the baseline samples, drugs were directly perfused into the dorsal striatum through the dialysis probe. To establish the dose–response curves for the capacity of L-AP4 or MPPG to alter extracellular dopamine content, three increasing concentrations of either agent were used. The drug at each concentration was passed through the probe for 45 min during which three 15-min dialysis samples were collected. To elucidate the effect of L-AP4 on amphetamine-stimulated dopamine release, one dose of L-AP4 selected from the above dose–response study was perfused through the dialysis probe for

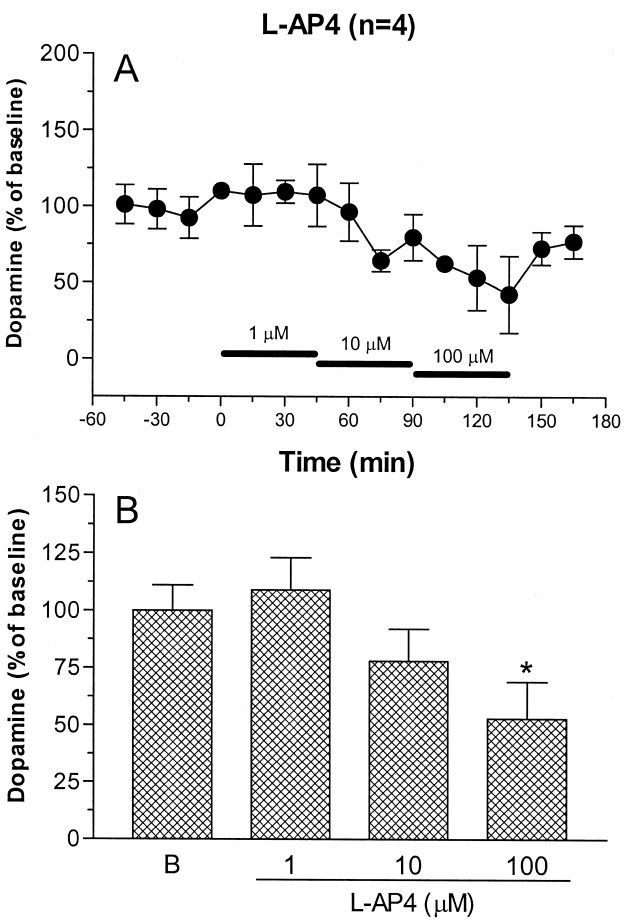

Fig. 1. (A) The effect of the group III mGlu receptor agonist L-AP4 on extracellular levels of dopamine in the dorsal striatum. Four baseline dialysis samples (15-min each) were collected before perfusion of three increasing concentrations of L-AP4 (1–100 μM) through the dialysis probe. Each concentration of L-AP4 was perfused for 45 min during which three dialysis samples were collected. (B) The histogram represents the average of three-dialysis samples at each concentration. The values are expressed as means ± S.E.M. $^{*}P < 0.05$ as compared to baseline.

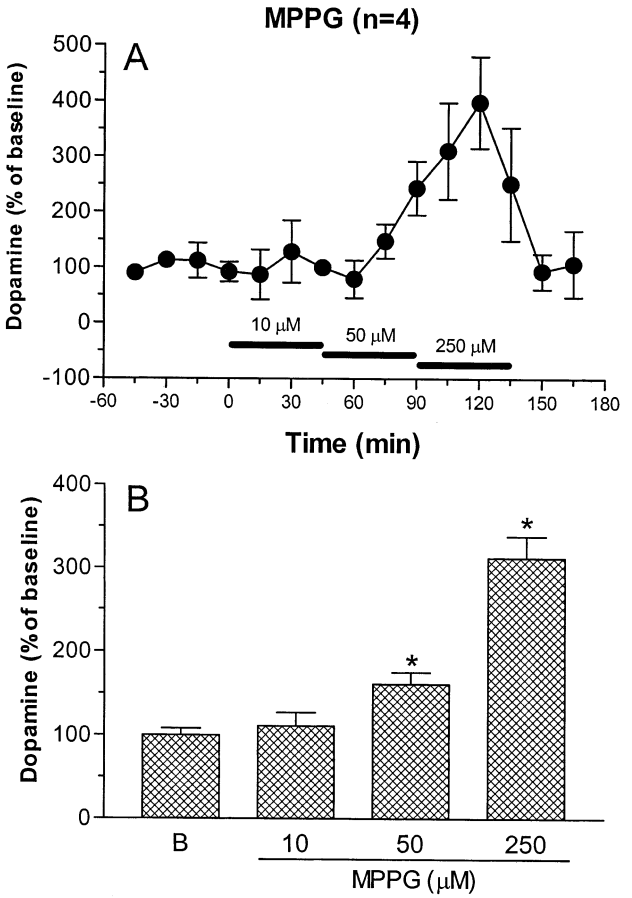

Fig. 2. (A) The effect of the group III mGlu receptor antagonist MPPG on extracellular levels of dopamine in the dorsal striatum. Four baseline dialysis samples (15-min each) were collected before perfusion of three increasing concentrations of MPPG through the dialysis probe. Each concentration of MPPG was perfused for 45 min during which three dialysis samples were collected. (B) The histogram represents the average of three-dialysis samples at each concentration. The values are expressed as means ± S.E.M. $^{*}P < 0.05$ as compared to baseline.

15 min after initial baseline samples were collected. L-AP4 was then continuously co-perfused with amphetamine for 1 h. Each change of drug solution perfusion was handled manually within 10–20 s. The estimated dead volume lag time for each probe was less than 30 s. All the dialysis samples were stored at −85°C until high performance liquid chromatography (HPLC) analysis.

2.4. Quantification of dopamine

Each collected fraction of microdialysate sample was injected into an HPLC (Waters, Milford, MA) equipped with a C18 reverse phase, 3 μm LUNA column (100 × 2.0 mm, Phenomenex, Torrance, CA). The sample was eluted by a mobile phase made of 25 mM NaH_2PO_4, 50 mM Na-citrate, 0.03 mM EDTA, 10 mM diethylamine HCl and 2.2 mM sodium octyl sulfate (pH 3.2), 30 ml/l methanol, and 22 ml/l dimethylacetamide at a flow rate of 0.4 ml/min. Dopamine peak was determined by electrochemi-

cal detection at a potential of 0.6 V. The dopamine content in the sample was calculated by extrapolating the peak area from a standard curve (ranging 1–200 pg of dopamine), which was constructed under the same conditions during each run by the Maxima Workstation (Waters, Milford, MA). The threshold level of dopamine detection was around 0.5–1 pg in this study.

A 100 pg/30 μl dopamine standard was used for detection of in vitro probe recovery. Percent recovery was 15% calculated by dividing the collected dopamine concentration by the known concentration within the standards and multiplying this value by 100. Fresh standards were compared with frozen standards and no differences were found between them.

2.5. Histology

At the end of dialysis experiment, rats were euthanized with an overdose of 4% chloral hydrate (> 240 mg/kg, i.p.) and decapitated after the dialysis experiment. Brains were removed and placed in 10% formalin for 5–7 days before slicing at the level of the caudoputamen with a cryostat (coronal sections, 40 μm). The sections were mounted on gelatin-coated slides and stained with 0.1% thionin. Dialysis probe locations and histological damage surrounding probe tips were determined microscopically according to the atlas of Paxinos and Watson (1986) by an examiner without knowing the drug treatments.

2.6. Drugs

D-amphetamine sulfate was purchased from Sigma (St. Louis, MO). L-AP4 and MPPG were purchased from Tocris Cookson (Ballwin, MO). All drugs were dissolved in the ACSF on the day of perfusion experiments. Dose of amphetamine was calculated as the salt and L-AP4 and MPPG as the base.

2.7. Data and statistics

All data are presented as the means ± S.E.M. In each experiment, the baseline dopamine is calculated as an average of four baseline samples and this average is considered as 100%. The effect of an administered drug on the dialysate dopamine level is expressed as a percent of the baseline value. To analyze the overall change in basal dopamine levels by L-AP4 or MPPG at different concentrations, the three samples obtained from each concentration of the drug were averaged and compared to the average of the four baseline samples. Areas under curve values were calculated for the data obtained from the experiments with amphetamine and plotted in histograms. A one-way analysis of variance (ANOVA) was applied (dose or drug treatment). If a significant F score ($P < 0.05$) was identified, post-hoc comparisons between two groups were conducted using the Tukey's test. Probability levels of less than 0.05 were considered statistically significant.

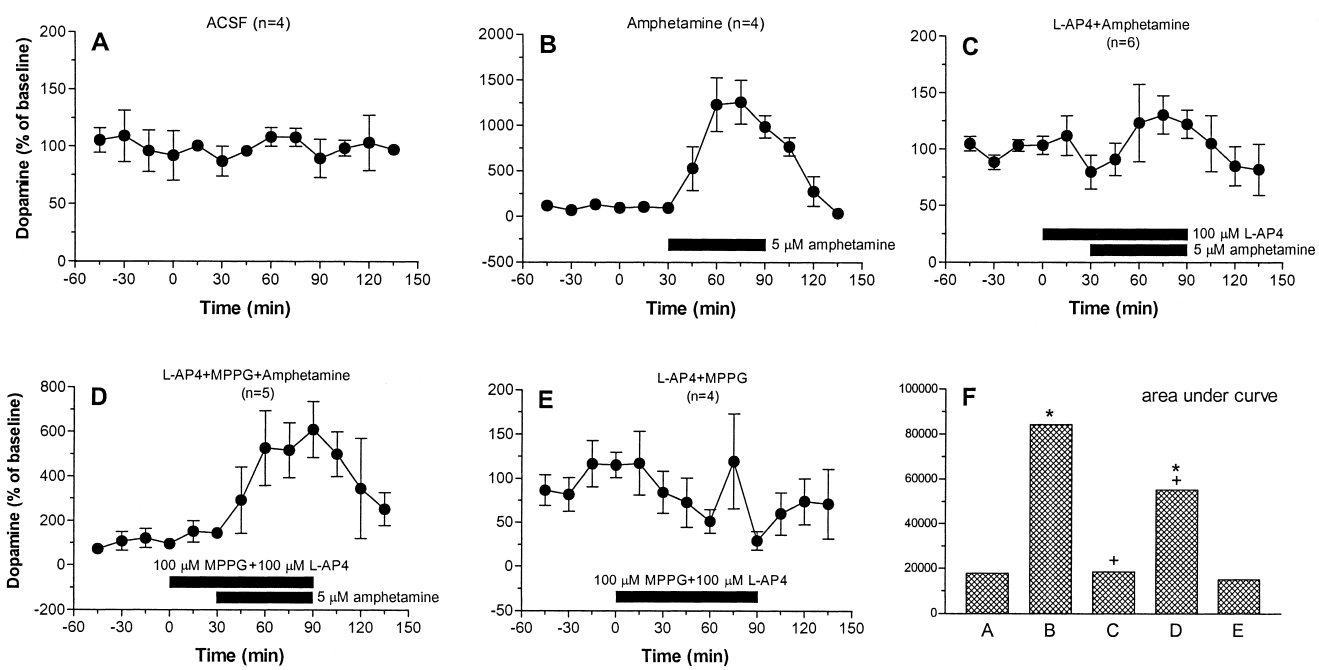

Fig. 3. The effect of activation of group III mGlu receptors on elevation of extracellular dopamine levels induced by amphetamine. (A) Continuous perfusion of ACSF caused minimal fluctuations of extracellular dopamine. (B) Amphetamine increased dopamine levels in the dorsal striatum. (C) L-AP4 activation of group III mGlu receptors blocked the stimulative effect of amphetamine on dopamine release. (D) MPPG blockade of group III mGlu receptors partly reversed L-AP inhibition of amphetamine action. (E) Co-perfusion of MPPG and L-AP4 did not significantly alter basal levels of dopamine. (F) Presentation of data in (A), (B), (C), (D), and (E) in the area under curve. The values are expressed as means ± S.E.M. $^{*}P < 0.05$ as compared to the group of rats treated with ACSF. $^{+}P < 0.05$ as compared to the group of rats treated with amphetamine.

3. Results

The average amount of basal extracellular dopamine in dialysates collected prior to drug perfusion (30 μl per HPLC injection) were 7.34 ± 1.21 pg (equivalent to 1.59 fmol/μl) in the dorsal striatum. Throughout 4-h perfusion of ACSF, which all experiments in this study were confined to, basal levels of dialysate dopamine showed only minimal fluctuations (see Fig. 3A).

3.1. Effects of the group III mGlu receptor agonist and antagonist on basal levels of extracellular dopamine

The effect of the group III agonist L-AP4 (1–100 μM) perfused through the dialysis probe on extracellular dopamine was tested in four rats, and the results are illustrated in Fig. 1. L-AP4 at the lowest concentration (1 μM) did not cause any detectable change in extracellular dopamine content as compared to baseline levels. At 10 μM, L-AP4 elicited a 23% reduction of extracellular dopamine from the baseline although this reduction is not significantly different from baseline levels ($P > 0.05$). A profound reduction of extracellular dopamine (48% of baseline levels, $P < 0.05$) was induced following administration of the drug at its highest concentration (100 μM).

A clear concentration-dependent elevation of extracellular dopamine was observed after perfusion with the group III antagonist MPPG (10–250 μM) through the dialysis probe in a separate group of four rats (Fig. 2). While MPPG at 10 μM did not alter dopamine levels, an intermediate concentration (50 μM) induced a 56% increase in extracellular dopamine over the baseline ($P < 0.05$). The increase was more evident after subsequent perfusion of the drug at the highest concentration (250 μM). Within the perfusion of 250 μM MPPG, the increased dopamine levels reached a peak at the moment when second dialysate sample was measured. A quick and complete recovery of elevated extracellular dopamine to baseline levels was observed 15 min after the cessation of drug perfusion (Fig. 2A).

3.2. Effects of the group III mGlu receptor agonist on amphetamine-induced increase in extracellular dopamine

The effect of L-AP4 on amphetamine-stimulated dopamine levels in the dorsal striatum was detected in a series of studies. Perfusion of ACSF throughout the experiment did not cause any significant shift of the basal levels of extracellular dopamine (Fig. 3A). Perfusion of amphetamine (5 μM) for 60 min through the dialysis probe induced a rapid and profound increase in extracellular dopamine content (Fig. 3B). The maximal increase of dopamine was about 1164% above the baseline level, and this increase gradually declined to normal levels after the termination of amphetamine perfusion. In a separate group of rats ($n = 6$), perfusion of L-AP4 (100 μM, selected from the above study) was made before co-perfusing 5-μM amphetamine to determine the effect of L-AP4 on amphetamine action. Fig. 3C shows that amphetamine did not cause significant changes in extracellular dopamine levels in the presence of L-AP4. To further determine if L-AP4 effect was mediated by its interaction with group III receptors, a mixed solution of the selective group III antagonist MPPG (100 μM) and agonist L-AP4 (100 μM) was perfused 30 min before co-perfusing 5 μM amphetamine for 1 h. In the presence of both antagonist and agonist, amphetamine restored its ability to elevate extracellular dopamine levels by approximately 501% (Fig. 3D). Perfusion of L-AP4 and MPPG together did not cause significant alterations in dopamine levels (Fig. 3E). Fig. 3F illustrates the data shown in Fig. 3A–E, converted to the area under curve. As can be seen, amphetamine (5 μM) alone increased dopamine levels by 3.7-fold over the baseline. This increase was completely blocked by L-AP4 (100

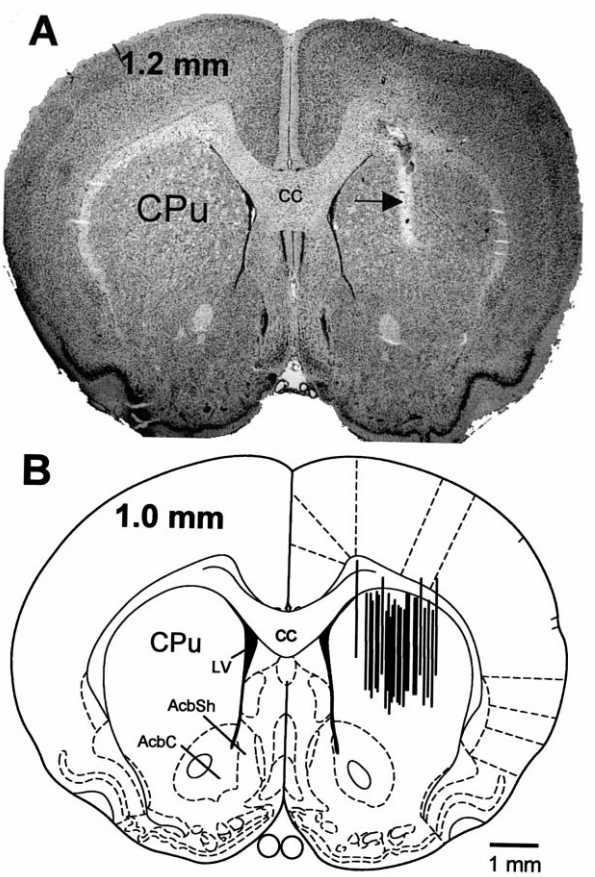

Fig. 4. (A) A representative histology of a dialysis probe location in the dorsal striatum. A thionin-stained coronal section at ~1.2-mm rostral to bregma demonstrates the typical histological pattern surrounding the probe track and tip. The arrow points to the probe track. (B) A drawing reconstruction of anatomical placements of all probes used in the study. The drawing is a standard tissue section taken from the atlas of Paxinos and Watson (1986). The numbers on the upper left corner indicate mm rostral to bregma. AcbC, core of nucleus accumbens; AcbSh, shell of nucleus accumbens; cc, corpus callosum; CPu, caudate–putamen; LV, lateral ventricle.

μM). MPPG, at a submaximal dose of 100 μM, partly reversed the L-AP4 blockade of amphetamine-stimulated extracellular dopamine levels.

3.3. Histology

Microdialysis probe sites in the striatum were examined microscopically, and the results are illustrated in Fig. 4. No apparent neurological damage to striatal neurons beyond the mechanical destruction produced by insertion of the dialysis probe was evident. A representative thionin-stained section perfused with 5 μM amphetamine is shown in Fig. 4A. The similar result was seen in striatal tissue perfused with other drugs in this study. The majority of dialysis probes were located in the central part of the dorsal striatum (Fig. 4B). The predominant portion of the active membrane region of dialysis probes was confined within the caudoputamen nucleus (Fig. 4B).

4. Discussion

A series of experiments with in vivo microdialysis technique was conducted in this study to examine alterations in extracellular dopamine in the dorsal striatum in response to pharmacological manipulations of group III mGlu receptors in normal and amphetamine-treated rats. We found that stimulation of group III mGlu receptors with a group III agonist reduced basal levels of extracellular dopamine in a dose-dependent manner. In contrast, blockade of group III mGlu receptors with a group III selective antagonist caused a dose-dependent increase in extracellular dopamine. More interestingly, co-perfusion of the group III agonist substantially attenuated amphetamine-stimulated dopamine release. These data indicate the existence of endogenous glutamatergic tone on group III mGlu receptors in the dorsal striatum controlling the basal and stimulated release of dopamine.

4.1. Subgroup-specific regulation of extracellular dopamine content by mGlu receptors

The regulation of extracellular dopamine levels by mGlu receptors has been intensively investigated initially with a non-subgroup selective agonist, 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD). Perfusing ACPD into the dorsal striatum through the dialysis probe, most investigators have recorded an increase in extracellular dopamine (Arai et al., 1996; Ohno and Watanabe, 1995; Verma and Moghaddam, 1998). However, ACPD caused a biphasic alteration in extracellular dopamine in the ventral striatum, depending upon the dose surveyed (Taber and Fibiger, 1995). At the lower dose (100 μM), ACPD induced a reduction of dopamine levels. Moreover, ACPD seemed to reduce dopamine release in the dorsal or ventral striatum induced by handling (Feenstra et al., 1998), electrical cortical stimulation (Taber and Fibiger, 1995), feeding (Taber and Fibiger, 1997), or a high concentration of potassium (Verma and Moghaddam, 1998). The divergent data from the above studies with ACPD are partially due to the dose and area where the drug is administered. More importantly, the three different groups of mGlu receptors may have distinct effects on striatal dopamine release given the diversity of these groups in coupling to intracellular effectors (Conn and Pin, 1997). Using the group-specific agents that are available just recently, one study reported an increase in extracellular dopamine after perfusion of an agonist relatively selective for the group I mGlu receptors, 3,5-dihydroxyphenylglycine, into the nucleus accumbens (Bruton et al., 1999). As opposed to the facilitatory effect of the group I agonist, the group III agonist L-AP4 decreased extracellular dopamine in the same area (Hu et al., 1999). In this study, L-AP4 similarly decreased extracellular dopamine content in the dorsal striatum. This indicates that enhancement of group III glutamatergic transmission can exert an inhibitory modulation of dopamine release in the dorsal striatum as it does in the ventral striatum (Hu et al., 1999). The inhibitory effect was sensitive to blockade of group III receptors with the selective group III antagonist MPPG. MPPG alone elevated extracellular dopamine content in a dose-dependent fashion. This latter observation implies the presence of a tonically active glutamatergic input at the site of the group III mGlu receptors to pre-synaptically inhibit dopamine release under normal physiological conditions.

4.2. Possible mechanisms underlying regulation of dopamine release by group III mGlu receptors

Moderate to high levels of mRNA encoding mGlu7 receptors are present in the ventral mesencephalic region containing dopaminergic neurons that project to the caudate nucleus (Ohishi et al., 1995). Dense mGlu4-like and mGlu7-like immunocytochemical staining is also found on axonal terminals throughout the dorsal striatum (Bradley et al., 1999; Kosinski et al., 1999). These anatomical data indicate the presence of group III mGlu receptors on intrastriatal dopaminergic terminals. Thus, direct activation or blockade of these receptors with exogenous L-AP4 or MPPG, respectively, could alter glutamatergic tone at the terminals to regulate dopamine release pre-synaptically. Alternatively, group III mGlu receptors may indirectly regulate dopamine release via a mechanism involving the excitatory amino acid glutamate. Glutamate has long been recognized to facilitate dopamine release (for a review, see Wang and McGinty, 1999). Recent studies show that mGlu receptors can exert profound influence over pre-synaptic glutamate release, presumably through autoreceptor-like effects. For example, ACPD increases glutamate release in the striatum in vivo (Cozzi et al., 1996; Liu and Moghaddan, 1995; Samuel et al., 1996). This increase is most likely induced by selective stimulation of group I mGlu

receptors because the group I agonist facilitates, whereas the group II agonist inhibits, glutamate release (Cozzi et al., 1997; Moroni et al., 1998). If L-AP4 can pharmacologically remove glutamatergic tone that is prerequisite for basal dopamine release, L-AP4 inhibition of glutamate release can in turn result in a sequential suppression of dopamine release. In order to clarify this hypothesis, the effect of group III drugs on striatal glutamate release is currently being investigated in this laboratory.

4.3. Inhibition of amphetamine-stimulated dopamine release by the group III agonist

The important finding in this study is the inhibition of amphetamine-stimulated dopamine release by L-AP4. Amphetamine produces its biological actions primarily via stimulation of catecholamine release in the mesostriatal and mesolimbic systems. Inhibition of amphetamine-induced dopamine release by L-AP4 in this in vivo microdialysis study explains the results from our previous behavioral study showing that L-AP4 effectively antagonized amphetamine-stimulated motor activity (Mao and Wang, 2000a). However, the molecular characteristics of terminal dopamine release underlying amphetamine/L-AP4 interaction are still unknown. While amphetamine-stimulated dopamine release is primarily Ca^{2+}-independent (Fisher and Cho, 1979; Seiden et al., 1993; Gray et al., 1999), it is unclear how adenylate cyclase-dependent group III mGlu receptors could directly affect amphetamine effects on dopamine release. The NMDA antagonist MK-801 is shown to suppress methamphetamine-stimulated dopamine release (Weihmuller et al., 1991). Moreover, acute systemic or central administration of amphetamine was found to increase the extracellular concentration of glutamate in the striatum of awake rats in the recent reports (Del Arco et al., 1999; Gray et al., 1999; Rawls and McGinty, 2000; Miele et al., 2000). These data indicate the presence of significant glutamatergic tone essential for stimulation of dopamine release by psychostimulant. It is then possible that L-AP4 antagonized stimulated dopamine release by inhibition of glutamatergic transmission. In addition, a recent study shows that amphetamine-evoked striatal dopamine release was partially calcium-dependent, and co-infusion of an opioid kappa receptor agonist blocked the calcium-dependent component of amphetamine-stimulated dopamine release (Gray et al., 1999). Thus, the group III agonist may regulate amphetamine-stimulated dopamine release through unknown cAMP/calcium signal interactions.

Differences exist in dose and duration of intrastriatal infusion of amphetamine in producing increases in local dopamine release. Heidbreder and Feldon (1998) reported that 60 min infusion of amphetamine at the low (0.05–0.1 μM) and high (1 μM) concentrations caused a decrease and increase in dopamine release in the nucleus accumbens, respectively. Similarly, amphetamine infusion at 1 and 10 μM for 100 min each induced a dose-dependent increase in extracellular dopamine levels in this brain area (Pierce and Kalivas, 1997). Therefore, in this study, we selected to perfuse amphetamine at 5 μM for 60 min according to the above reports. This perfusion was confirmed to induce reliable and significant increases in extracellular dopamine levels in the dorsal striatum.

4.4. Correlation of extracellular dopamine content and behavioral expression

Motor modulation by group I and group II mGlu receptors is closely correlated with the levels of extracellular dopamine in the striatum. This is evidenced by the fact that the group I agonist induces parallel increases, whereas the group II agonist induces parallel decreases, in striatal dopamine release and behavior (Bruton, et al., 1999; Hu et al., 1999; Mao and Wang, 1999, 2000a; Wang and Mao, 2000). On the contrary, the group III mGlu receptor agonist L-AP4, which lowers basal extracellular dopamine causes either no or an increase in the animal spontaneous motor activity (Hu et al., 1999; Swanson and Kalivas, 2000; Mao and Wang, 2000b). The unexpected dissociation between peripheral behavior and striatal dopamine levels brought by the group III mGlu receptor agonist may suggest a complex receptor arrangement and interaction involved among multiple transmitter systems present in the striatum that regulate behavior. L-AP4 may possess additional pharmacological actions to facilitate the release of other excitatory transmitters and/or suppress the release of other inhibitory transmitters, which, together with the effect of L-AP4 on dopamine release, determines the final outcome of motor responses.

In summary, this study investigated the effects of a group III mGlu receptor agonist L-AP4 and antagonist MPPG perfused through the dialysis probe on basal and amphetamine-stimulated dopamine release in the rat dorsal striatum using in vivo microdialysis technique. It was found that L-AP4 concentration-dependently reduced basal extracellular dopamine whereas MPPG caused a concentration-dependent increase in dopamine levels. Moreover, L-AP4 protected against amphetamine-induced dopamine release. These results indicate a strong inhibitory modulation of dopaminergic transmission in forebrain under normal and amphetamine-stimulated conditions by group III mGlu receptors.

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