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Distinct inhibition of acute cocaine-stimulated motor activity following microinjection of a group III metabotropic glutamate receptor agonist into the dorsal striatum of rats

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Abstract

Group III metabotropic glutamate receptors (mGluRs) are negatively coupled to adenylate cyclase through G-proteins. Activation of this group of mGluRs shows an inhibition of dopaminergic transmission in the forebrain. To define the role of striatal group III mGluRs in the regulation of basal and dopamine-stimulated motor behavior, the recently developed agonist and antagonist relatively selective for group III mGluRs were utilized to pharmacologically enhance and reduce group III mGluR glutamatergic tone in the dorsal striatum of chronically cannulated rats. Bilateral injections of a group III agonist, L-2-amino-4-phosphonobutyrate (L-AP4), did not alter basal levels of motor activity at three doses surveyed (1, 10, and 100 nmol). Neither did intracaudate injection of a group III antagonist, α-methyl-4-phosphonophenylglycine (MPPG), at 10, 30, and 100 nmol. However, pretreatment with L-AP4 (10 and 100 nmol) dose dependently blocked hyperlocomotion induced by acute injection of cocaine (20 mg/kg, i.p.), amphetamine (2.5 mg/kg, i.p.), or apomorphine (1 mg/kg, s.c.). The behavioral activity induced by cocaine was much more sensitive to L-AP4 than that induced by amphetamine and apomorphine. At 100 nmol, L-AP4 completely blocked cocaine effect whereas amphetamine- and apomorphine-stimulated behaviors were blocked only by 28% and 31%, respectively. The blocking effect of L-AP4 on cocaine action was reversed by pretreatment with MPPG. MPPG itself did not modify behavioral responses to cocaine, amphetamine, or apomorphine. These data indicate that the glutamatergic tone on the group III mGluRs is not active in the regulation of basal and acute dopamine-stimulated motor activity. However, enhanced group III mGluR glutamatergic transmission by an exogenous ligand is capable of suppressing behavioral responses to acute exposure of dopamine stimulants.

Keywords: Behavior; Motor activity; Basal ganglia; Caudoputamen; Dopamine; L-AP4; MPPG; Amphetamine; Apomorphine

1. Introduction

Group III metabotropic glutamate receptors (mGluRs), which include mGluR4, mGluR6, mGluR7, and mGluR8 subtypes, are negatively coupled to adenylate cyclase and cAMP formation [9,23,28,32,36,37]. Like the other groups of mGluRs, group III mGluRs are highly heterogeneous in their distribution in the central nervous system and physiological and pharmacological profiles. Recent morphological evidence shows that group III mGluRs are densely distributed in the striatum, a central part of basal ganglia controlling motor movement. Studies with in situ hybridization reveal moderate levels of mGluR4 and mGluR7 mRNAs in the medium spiny projection neurons of rat striatum [45]. An immunocytochemical study finds positive staining of group III mGluR-like immunoreactivity on the cell bodies of striatal neurons [5]. Autoradiographic receptor binding with the [^{3}H]-labeled L-2-amino-4-phosphonobutyrate (L-AP4), an agonist selective for group III mGluRs [46], exhibits specific [^{3}H]-L-AP4 bindings throughout the rat striatum [19]. Although subcellular and pre- and postsynaptic distributions of group III mGluRs in the striatum are far from clear, intensive distribution of the receptors in this region indicates a functional role in the regulation of striatal neuronal activity.

With the availability of the agonists and antagonists relatively selective for group III mGluRs, functional studies

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on physiological roles of striatal group III mGluRs have emerged in the recent several years. First, the group III mGluR agonists, L-AP4 and (*R*,*S*)-4-phosphonophenylglycine [15], were found to protect cortical and striatal neurons from NMDA- or quinolinic acid-induced lesions in vivo and in vitro [7,15,17]. Second, selective activation of group III mGluRs with i.c.v., i.p., or i.v. injection of these agonists produced anticonvulsive and antiepileptogenic effects in rats or mice [1,15,16,43]. Deficiency of mGluR7 in knock-out mice caused spontaneous epileptic seizures [15]. Finally, with in vivo microdialysis, a recent report shows that intrastriatal perfusion of L-AP4 reduced basal levels of extracellular dopamine in the nucleus accumbens [18]. On the contrary, perfusion of α-methyl-4-phosphonophenylglycine (MPPG), an antagonist selective for the group III mGluRs [4,35], elevated basal dopamine levels in this area [18]. Apparently, there exists a significant in vivo glutamatergic tone on group III mGluRs controlling basal dopaminergic transmission in the forebrain. However, at present, no attempt has been made to define putative involvement of group III mGluRs in the modulation of spontaneous motor activity and behavioral responses to the stimulation of mesolimbic and mesostriatal dopaminergic transmission.

Acute administration of the indirect dopamine receptor agonists, cocaine and amphetamine, induces locomotion and stereotypical behaviors in experimental animals. The behavioral changes induced by these psychostimulants primarily result from their actions to elevate extracellular dopamine levels in the striatum. However, difference seems to exist in elevating dopamine levels between the two stimulants. Cocaine increases extracellular dopamine levels primarily by blocking dopamine uptake through inhibiting dopamine transporters [44], whereas amphetamine directly stimulates presynaptic dopamine release with a little effect on dopamine transporters [14].

To investigate the possible involvement of striatal group III mGluRs in the regulation of basal and phasic motor activity, the group III agonist L-AP4 and antagonist MPPG were utilized in this study. Effects of the two agents injected into the dorsal striatum on normal behavior and hyperlocomotion induced by acute administration of cocaine, amphetamine, or a mixed D1/D2 dopamine receptor agonist apomorphine were examined in chronically cannulated rats.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (225–249 g, Charles River, New York, NY) were used in this study. Animals were individually housed in a controlled environment at a constant temperature of 23°C and humidity of 50 ± 10% with food and water available ad libitum. Animal room was on a 12L/12D cycle, with lights on at 07:00 hours. All animal use procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee.

2.2. Surgery and drug administration

Rats were anesthetized with 4% chloral hydrate (400 mg/kg, i.p.) and placed in a David Kopf stereotaxic holder. Two 24-ga stainless-steel guide cannulas (10 mm in length) were bilaterally implanted to the central part of dorsal striatum (1 mm anterior to bregma, 2.5 mm lateral to midline and 3 mm below surface of skull). The guide cannulas were sealed with stainless steel wires of the same length (10 mm). Rats were allowed at least 5 days for recovery from surgery. On the day of the experiment, the inner steel wires were replaced by 30-ga stainless steel injection cannulas with a length of 12.5 mm that protruded 2.5 mm beyond the guide cannulas. Through the injection cannulas, drugs were infused into the target area in a volume of 1 μl/side at a rate of 0.4 μl/min in freely moving animals in the quiet (low stress) home cage. Progress of injection was monitored by observing movement of a small air bubble through a length of precalibrated PE-10 tubing inserted between the injection cannula and a 5-μl Hamilton microsyringe. After completing each injection, the cannula was left in place for an additional 3 min to reduce any possible backflow of the solution along the injection track. Each rat received one intrastriatal injection.

Systemic administration of cocaine (i.p.), amphetamine (i.p.), or apomorphine (s.c.) was made in a volume of 1.2 (i.p.) or 0.8 (s.c.) ml/kg. Intrastriatal injection of L-AP4, MPPG, or vehicle control was made 5 min prior to systemic injection of the drugs.

2.3. Behavioral observation

Behavioral activities in response to the drug treatments were continuously observed and rated in animal's home cage. Two trained observers, blind to the drug treatments, rated the behavior of each rat, using an eight-point rating scale that was modified by Ellinwood and Balster [11] and utilized in our previous studies [27]: (1) sedation (insensitive to touch, loss of righting reflex); (2) asleep, inactive; (3) normal activities (normal grooming, chewing and alert); (4) locomotion I (hyperactive running, jumping, rearing, sniffing or jerky movement); (5) locomotion II (slow patterned repetitive exploration with normal levels of activity; (6) locomotion III (fast patterned repetitive exploration with hyperactivity); (7) stereotypy (repetitive sniffing/rearing in one location to the exclusion of other activities), and (8) dyskinesia, seizures.

2.4. Experimental protocols

A series of three experiments was conducted in this study. Experiment I investigated the effects of pharmacolo-

gical activation and blockade of striatal group III mGluRs with intracaudate injection of the group III agonist and antagonist, respectively, on basal levels of motor activity. Experiment II detected the effects of intracaudate injection of the group III agonist and antagonist on motor activities induced by dopamine stimulants. Finally, the effects of blockade of group III mGluRs on the inhibition of cocaine-stimulated behaviors induced by intrastriatal injection of the group III agonist were tested in experiment III to verify the specificity of group III mGluRs in the mediation of group III agonist-induced inhibition.

2.5. Histology

At the end of each experiment, 0.5 μl of 4% methylene blue dye was applied to the injection site. Animals were deeply anesthetized with 4% chloral hydrate (400 mg/kg, i.p.) and then decapitated. The brains were removed rapidly from skull and immediately frozen in isopentane at −40°C and stored at −70°C until they were sectioned for histological examination. Frozen serial sections (40 μm) of the forebrain were cut at the injection level and thaw-mounted onto the gelatin-coated slides. The sections were fixed, defatted, and stained for Nissl substance with 0.1% Thionin. Accurate injection sites and morphological structure of the tissue surrounding the injection site were checked by referring to the Paxinos and Watson atlas [31]. Locations of the blue spots in the dorsal striatum were plotted onto copies of standard sections of the striatum taken from the atlas.

2.6. Drugs

Cocaine hydrochloride, D-amphetamine sulfate and *R*(−)-apomorphine were purchased from Sigma (St. Louis, MO). L-AP4 and MPPG were purchased from Tocris Cookson (Ballwin, MO). All drugs were freshly prepared at the day when intracranial injections were made. Cocaine and amphetamine were dissolved in physiological saline. Apomorphine was dissolved in distilled H_2O. L-AP4 and MPPG were dissolved in artificial cerebrospinal fluid (aCSF) (in mM: NaCl 123, $CaCl_2$ 0.86, KCl 3.0, $MgCl_2$ 0.89, Na_2HCO_3 25, NaH_2PO_4 0.50, and Na_2HPO_4 0.25 aerated with 95% O_2–5% CO_2) to the appropriate doses, and neutralized to pH 7.2–7.6 with 1 N NaOH if needed. Doses of the drugs injected intrastriatally were changed with altering concentration but not volume. The doses of cocaine, amphetamine, and apomorphine were calculated as the salt, whereas the doses of L-AP4 and MPPG were referred to their free bases.

2.7. Statistics

The results are shown as means ± 1 SEM. Data were analyzed by a two-way analysis of variance (ANOVA), followed by a group comparison using least-square-adjusted means on the area-under-curve (AUC) values calculated from plotting behavioral ratings against time. $p < 0.05$ was taken as a significant level of difference.

3. Results

3.1. Experiment I. Behavioral responses to intracaudate injection of L-AP4 or MPPG

To test the role of striatal group III mGluRs in the regulation of normal motor activity, three doses of L-AP4 or MPPG were bilaterally infused into the dorsal striatum. As can be seen in Fig. 1A, local infusion of vehicle control did not induce any alteration in basal motor activity. Similarly, L-AP4 at all three doses tested (1, 10, and 100 nmol) showed no detectable difference in behavioral ratings compared to the vehicle group, $F(3,19) = 1.16$, $p > 0.05$. Like L-AP4, behavioral ratings from the rats treated with MPPG at three doses (10, 30, and 100 nmol) were not significantly different from that observed in the rats treated with vehicle (Fig. 1B), $F(3,15) = 0.89$, $p > 0.05$.

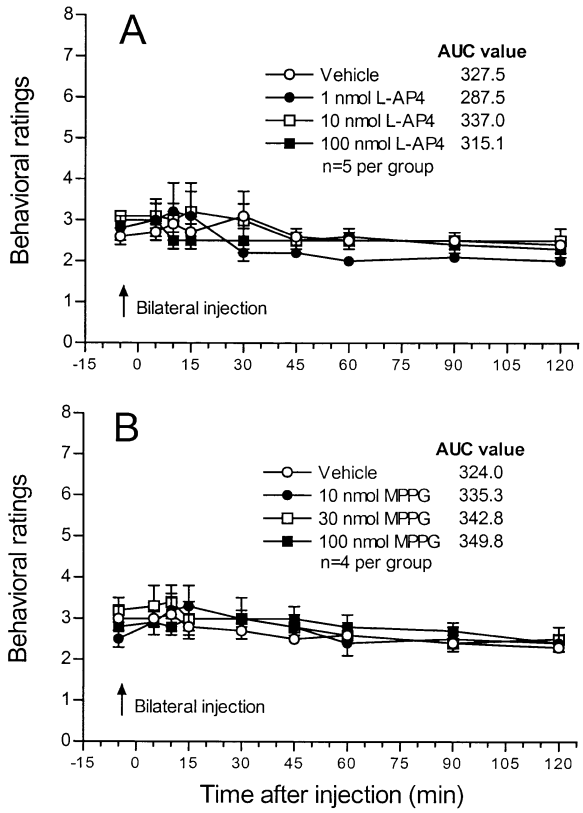

Fig. 1. Effects of bilateral injections of the group III agonist L-AP4 (A) and antagonist MPPG (B) into the dorsal striatum on basal behavioral activity. The behavioral ratings are expressed as means ± SEM.

3.2. Experiment II. Effects of intrastriatal injection of L-AP4 or MPPG on cocaine, amphetamine, or apomorphine-stimulated motor behaviors

Similar to the results from the above experiment, L-AP4 (10 and 100 nmol) and MPPG (100 nmol) bilaterally injected into the dorsal striatum in combination with i.p. injection of saline did not alter basal motor activity (Fig. 2A). Acute injection of cocaine (20 mg/kg, i.p.) induced the typical cocaine-like locomotor activities, i.e., sniffing, exploration, and rearing (Fig. 2B). In two rats treated with cocaine, transient stereotypical behavior (continuous sniffing in one location of cage) was seen between 30 and 45 min after cocaine injection. Pretreatment of rats with bilateral injections of 10 nmol L-AP4 into the dorsal striatum partially blocked cocaine-stimulated behavioral activities as demonstrated by a significantly less increase in behavioral activity in the presence of 10 nmol L-AP4 (Fig. 2B). A complete blockade of cocaine-stimulated motor behavior was observed following L-AP4 injection at a higher dose (100 nmol) in seven rats surveyed, $F(7,35) = 18.41$, $p < 0.001$. In all seven rats, subsequent injection of cocaine alone at the same dose (20 mg/kg) on the next day produced increases in motor behavior comparable to that observed in vehicle + cocaine group (data not shown). Unlike L-AP4, MPPG at 100 nmol showed no effect on cocaine-stimulated behavioral activity (Fig. 2B).

Acute amphetamine injection at a moderate dose (2.5 mg/kg, i.p.) caused locomotion, including sniffing, exploration, rearing, and running (Fig. 2C). L-AP4 at 10 nmol did not modify amphetamine-stimulated motor activities (Fig. 2C). Only at 100 nmol was amphetamine-stimulated behaviors blocked partially, $F(7,35) = 12.54$, $p < 0.001$. In the rats treated with MPPG (100 nmol), amphetamine induced strong behavioral responses similar to that seen in vehicle + amphetamine group (Fig. 2C).

Acute apomorphine injection (1 mg/kg) increased locomotor activities (Fig. 2D). Intrastriatal injection of 10 nmol L-AP4 produced no significant influence on apomorphine-stimulated motor activities (Fig. 2D). However, L-AP4 at 100 nmol diminished behavioral responses to apomorphine by a small portion, $F(7,37) = 7.11$, $p < 0.001$. In the pre-

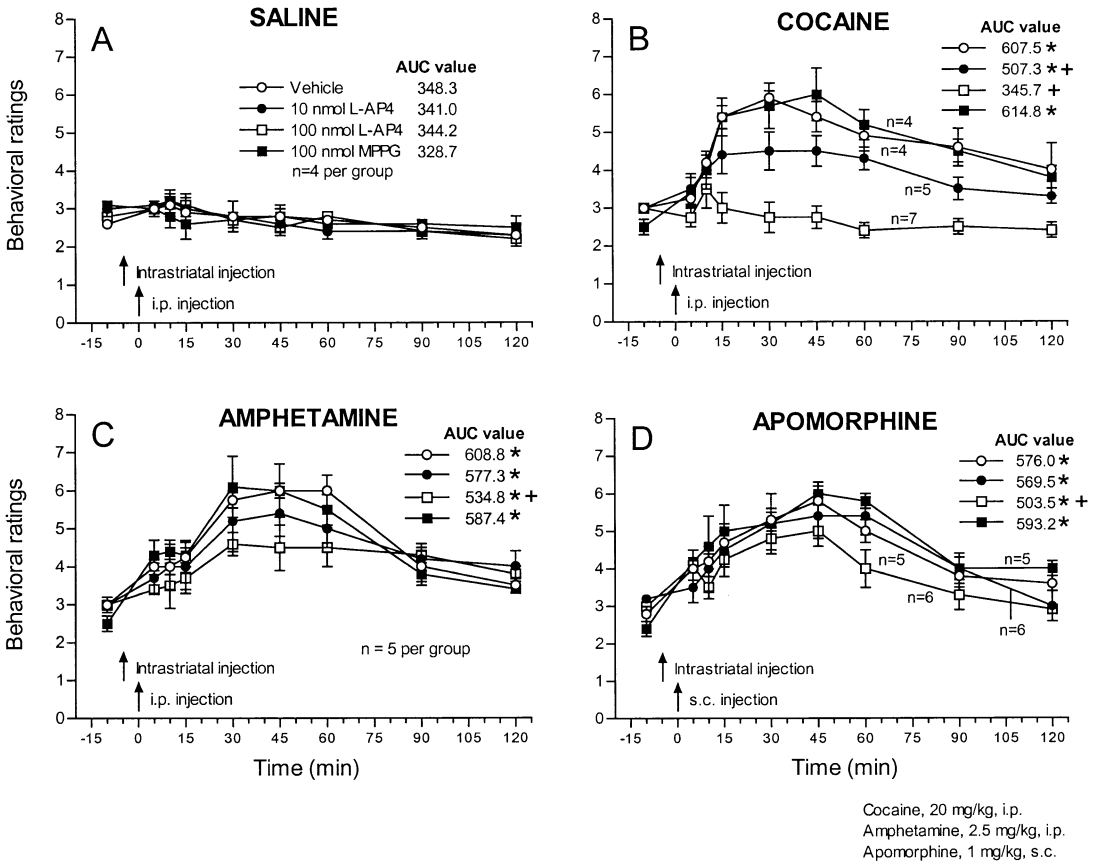

Fig. 2. Effects of intracaudate injection of the group III agonist L-AP4 and antagonist MPPG on basal (A) and stimulated behavioral activities by acute systemic injection of cocaine (B), amphetamine (C), and apomorphine (D). Bilateral injections of L-AP4 (10 and 100 nmol/1 μl/side) or MPPG (100 nmol/1 μl/side) into the dorsal striatum were made 5 min prior to injection of cocaine (20 mg/kg, i.p.), amphetamine (2.5 mg/kg, i.p.), or apomorphine (1 mg/kg, s.c.). The behavioral ratings are expressed as means ± SEM. * $p < 0.05$ compared with vehicle + saline, $^{+}$ $p < 0.05$ compared with vehicle + cocaine (B), vehicle + amphetamine (C), and vehicle + apomorphine (D).

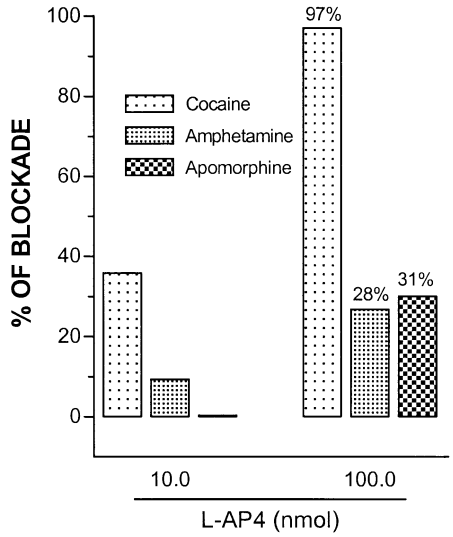

Fig. 3. Blockade of cocaine-, amphetamine-, and apomorphine-stimulated motor behaviors following intracaudate injection of the group III mGluR agonist L-AP4 (10 and 100 nmol). The percentages of blockade were calculated according to the formula: [[(AUC value of vehicle + stimulant group − AUC value of vehicle + saline group) − (AUC value of L-AP4 + stimulant group − AUC value of vehicle + saline group)]/(AUC value of vehicle + stimulant group − AUC value of vehicle + saline group)] × 100%.

sence of MPPG (100 nmol), apomorphine preserved its full potency of stimulating motor activity (Fig. 2D).

Fig. 3 compares the potency of L-AP4 in blocking cocaine-, amphetamine-, and apomorphine-stimulated motor behaviors. Behavioral stimulation by amphetamine and apomorphine was not affected by 10 nmol L-AP4 and was largely preserved after 100 nmol L-AP4 injection (only 28% blockade for amphetamine and 31% blockade for apomorphine). In sharp contrast to amphetamine and apomorphine, behavioral responses to cocaine were sensitive to L-AP4. A total of 36% and 97% of cocaine-stimulated behavioral activity were blocked by L-AP4 at 10 and 100 nmol, respectively.

3.3. Experiment III. Effects of MPPG on L-AP4-induced blockade of cocaine-stimulated behaviors

The specificity of group III mGluRs in the mediation of blocking effect of L-AP4 was clarified in eight groups of rats, and the results from this study are illustrated in Fig. 4. From the first four groups of rats pretreated with bilateral injections of vehicle (Fig. 4A), behavioral changes resembled those described in the above study (Fig. 2A and B). From the four other groups of rats pretreated with bilateral injections of 100 nmol MPPG (Fig. 4B), L-AP4 no longer attenuated cocaine-stimulated motor activity (Fig. 4B). The behavioral ratings from MPPG + L-AP4 + cocaine group were not significantly different from those from MPPG + vehicle + cocaine group (Fig. 4B). As a matter of fact, MPPG seemed to augment cocaine-stimulated behaviors (MPPG + vehicle + cocaine vs. vehicle + vehicle + cocaine). However, this augmentation did not reach a statistically significant level (644.5 vs. 591.8, $p>0.05$).

3.4. Histology

Distributions of the injection sites in the dorsal striatum from the above experiments are illustrated in Fig. 5. The injection sites from the experiments I (Fig. 5A) and III (Fig. 5C) were concentrated in the central part of the caudoputamen. All injection sites from the experiment II were also located in the dorsal striatum, and these sites from the data illustrated in Fig. 2B are shown in Fig. 5B. There is no

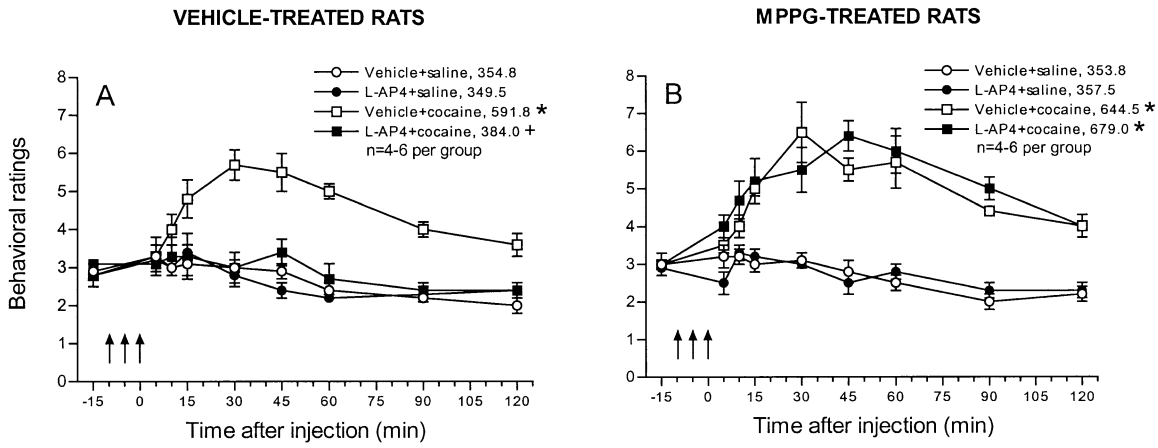

Fig. 4. Effects of the group III mGluR antagonist MPPG on L-AP4-induced blockade of acute cocaine-stimulated behaviors. Bilateral intracaudate injections of vehicle (A) or 100 nmol MPPG (B) were made 5 min prior to bilateral injections of L-AP4 (100 nmol) into the same sites. Injection of saline or cocaine (20 mg/kg, i.p.) was made 5 min after L-AP4 injection. The values are expressed as means ± SEM. The numbers on the top-right legend of each panel represent the AUC values. * $p < 0.05$ compared with vehicle + vehicle + saline. $^{+}$ $p < 0.05$ compared with vehicle + vehicle + cocaine.

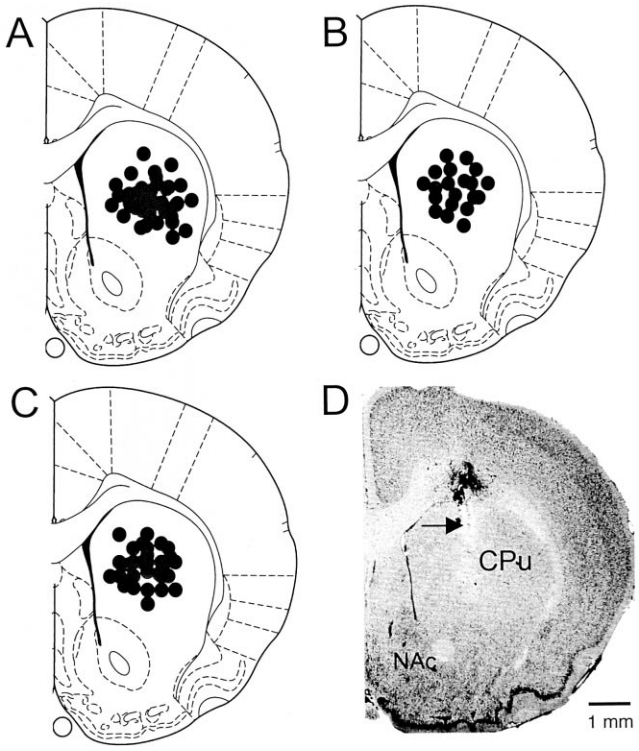

Fig. 5. Reconstruction of microinjection sites in the rat dorsal striatum wherein intrastriatal injections of drugs were made. (A) Intrastriatal injection sites from Experiment I. (B) Intrastriatal injection sites from the data illustrated in Fig. 2B in Experiment II. (C) Intrastriatal injection sites from Experiment III. The injection sites were marked on one side of the striatum. (D) A photomicrograph of the half brain section from a rat treated with vehicle + cocaine. Arrow marks intrastriatal injection track. CPu, caudate putamen; NAc, nucleus accumbens.

detectable loss or damage of neurons surrounding the cannula track (Fig. 5D).

4. Discussion

A series of experiments was conducted in this study to define the role of group III mGluRs in the striatum in the regulation of normal and stimulated motor activity. We found that selective activation or blockade of striatal group III mGluRs with bilateral injections of the agonist L-AP4 and antagonist MPPG, respectively, did not modify basal motor behavior. This indicates that glutamatergic inputs on group III mGluRs are not involved in normal motor modulation. However, activation of group III mGluRs with exogenous L-AP4 exerted a distinct inhibition of cocaine-stimulated behaviors. This indicates that group III mGluR glutamatergic transmission possesses an inhibitory power to control excitatory responses of striatal neurons and thus behavioral activity to dopamine stimulation.

The implication of mGluRs in extrapyramidal motor modulation was first demonstrated in the early behavioral studies with the nongroup selective agents. Schoepp's group first reported that a unilateral injection of a nongroup selective agonist, ACPD, into the rat dorsal striatum caused turning behavior (rotation) contralateral to the injection side [33,34]. This finding has generally been replicated afterwards by others [12,13,20,33,39]. With the recently available agonists and antagonists relatively selective for subgroups of mGluRs, motor modulation by mGluRs is demonstrated to be a group-specific event. Activation of group I mGluRs (mGluR1 and mGluR5) induced ACPD-like contraversive turning or locomotion, which was sensitive to coadministration of a group I, but not a group II/III, antagonist [13,49]. In contrast to the behavioral stimulation by the group I agonist, the group II (mGluR2 and mGluR3) agonist DCG-IV reduced both basal and amphetamine-stimulated motor activity [26]. In this study, the group III agonist attenuated dopamine-stimulated motor behaviors. It appears that the mGluRs that are coupled to stimulation of phosphoinositide hydrolysis (group I) facilitate, whereas the mGluRs that coupled to inhibition of cAMP formation (group II/III) suppress, basal and/or stimulated behavioral activity.

L-AP4 and MPPG have been shown to decrease and increase extracellular dopamine levels in the nucleus accumbens [18] and dorsal striatum (Mao et al., submitted), respectively. The increase in dopamine levels after MPPG suggests a significant glutamatergic tone on the group III mGluRs to inhibit dopamine release under the normal physiological conditions. However, behavioral data from the current study seem to provide no reflection of this tonically active group III regulation of basal dopamine release because no significant alteration in spontaneous motor activity was observed following intrastriatal injection of L-AP4 or MPPG. Perhaps, changes in dopamine release, if there was any, in response to L-AP4 or MPPG injection, did not reach the degree high enough to be translated into behavioral manifestation. Alternatively, group III mGluRs may also regulate releasing activity of other transmitters, such as acetylcholine, a rich transmitter in the striatum generally antagonizing dopamine influence on striatal neurons [50,51,53]. If L-AP4 inhibits basal release of both acetylcholine and dopamine to a comparable degree, striatal neuronal activity may remain unchanged as a result of parallel withdrawal of the two opposing driving forces on striatal neurons.

Among the three dopamine stimulants surveyed in this study, cocaine-stimulated motor activity was particularly susceptible to L-AP4 blockade. Although precise mechanism(s) underlying the distinctive blockade of cocaine action by L-AP4 is unclear, it can be first assumed that the inhibition of dopamine release by L-AP4 [18] may play a role in this event (see Fig. 6). It is well known that cocaine increases extracellular dopamine levels by blocking dopamine transporters [44]. If dopamine release is inhibited by L-AP4, opportunity for cocaine to increase extracellular dopamine by blocking dopamine uptake can be diminished

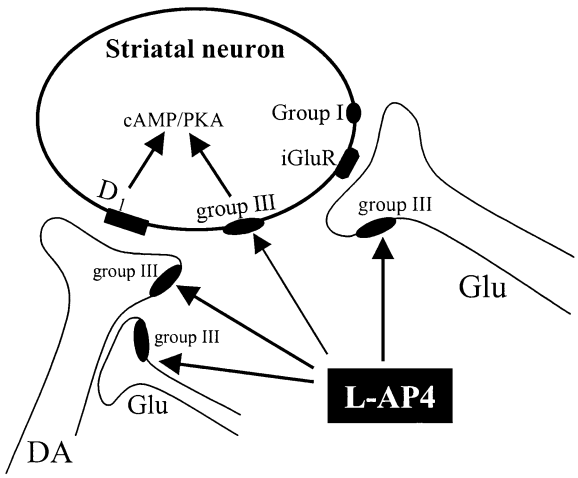

Fig. 6. Schematic diagram illustrating mechanisms underlying the inhibitory modulation of glutamatergic and dopaminergic transmission by group III mGluR activation. Presynaptically, the group III selective agonist L-AP4 can inhibit dopamine release by interacting with presynaptic heteroreceptors. More importantly, L-AP4 suppresses presynaptic glutamate release via group III autoreceptors. This may, in turn, result in a reduced dopamine release and a suppressed neuronal response to cellular stimulation if active glutamate transmission has a permissive role in dopamine release and responsiveness of striatal neurons to dopamine stimulation. Postsynaptically, L-AP4 binds to postsynaptic group III mGluRs that are negatively coupled to adenylate cyclase to antagonize stimulative effect of D1 receptor on cAMP/PKA pathway. Abbreviation: D1, dopamine D1 receptor; DA, dopamine; Glu, glutamate; iGluR, ionotropic glutamate receptor; PKA, protein kinase A.

accordingly. As to how L-AP4 inhibits dopamine release, two presynaptic mechanisms can be considered. First, L-AP4 suppresses dopamine release through activating presynaptic group III mGluRs on dopaminergic terminals if the presence of these heteroreceptors can be proven. Second, L-AP4 can indirectly reduce dopamine release by affecting glutamatergic transmission (Fig. 6). There exists a feedback depression of glutamate release through presynaptic group III autoreceptors [38], likely via reduction of cAMP levels and resultant inhibition of voltage-gated calcium entry [9,47].

Because endogenously released L-glutamate has a permissive role on tonic and phasic dopamine release [3,29,41,48], L-AP4 inhibition of glutamate release may subsequently result in reduction of dopamine release. Additionally, L-AP4 inhibition of glutamate release may reduce postsynaptic responsiveness of striatal neurons to dopamine stimulation because coactivation of glutamate receptors appears to be prerequisite for dopamine receptors to functionally interact with ligand binding (Fig. 6). This was demonstrated in dissociated striatal neurons in vitro that glutamate at the level insufficient to induce c-*fos* immediate early gene expression, a presumed indicator of neuronal activity, significantly enhances dopamine-stimulated c-*fos* expression [24]. Lastly, because striatal neurons express mGluR4 and mGluR7 mRNAs [42,45], L-AP4 may suppress excitatory responses of the neurons to dopamine receptor stimulation by directly interacting with postsynaptic group III mGluRs (Fig. 6). However, this postsynaptic mechanism is believed to be less significant in antagonizing dopamine stimulation compared with the presynaptic action of this drug. A recent study conducted in this laboratory found that intrastriatal injection of a group II agonist DCG-IV also inhibited amphetamine-stimulated motor activity [26]. Because group II mGluRs are negatively coupled to adenylate cyclase like group III mGluRs, the mechanisms illustrated in Fig. 6 generally apply to the effects of DCG-IV on dopamine-stimulated behaviors as well.

In contrast to strong inhibition of cocaine-stimulated behaviors, amphetamine-stimulated motor activity showed a strong resistance to L-AP4 injection. Only at a higher dose (100 nmol) were amphetamine-stimulated behaviors blocked by a relatively small percentage (28%). The insensitivity of amphetamine action to L-AP4 may be due to a different mechanism that underlies amphetamine stimulation of dopaminergic transmission. Unlike cocaine, amphetamine increases extracellular dopamine concentrations primarily through a direct stimulation of dopamine release from dopaminergic terminals in a Ca^{2+}-independent fashion [14]. Thus, L-AP4 has to alter a Ca^{2+}-independent pathway to affect the capacity of amphetamine to stimulated dopamine release [47]. Blockade of a small portion of amphetamine-stimulated behavior by L-AP4 may result from postsynaptic inhibition of striatal projection neurons. This is because (1) the projection neurons indeed express group III receptors [5,42,45]; and (2) L-AP4 produced a comparable blockade (31%) of behavior induced by direct apomorphine stimulation of dopamine receptors postsynaptically located on the projection neurons.

It should be pointed out that in this study, activation of group III mGluRs was achieved by exogenous administration of the agonist. To detect whether group III mGluRs are activated by endogenous ligand (glutamate) in response to dopamine stimulation, effects of a group III blocker on cocaine-stimulated behavior was examined. It was found that blockade of striatal group III mGluRs with MPPG did not augment acute cocaine-stimulated behaviors even though cocaine was deliberately given at a moderate dose (20 mg/kg) to leave room for further behavioral augmentation. This suggests that MPPG-sensitive group III mGluRs are less likely operative to play any type of role in the regulation of dopamine-stimulated motor activity. However, it remains to be tested whether group III mGluRs can be ignited when stronger dopamine stimulation is applied by higher doses of psychostimulants or, more interestingly, by repeated drug injections. Under both of the conditions, glutamatergic transmission is usually largely enhanced. Group III glutamatergic tone may therefore be increased to exert a compensatory modulation of long-term addiction properties of the drugs [50,53].

The mesolimbic dopamine terminal field, nucleus accumbens, is an important area mediating behavioral proper-

ties of dopamine stimulants. A great deal of evidence also demonstrates that the adjacent nigrostriatal dopamine projection area, the dorsal striatum (caudate putamen), is involved in the mediation of the stimulative action of the drugs. First, cocaine and amphetamine markedly increase extracellular dopamine in the dorsal striatum [6,30], although the drugs seem to preferentially elevate extracellular dopamine in the ventral striatum [8,10]. Second, pharmacological blockade of dopamine receptors (D1, D2, or both) by injecting the selective antagonists into the dorsal striatum completely blocks hyperlocomotion and especially stereotypy induced by cocaine or amphetamine [25,51]. Besides dopamine, there exist the other transmitters in the dorsal striatum that are profoundly involved in the mediation of drug actions. For instance, the NMDA or 5-HT_3 receptor antagonists injected into the dorsal striatum substantially antagonize amphetamine-stimulated motor activity [21,22]. A muscarinic receptor agonist or an antagonist injected into the dorsal striatum inhibits or augments amphetamine-stimulated behavior, respectively [52]. These data indicate that the dorsal striatum plays an essential role as the ventral striatum in processing drug stimulation of motor behavior, and pharmacological manipulations of the responsible transmitter systems in the localized dorsal striatum can substantially affect actions of dopamine stimulants. However, the preference (heterogeneity) of the dorsal vs. ventral striatum exists in processing different types of stimulated behaviors (locomotion, stereotypy, rewarding, etc.) caused by different drugs at different doses [2]. It would be interesting to define the capacity of the group III agonist to affect behavioral effects of the drugs tested in this study.

In summary, this study examined the role of striatal group III mGluRs in the regulation of normal and dopamine-stimulated motor activity with the agonist and antagonist relatively selective for group III mGluRs. It was found that intrastriatal injection of the group III agonist L-AP4 or antagonist MPPG had no significant effects on basal levels of behavioral activity. This indicates the lack of the glutamatergic tone on group III mGluRs controlling basal motor activity. However, L-AP4 dose dependently blocked behavioral activities induced by acute administration of cocaine, amphetamine, and apomorphine. The blocking effect of L-AP4 was attenuated by pretreatment with MPPG. Thus, the group III mGluR glutamatergic transmission in the striatum shows a potential to regulate dopamine-stimulated motor activity in an inhibitory manner.

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