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Characterization of a metabotropic glutamate receptor: Direct negative coupling to adenylyl cyclase and involvement of a pertussis toxin-sensitive G protein

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ABSTRACT **We have characterized a G-protein-coupled glutamate receptor in primary cultures of striatal neurons. Glutamate, quisqualate, or *trans*-1-aminocyclopentane-1,3-dicarboxylate inhibited by 30–40% either forskolin-stimulated cAMP production in intact cells or forskolin plus vasoactive intestinal peptide-activated adenylyl cyclase assayed in neuronal membrane preparations. These inhibitory effects were suppressed after treatment of striatal neurons with *Bordetella pertussis* toxin, suggesting the involvement of a heterotrimeric guanine nucleotide-binding protein (G protein) of the G_i/G_o subtype. The pharmacological profile of this glutamate receptor negatively coupled to adenylyl cyclase was different from that of the metabotropic Qp glutamate receptor coupled to phospholipase C in striatal neurons and from that of the recently cloned "mGluR2" glutamate receptor, which is negatively coupled to adenylyl cyclase when expressed in nonneuronal cells.**

There has been a growing interest in the pharmacology and mechanisms of action of excitatory amino acids (EAAs) (1). EAA receptors, like many other neurotransmitter receptors, can be divided into two categories: those which are ionic channels (ionotropic receptors) and those coupled to G proteins (metabotropic receptors). Three types of ionotropic glutamate receptors have been described: *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate receptors (1). In primary cultures of striatal neurons, we showed that several EAAs, and in particular quisqualate, were able to stimulate phospholipase C (PLC) by acting via another type of glutamate receptor, which we termed the metabotropic Qp receptor (2–4). It is now more commonly called the metabotropic glutamate receptor (mGluR) or *t*-ACPD receptor, because *trans*-1-aminocyclopentane-1,3-dicarboxylate (*t*-ACPD) is the most specific agonist at this receptor (1, 5).

In a recent review, Miller pointed out that mGluR and muscarinic receptors have, in addition to PLC activation, other common transduction mechanisms (6). Indeed, mGluR, like muscarinic receptors, blocks K^+ conductances (I_M, I_{AHP}), spike accommodation, and Ca^{2+} channels and activates Ca^{2+}-dependent K^+ conductance (7–12). In addition, muscarinic m2 and m4 receptors inhibit adenylyl cyclase (11, 12).

A mGluR that stimulates PLC has been cloned (mGluR1) and is expressed as two spliced variants (13, 14). Tanabe *et al.* (15) described a mGluR family (mGluR1–4). In CHO cells transfected with mGluR2 receptors, glutamate and other EAAs inhibited forskolin-induced cAMP formation. This suggests that a mGluR negatively coupled to adenylyl cyclase exists in genuine neurons.

In a preliminary study, we observed that *t*-ACPD inhibited forskolin-induced cAMP formation in striatal neurons in primary culture (16). This led us to examine the possible existence of a mGluR directly and negatively coupled to adenylyl cyclase in cultured striatal neurons.

EXPERIMENTAL PROCEDURES

Materials. *t*-ACPD, quisqualate, AMPA, and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were from Tocris Neuramin (Essex, U.K.). [2-^{3}H]Adenine was from Amersham and [α-^{32}P]ATP from NEN. The 12-well tissue culture cluster plates were from Costar; Dulbecco's modified Eagle's medium and Ham's F-12 nutrient from GIBCO; and 3-isobutyl-1-methylxanthine, *N*-methyl-D-aspartate, kainate, phorbol 12,13-dibutyrate (PBt_2), and forskolin from Sigma. Pertussis toxin (PTX) was from List Biological Laboratories (Campbell, CA). MK-801 was a gift from Merck Sharp & Dohme. All other compounds were of the highest possible grade from commercial sources. Both (1*R*,3*S*)- and (1*S*,3*R*)-*t*-ACPD were prepared and separated as described (17).

Striatal Neuronal Cultures. Primary cultures of striatal neurons were prepared in serum-free medium (18). Pregnant mice (Iffa Credo, Lyon, France) were decapitated and their 14- to 15-day embryos were removed. The striata were then dissected, and cells were gently dissociated in culture medium with a fire-narrowed Pasteur pipette and plated (10^6 cells per well, 1 ml per well) in 12-well cluster plates coated successively with poly(L-ornithine) (15 μg/ml; M_r 40,000) and medium containing 10% fetal bovine serum. Culture medium was a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 nutrient, supplemented with glucose (30 mM), glutamine (2 mM), sodium bicarbonate (3 mM), and Hepes (5 mM). Under these conditions, the cultures are highly enriched in neurons that form many mature and functional synapses (18). Only 7% of the cells have been identified as astrocytes (representing 5% of the surface area) after 12 days *in vitro*.

To avoid the inhibitory effects of γ-aminobutyrate, which is released upon glutamate stimulation during measurements of cAMP formation, we performed our experiments on striatal neurons after 6 days *in vitro*. These immature neurons are

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Abbreviations: *t*-ACPD, *trans*-1-aminocyclopentane-1,3-dicarboxylate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP3, 2-amino-3-phosphonopropanoate; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EAA, excitatory amino acid; mGluR, metabotropic glutamate receptor; PBt_2, phorbol 12,13-dibutyrate; PKC, protein kinase C; PLC, phospholipase C; PTX, pertussis toxin; VIP, vasoactive intestinal peptide.

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unable to release γ-aminobutyrate or other neurotransmitters (18).

Formation of cAMP. The cAMP content of cells was measured by a prelabeling technique (19). On day 6, the cells were washed and incubated at 37°C (5% CO_2/95% air mixture) with [^{3}H]adenine (2 μCi/ml, 24 Ci/mol; 1 Ci = 37 GBq). After 2 hr, the cultures were washed and incubated with 0.75 mM 3-isobutyl-1-methylxanthine and test agents in 1 ml of Hepes-buffered saline (146 mM NaCl/4.2 mM KCl/0.5 mM $MgCl_2$/0.1% glucose/20 mM Hepes, pH 7.2) for 10 min at 37°C in the presence of forskolin (10 μM). In the incubation buffer, Ca^{2+} was omitted to avoid Ca^{2+} influx. Antagonists, phorbol ester, and staurosporine were preincubated 5 min before the beginning of the reaction. The reaction was stopped by aspiration of the medium and addition of 1 ml of ice-cold 5% (wt/vol) trichloroacetic acid. Cells were loosened with a rubber scraper and 100 μl of 5 mM ATP/5 mM cAMP was added to the mixture. Cellular protein was centrifuged at 5000 × *g*. [^{3}H]ATP and [^{3}H]cAMP were separated by sequential chromatography on Dowex and alumina columns. cAMP formation is expressed as percent conversion of [^{3}H]ATP to [^{3}H]cAMP: ([^{3}H]cAMP × 100)/[^{3}H]cAMP + [^{3}H]ATP.

Assay of Adenylyl Cyclase *in Vitro*. After removal of the culture medium, the cells were washed three times with 5 ml of 10% (wt/vol) sucrose/1 mM EGTA/5 mM EDTA/20 mM Tris·HCl, pH 7.4. They were loosened with a rubber scraper and centrifuged for 10 min at 39,000 × *g* at 4°C. The pellet was suspended in 2 ml of the same medium.

Adenylyl cyclase was assayed at 30°C in 100 μl of incubation medium containing 80 mM Tris·HCl (pH 7.4), 2 mM $MgCl_2$, 50 μM ATP, 20 mM creatine phosphate, 20 μg of creatine kinase, 1 mM IBMX, 1 mM cAMP, 50 μM GTP, 1 μCi of [α-^{32}P]ATP and 10 nCi of [^{3}H]cAMP. The membranes were incubated for 10 min and the reaction was stopped by addition of 900 μl of 5.5 mM Tris·HCl, pH 7.4/0.4 mM ATP/0.6 mM cAMP/0.1 M HCl/10 mM $CaCl_2$. cAMP was isolated as described (20).

ADP-Ribosylation. The cells were incubated overnight with the indicated concentrations of PTX diluted in serum-free medium. The cells were then loosened with a rubber scraper and the membrane preparations were pelleted for 15 min at 4°C in an Eppendorf centrifuge and suspended in 20 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM dithiothreitol/0.05% Lubrol PX. Samples were then incubated, unless otherwise indicated, for 60 min at 30°C in 70 mM Tris·HCl (pH 8.0) with 0.5 μM NAD, 1.5 μCi of [α-^{32}P]NAD (800 Ci/mmol), 1 mM ATP, 0.1 mM GTP, 10 mM thymidine, 1 mM EDTA, 0.1 mM $MgCl_2$, 120 μg of L-α-phosphatidylcholine (dimyristoyl), 10 mM nicotinamide, and 25 mM dithiothreitol in a final volume of 60 μl. The procedure was then followed as described (21).

PTX Treatment of Cells. On day 5, cells were incubated with PTX diluted in serum-free medium at 10–1000 ng/ml. After 12 hr, cells were washed and incubated with [^{3}H]adenine for cAMP determination.

RESULTS

EAAs Inhibit Forskolin-Induced cAMP Formation in Cultured Striatal Neurons. Striatal neurons were used after 6 days *in vitro* in all experiments. At this developmental stage, synapses, synaptic vesicles, and neurotransmitter release are almost nonexistent (18). To avoid the effect of ionotropic glutamate receptors on cAMP production, we performed experiments in the presence of 10 μM MK-801 (a noncompetitive *N*-methyl-D-aspartate receptor antagonist) and 30 μM CNQX (a specific competitive antagonist of kainate/AMPA receptors). Finally, we used nominally Ca^{2+}-free medium (in such a medium, we were unable to detect any agonist-induced intracellular Ca^{2+} increase, measured by fura-2 ratio-imaging; data not shown) and tetrodotoxin (3 μM) to reduce indirect effects on cAMP production that could be mediated by Ca^{2+} influxes and cell depolarization, respectively. Under these conditions, glutamate inhibited forskolin (10 μM)-induced cAMP formation in a dose-dependent manner (Fig. 1*A*). The maximal inhibition was 48.5 ± 4.3% (n = 29) and the EC_{50} of glutamate was 53 ± 19 μM (n = 4).

That glutamate could inhibit cAMP formation in the presence of MK-801, CNQX, tetrodotoxin, and low external Ca^{2+} suggested that a nonionotropic glutamate receptor was implicated. We therefore tested the pharmacological profile of this inhibition, obtaining complete dose–response curves for quisqualate, kainate, AMPA, ibotenate, and *t*-ACPD. Quisqualate and *t*-ACPD induced dose-dependent inhibition [EC_{50} of 27 ± 10 μM and 156 ± 38 μM (n = 5), respectively; Fig. 1 *B* and *C*]. Kainate, ibotenate, and AMPA were inhibitory only at very high concentrations (>1 mM) (data not shown; see Fig. 6*B*). To ensure that the effects of EAAs were

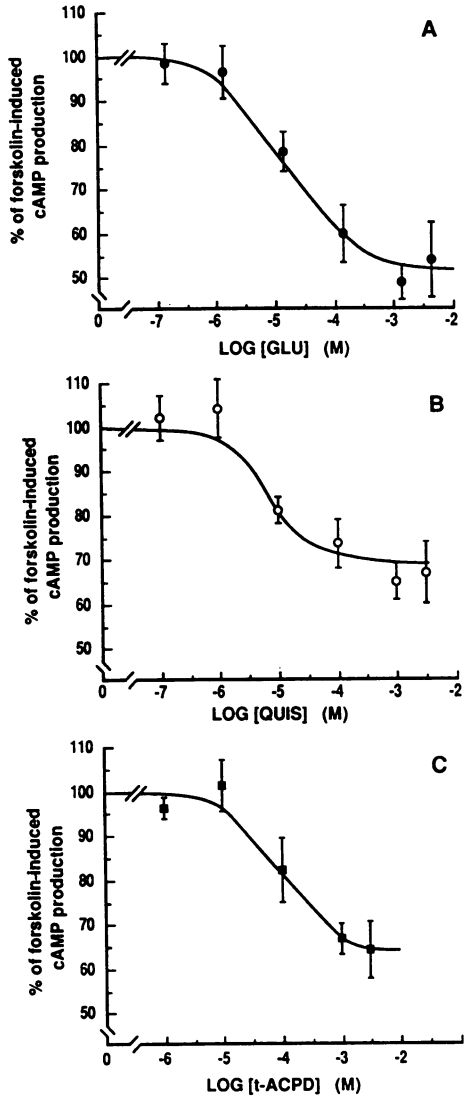

FIG. 1. Concentration-dependent inhibition of forskolin-stimulated cAMP production in striatal neurons by EAAs. Neurons were exposed for 10 min to the indicated concentrations of the agonists glutamate (GLU) (*A*), quisqualate (QUIS) (*B*), and *t*-ACPD (*C*) in the presence of forskolin (10 μM). In the presence and the absence of forskolin, conversion of [^{3}H]ATP to [^{3}H]cAMP was 2.2 ± 0.18% (n = 11) and 0.3 ± 0.02% (n = 6), respectively. These results are representative of four other experiments. Each value is the mean ± SEM of triplicates.

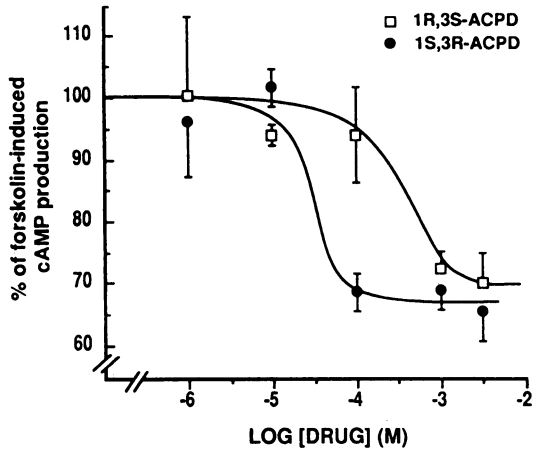

FIG. 2. Inhibitory effect of *t*-ACPD enantiomers on forskolin-induced cAMP accumulation in striatal neurons. Neurons were exposed for 10 min to the indicated concentrations of the 1*R*,3*S* (□) or 1*S*,3*R* (●) enantiomer in the presence of forskolin (10 μM). In the presence and absence of forskolin, conversion of [^{3}H]ATP to [^{3}H]cAMP was 2.97 ± 0.4% and 0.295 ± 0.06%, respectively. These results are representative of five other experiments. Each value is the mean ± SEM of triplicates.

all mediated by the same saturable receptor molecule, we verified that their effects were not additive with those of *t*-ACPD (data not shown).

Differential effects of *t*-ACPD enantiomers on agonist-induced inositol phosphate formation have been described (4). We found that (1*S*,3*R*)-*t*-ACPD was the most potent enantiomer (EC_{50} of 70.4 ± 17.5 μM, n = 4), whereas (1*R*,3*S*)-*t*-ACPD induced a significant effect only when present at >0.1 mM (Fig. 2).

EAAs Inhibit Adenylyl Cyclase Activity in Neuronal Membrane Preparations. To show that the inhibition of cAMP formation in striatal neurons was due to a direct and not to an indirect inhibition of adenylyl cyclase, we measured the effects of glutamate, quisqualate, and (1*S*,3*R*)-*t*-ACPD on adenylyl cyclase activity in membrane preparations. The activity was stimulated ≈10-fold by coapplication of 1 μM forskolin and 0.1 μM vasoactive intestinal peptide (VIP). Glutamate, quisqualate, and (1*S*,3*R*)-*t*-ACPD inhibited in a dose-dependent manner the forskolin/VIP-stimulated adenylyl cyclase activity with EC_{50} of 14.9 ± 9.9 μM, 44.7 ± 2 μM, and 260 ± 40 μM, respectively (n = 3). The maximal inhibitions were 26 ± 7%, 19 ± 3%, and 13 ± 2% (n = 3) for 1 mM glutamate, quisqualate, and (1*S*,3*R*)-*t*-ACPD, respectively (Fig. 3).

Glutamate-Induced Adenylyl Cyclase Activity Is Not Mediated by Protein Kinase C (PKC) Activation. Metabotropic receptors coupled to PLC in striatal neurons can stimulate and be inhibited by PKC (22). PKC activation (with phorbol esters) can induce changes to either facilitate or inhibit cAMP accumulation elicited by forskolin (23). We tested the effects of PBt_2, a phorbol ester, and staurosporine, a widely used PKC inhibitor, on glutamate-induced adenylyl cyclase inhibition. Pretreatment of neurons for 5 min with 0.1 μM PBt_2 or 0.1 μM staurosporine had no effect on inhibition by glutamate, *t*-ACPD, or quisqualate (Fig. 4).

Insensitivity to 2-Amino-3-Phosphonopropanoate (AP3). AP3 has been shown to be a partial agonist or an antagonist of some but not all mGluR subtypes coupled to PLC, depending on the model tested (24, 25). AP3 (1 mM), tested either as an antagonist or an agonist, was completely inactive on this glutamate receptor coupled to adenylyl cyclase (Fig. 5).

Sensitivity to PTX. Overnight preincubation of neurons with various doses of PTX led to a suppression of glutamate-induced inhibition (Fig. 6*A*). Complete reversion was observed with PTX at 1000 ng/ml, a dose necessary to achieve almost complete *in vivo* ADP-ribosylation of G_i and G_o proteins in these neurons (Fig. 6*B*).

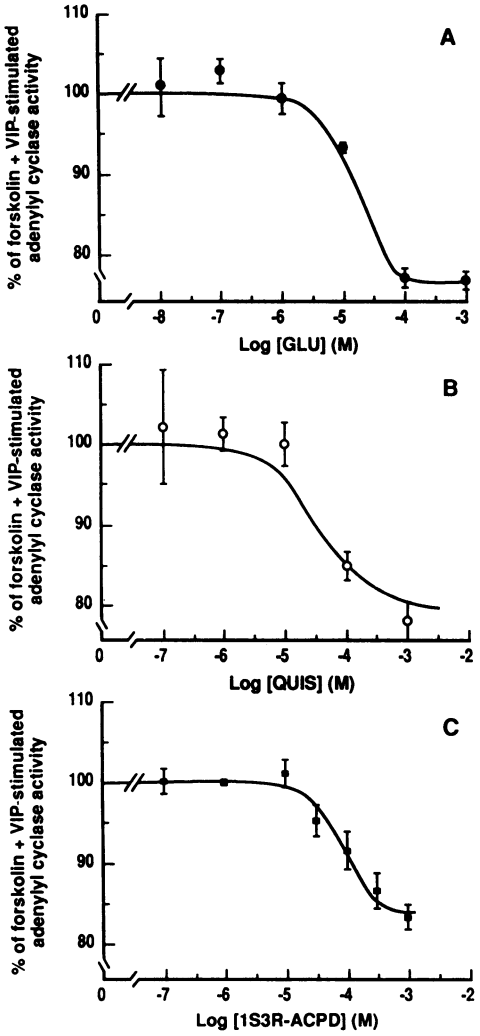

FIG. 3. Concentration-dependent inhibition of forskolin/VIP-stimulated adenylyl cyclase activity in striatal neuron membranes by EAAs. Adenylyl cyclase activity in membrane preparations was assayed for 5 min in the presence of the indicated concentrations of the agonists glutamate (*A*), quisqualate (*B*), and (1*S*,3*R*)-*t*-ACPD (*C*). In the presence of 1 μM forskolin and 0.1 μM VIP, formation of cAMP from ATP was 133.3 ± 2.7 pmol per min per mg of protein, compared with 29.3 ± 8.6 pmol per min per mg in the absence of stimulating agents. Each value is the mean ± SEM of triplicates. This experiment is representative of two others.

When neurons were preincubated overnight with a submaximal concentration of PTX (500 ng/ml), maximal inhibitory effects of *t*-ACPD and quisqualate were greatly reduced, demonstrating that a PTX-sensitive G protein was involved in these responses (Fig. 6*C*). Interestingly, the effects of AMPA (1 mM) and kainate (1 mM) (Fig. 6*C*) as well as ibotenate (data not shown) were also reduced by the same concentration of PTX, suggesting that high concentrations of these EAAs could specifically inhibit adenylyl cyclase via stimulation of a mGluR.

DISCUSSION

We describe here a mGluR negatively and directly coupled to adenylyl cyclase via a transduction G protein of the G_i/G_o subtype.

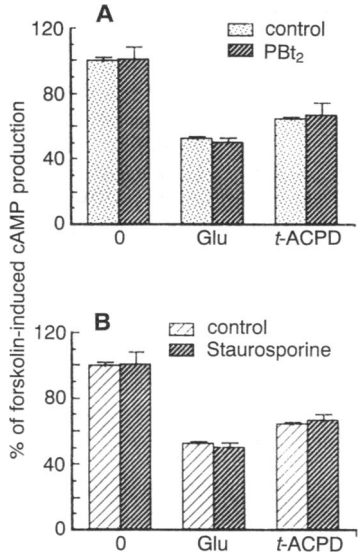

FIG. 4. PBt_2 (0.1 μM) and staurosporine (0.1 μM) do not affect glutamate (1 mM)- and *t*-ACPD (1 mM)-induced inhibition of forskolin-stimulated cAMP production. Neurons were treated with PBt_2 and staurosporine as described in *Experimental Procedures*. In the presence of forskolin alone (0), conversion of [^{3}H]ATP to [^{3}H]cAMP was 3.17 ± 0.4% (n = 10). Each value is the mean ± SEM of nine independent experiments.

Tanabe *et al.* (15) reported that activation of mGluR2 cloned from a rat brain cDNA library and expressed in transfected CHO cells led to inhibition of the forskolin-induced cAMP accumulation, strongly suggesting (without demonstrating) that such a receptor is expressed in neurons. In contrast, we and another group reported preliminary studies indicating that *t*-ACPD could inhibit cAMP formation in striatal neurons and brain slices, respectively (16, 24). However, neither the demonstration of a direct coupling of a glutamate receptor to adenylyl cyclase nor the demonstration of its neuronal localization has been provided. Indeed, a wide range of glutamate receptor subtypes coupled either to ion channels or to enzymatic processes (for review see refs. 1, 3, and 4) have been described; thus the quest for new glutamate receptor subtypes has become a challenging task for researchers using "integrated" models (e.g., brain slices or mixed neuron/glial cell cultures). In such systems, complex

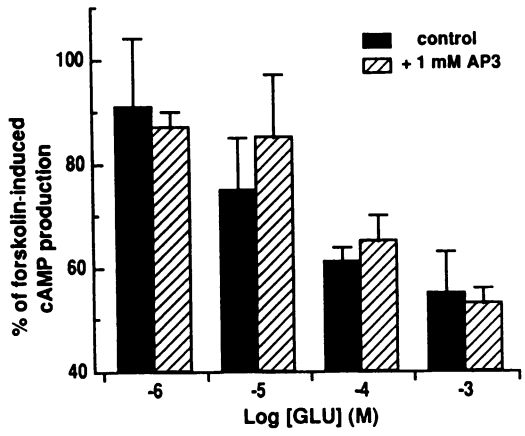

FIG. 5. AP3 does not affect glutamate-induced inhibition of forskolin-stimulated cAMP production. Neurons were treated with AP3 (1 mM) for 5 min prior glutamate application. In the presence of forskolin alone, conversion of [^{3}H]ATP to [^{3}H]cAMP was 3.4 ± 0.5% (n = 5). Each value is the mean ± SEM of six independent experiments.

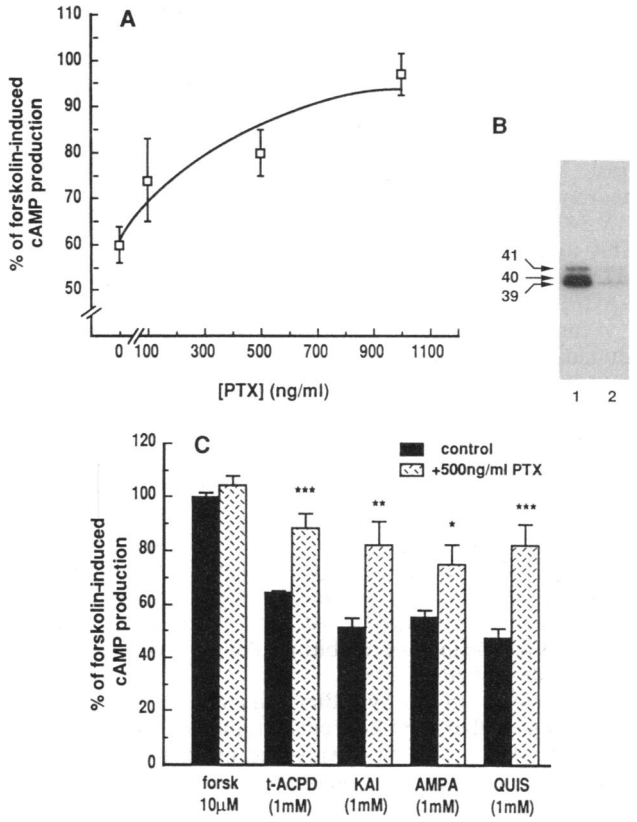

FIG. 6. Effects of PTX on EAA-induced inhibition of forskolin stimulated cAMP production. (*A*) Concentration-dependent inhibition of glutamate-induced effect by PTX. Neurons were pretreated with PTX as described in *Experimental Procedures*. In the presence of forskolin, conversion of [^{3}H]ATP to [^{3}H]cAMP was 2.8 ± 0.5% (n = 6). Each value is the mean ± SEM of four independent experiments. (*B*) Pertussis toxin-catalyzed ADP-ribosylation of striatal neuron membrane preparations. Lane 1, untreated membranes; lane 2, membranes prepared from neurons treated overnight with PTX (1000 ng/ml). Arrows indicate apparent molecular mass in kilodaltons. Only the relevant part of the autoradiogram is shown. Bands were cut out for liquid scintillation spectrometry; values were 1829 ± 37 cpm and 196 ± 9 cpm (n = 3) for control and PTX-treated membranes, respectively. (*C*) Blockade of the inhibitory effect of several EAAs on forskolin-induced cAMP production by PTX. In the presence of forskolin (10 μM), the conversion of [^{3}H]ATP to [^{3}H]cAMP was 1.8 ± 0.2% (n = 11). Treatment with PTX was as described. Each value is the mean ± SEM of 10 independent experiments. A Student's t test was performed between the cAMP productions obtained for each agonist in neurons treated with or without PTX. ***, $P < 0.001$; **, $P < 0.03$; *, $P < 0.05$. KAI, kainate; QUIS, quisqualate.

interactions (between several neuronal populations, receptors, or uptake/release mechanisms) are very likely to occur, making any conclusion uncertain.

Using primary cultures of striatal neurons allows us to perform experiments in neuronal cells almost devoid of glial–neuron interactions (18), in conditions where Na^+-mediated depolarizations or Ca^{2+}-influxes are unlikely to occur (in the presence of TTX and in the absence of external Ca^{2+}) and in the presence of agents that selectively inhibit ionotropic Glu receptors (MK-801 and CNQX). Such features make primary cultured striatal neurons a suitable model for the functional and pharmacological characterization of mGluR subtypes.

Measuring intracellular cAMP accumulation (upon forskolin stimulation), we have pharmacologically characterized the glutamate-inhibitory effects. Glutamate, quisqualate, and *t*-ACPD were the most potent agonists, whereas kainate,

AMPA and ibotenate had very weak potencies (EC_{50} > 1 mM). Two *t*-ACPD enantiomers have been described, 1*S*,3*R* and 1*R*,3*S* (4). In rat hippocampal slices and in cerebellar and striatal cultures after 3 days *in vitro*, (1*S*,3*R*)-*t*-ACPD is far more potent than (1*R*,3*S*)-*t*-ACPD to stimulate inositol phosphate formation and intracellular Ca^{2+} increase (4). In contrast, in older (9–11 days *in vitro*) cerebellar and striatal neurons or in *Xenopus* oocytes injected with rat brain RNA, (1*R*,3*S*)-*t*-ACPD is more potent than (1*S*,3*R*)-*t*-ACPD (26). This suggests a heterogeneity of the mGluR coupled to PLC in these different systems. Here we have shown that (1*R*,3*S*)-*t*-ACPD is less potent than (1*S*,3*R*)-*t*-ACPD.

A further comparison of the pharmacological profile of this glutamate receptor negatively coupled to adenylyl cyclase with that of the PLC-coupled glutamate receptor that we previously described in the same striatal neurons in primary culture (2–4, 25) or with that of the mGluR2 clone expressed in cDNA-transfected CHO cells (15) does in fact reveal great differences. Indeed, the rank order of potencies is quisqualate ≥ ibotenate = glutamate > *t*-ACPD > kainate = AMPA for the mGluR coupled to PLC (16) and glutamate = *t*-ACPD > ibotenate > quisqualate for the cloned mGluR2 inhibiting cAMP production in CHO cells (15), whereas it is glutamate ≥ quisqualate > *t*-ACPD >> kainate = ibotenate = AMPA for the mGluR inhibiting adenylyl cyclase in striatal neurons described here.

Interestingly, *in situ* hybridization and Northern blot analysis using probes directed against mGluR2 revealed that mGluR2 mRNA is highly expressed in cerebral cortex, olfactory bulbs, cerebellum, and hippocampus but not in striatum (15).

In addition, biochemical features of the mGluR coupled to PLC and of the glutamate receptor negatively coupled to adenylyl cyclase are different. The mGluR coupled to PLC is blocked by phorbol esters in a staurosporine-sensitive manner (22). In the same cellular preparation, we found that glutamate's inhibitory actions were affected neither by PBt_2 nor by staurosporine pretreatment. Thus, PKC activation is not a necessary step in the transduction process leading to the inhibition of forskolin-induced cAMP formation, as it has been previously described in other systems (23).

Until now, the pharmacology of mGluRs has been in desperate need of a reliable antagonist. AP3 has been proposed as a rather selective antagonist of the mGluR coupled to PLC in several systems (4), whereas it is a partial agonist in other models (16). Here, AP3 was without effect (either as an antagonist or an agonist) on the glutamate-induced inhibition of cAMP formation.

mGluRs are coupled to their effectors through G proteins. We observed that the glutamate-induced inhibition of adenylyl cyclase was reduced in a dose-dependent manner by pretreatment with PTX. mGluR coupled to PLC has been found to be either sensitive or insensitive to PTX, depending on the system (4), and Tanabe *et al.* (15) described the "mGluR2 effect" to be blocked in CHO cells by low PTX doses (1 ng/ml). We found that PTX at 1000 ng/ml induced the maximal effect in striatal neurons. One can explain this discrepancy by the difference of penetration of PTX in these two cellular models (CHO cells and cultured rat striatal neurons). In striatal neurons, we showed that the same high concentrations of PTX were required to achieve complete ADP-ribosylation of G proteins in intact cells. Interestingly, the effects of kainate, ibotenate, and AMPA were also inhibited by PTX treatment, indicating that these ionotropic agonists were also stimulating glutamate receptors negatively coupled to adenylyl cyclase.

Finally, to ensure that glutamate inhibited cAMP formation directly via a receptor coupled to adenylyl cyclase, we measured the effects of quisqualate, glutamate, and *t*-ACPD on adenylyl cyclase activity in the membrane preparation. All three agonists inhibited adenylyl cyclase activity with similar potencies to those observed by intracellular cAMP formation.

In conclusion, our main finding is that, in genuine intact neurons, as well as in membrane preparations, glutamate, quisqualate, and *t*-ACPD can inhibit adenylyl cyclase activation via a PTX-sensitive G protein. The pharmacology of the receptor mediating this action is different from that of the other mGluRs (including the mGluR2 clone). The physiological functions and the possible identity of this receptor with one of the recently cloned metabotropic receptors remain to be determined.

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