Functional Switch from Facilitation to Inhibition in the Control of Glutamate Release by Metabotropic Glutamate Receptors*

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The probability of glutamate release at nerve terminals is regulated by presynaptic metabotropic glutamate receptors (mGluRs)1 coupled to the activation of different signal transduction pathways. Molecular cloning has revealed the existence of at least eight mGluRs (mGluR1–8) (Refs. 1–8; see Ref. 9 for a review). Group I receptors (mGluR1 and mGluR5) are selectively activated by 3,5-dihydroxyphenylglycine (DHPG) (10), and their expression in transfected cell lines has indicated the coupling of these receptors to phosphoinositide hydrolysis (2, 3, 11) and to the generation of the intracellular messengers diacylglycerol, active on protein kinase C (PKC), and inositol trisphosphate, which mobilizes intracellular calcium. On the other hand, Group II (mGluR2 and mGluR3) and Group III (mGluR4 and mGluR6–8) receptors are selectively activated by (2S,1'S,2'S)-2-carboxycyclopropylglycine (l-CCG-I) and l-2-amino-4-phosphonobutyrate (l-AP4), respectively. The expression of these receptors in non-neuronal cells has shown them to be negatively coupled to cAMP formation (4–8, 12). The activation of the adenylyl cyclase-linked mGluRs in neuronal preparations such as cerebral slices, cultured neurons, and nerve terminals reduces synaptic transmission (13–17), glutamate release (18, 19), and Ca2+ currents (17, 20–23). In cerebrocortical nerve terminals, it has been shown, in addition, that although the agonist l-AP4 reduces both the depolarization-induced increase in Ca2+ and forskolin-stimulated cAMP formation, only the reduction in [Ca2+]i, is responsible for the decrease in release (24).

In contrast to the negative modulation of neurotransmitter release by Group II and III mGluRs, the role of phosphoinositide-linked mGluRs in the control of glutamate release is not clear. In nerve terminals, the activation of these receptors facilitates glutamate release (25), an effect likely to be the result of diacylglycerol production (26) and protein kinase C activation (27). This facilitation of release is also consistent with the presence of a presynaptic PKC-dependent facilitatory pathway as revealed by synaptic transmission studies in slice preparations (28, 29). However, surprisingly, the activation of presynaptic phosphoinositide (PI)-linked mGluRs has shown a reduction of synaptic transmission due to decreased glutamate release (30, 31). Here we have found that PI-coupled mGluRs can mediate not only the facilitation, but also the inhibition of glutamate release depending on the response pathway activated. Under control conditions, the response to a first stimulation with the agonist is the facilitation of glutamate release, an effect likely to be due to diacylglycerol (DAG) production and PKC activation. However, following receptor activation, a reversible desensitization of both DAG production and release facilitation occurs, during which a second stimulation with the agonist inhibits the entry of Ca2+ and the release of glutamate. A complete switch to inhibition can be induced by a small (5 μM) tonic elevation of the extracellular glutamate concentration, suggesting that release facilitation, which may be necessary for synaptic plasticity, is limited to conditions under which an appropriate clearance of synaptic glutamate exists, a property likely important in avoiding the neurotoxic accumulation of glutamate in the synapse.

EXPERIMENTAL PROCEDURES

Synaptosomal Preparation—Cerebral cortices from male Wistar rats (2–3 months old) were isolated and homogenized in medium containing 320 mM sucrose, 0.5 mM EDTA, and 5 mM TES, pH 7.4. The homogenate was spun for 5 min at 900 × g at 4 °C, and the supernatants were spun...
again for 10 min at 17,000 × g. From the pellets thus formed, the white loosely compacted layer containing the majority of synaptosomes was gently resuspended in medium containing 250 mM sucrose and 5 mM TES, pH 7.4 (10 ml/four cortices), and the protein was determined by the biuret method. Pellets containing 1 mg of protein were stored on ice. Synaptosomes remained fully viable when stored as pellets for at least 6 h after preparation, as judged by the extent of KCl- and 4-aminopyridine (4-AP)-induced glutamate release.

**Glutamate Release**—Glutamate release was assayed by on-line fluorometry as described previously (32). Synaptosomal pellets were resuspended (0.67 mg/ml) in incubation medium containing 122 mM NaCl, 3.1 mM KH2PO4, 0.4 mM KH2PO4, 5 mM NaHCO3, 1.2 mM MgSO4, 10 mM glucose, and 20 mM TES, pH 7.4, and preincubated at 37 °C for 1 h in the presence of 16 μM bovine serum albumin (BSA) to bind any free fatty acids released from synaptosomes during the preincubation (33). After preincubation, the synaptosomes were pelleted and resuspended in fresh incubation medium both with and without BSA depending on whether release inhibition or facilitation was under investigation. An aliquot (1 ml) was transferred to a stirred cuvette containing 1 mM NADP+, 50 units of glutamate dehydrogenase, and 1.33 mM CaCl2 or 200 mM free Ca2+, and the fluorescence of NADPH was followed in a Perkin-Elmer LS-50 luminescence spectrometer at excitation and emission wavelengths of 340 and 510 nm. Data points were corrected for the Ca2+-independent release. Thus, the Ca2+-dependent release was calculated by subtracting the release obtained during 5 min at 200 mM free Ca2+ from the release at 1.33 mM CaCl2.

**Determination of Diacylglycerol**—Diacylglycerol was estimated as the amount of 32P incorporated from [γ-32P]ATP into phosphatidic acid in the presence of diacylglycerol kinase. Aliquots (0.5 ml) of the synaptosomal suspension (0.67 mg/ml) were added to 0.5 ml of chloroform/methanol (95:5, v/v) and rapidly frozen at −80 °C until all samples were collected. After thawing, the phases were separated by centrifugation, and the lower organic phase was collected and evaporated in a water bath at 60 °C. The tubes containing diacylglycerol were incubated in medium (final volume of 100 μl) containing 70 mM NaCl, 1.4 mM β-mercaptoethanol, 35 mM MgCl2, 0.7% Nonidet P-40, 0.14 mg/ml phosphatidylinerine, and 35 mM PIPES, pH 6.8. The reaction, at 30 °C for 1 h, was started by the addition of 60 milliunits of diacylglycerol kinase, 25 μM ATP, and 0.45 μCi of [γ-32P]ATP and stopped with 100 μl of a mixture containing 5% perchloric acid, 10 mM ATP, and 1 mM H2PO4. Phosphatidic acid was extracted with 0.7 ml of chloroform/methanol (95:5, v/v); the solvent was evaporated in a water bath at 60 °C, and radiolabel was estimated after the addition of 1.2 ml of scintillation liquid. 1,2-Diacylglycerol-sn-glycerol was used as a standard.

**Measurements of cAMP**—Synaptosomes were resuspended (2 mg/ml) in incubation medium containing 16 mM BSA and preincubated at 37 °C for 5 min. After this period, 1.33 mM CaCl2, adenosine deaminase (1 unit/mg of protein), and 1 mM 3-isobutyl-1-methylxanthine were added, and the incubation was continued for another 10 min. To test for an increase in cAMP formation, pharmacological agonists were added, and samples were taken 2 min later. In those experiments in which the ability of agonist to reduce forskolin-stimulated cAMP levels was tested, forskolin at 100 μM was added 10 min prior to the addition of the agonist. Agonists were then added, and 0.5-m1 aliquots were taken 1 min later. After acidic extraction and neutralization (34), the cAMP content was determined by radiomunnoassay with a commercially available cAMP kit (Amersham International, Buckinghamshire, United Kingdom).

**Cytosolic Free Ca2+ Concentration**—[Ca2+]i was measured with fura-2. Synaptosomes were resuspended (2 mg/ml) in incubation medium containing 16 mM BSA in the presence of 1.33 mM CaCl2 and 5 mM fura-2/acetoxymethyl ester and incubated at 37 °C for 20 min. After fura-2 loading, synaptosomes were pelleted and resuspended in fresh incubation medium with or without BSA depending on whether inhibition or facilitation of Ca2+ entry was being studied. An aliquot (1 ml) was transferred to a stirred cuvette containing 1.33 mM CaCl2, and the fluorescence was monitored at 340 and 510 nm. Data points were collected at 0.5-s intervals. [Ca2+]i was calculated using the equations described by Grynkiewicz et al. (35).

**Extracellular Glutamate Concentration** (Glui)—Synaptosomes in incubation medium containing BSA (0.67 mg/ml) were preincubated for 50 min and then treated with increasing concentrations (3–33 mM) of KCl for another 10 min. After this time, the suspension was centrifuged at 12,000 × g for 1 min, and the supernatant was saved for glutamate content, which was analyzed by the addition of 50-μl aliquots to a stirred cuvette containing 1.2 ml of incubation medium, 1 mM NADP+, and 50 units of glutamate dehydrogenase.

**Release Facilitation and Diacylglycerol Production**—The depolarization of nerve terminals with the K+ channel blocker 4-AP has been shown to open voltage-gated Ca2+ channels and to induce the release of vesicular glutamate (36). Fig. 1A shows that the prior addition of the PI-linked mGluR selective agonist DHPG (10) to cerebrocortical nerve terminals had no effect on the Ca2+-dependent release of glutamate induced by maximal depolarization with 4-AP at 1 mM. DHPG did, however, enhance the glutamate release induced by a suboptimal 4-AP concentration (50 μM), an effect that was abolished by the mGluR antagonist MCPG (Fig. 1B) (37). Fig. 1B shows that the PKC-prefering inhibitor staurosporine (38) abolished release facilitation, a result consistent with the involvement of protein kinase activity in the facilitatory pathway activated by the mGluR agonist.

Further evidence of the involvement of PI hydrolysis and protein kinase C activity in the potentiation of release by mGluRs was obtained from data showing that the addition of the agonist enhanced diacylglycerol formation in the nerve terminal preparation (Fig. 2 A, control). However, the change in DAG levels was transient since the response, which was maximal 15–20 s after agonist addition, then decayed toward basal levels. The DHPG-induced formation of diacylglycerol measured at the peak exhibited an EC50 value of 1.76 ± 0.92 μM (Fig. 2B) and was abolished not only by MCPG, but also by other mGluR antagonists such as (S)-4-carboxyphenylglycine (37). In contrast, the agonists of the adenyl cyclase-coupled mGluRs, l-CCG-I (39) and l-AP4, failed to either stimulate DAG production (Fig. 2C) or potentiate glutamate release (data not shown).
Release Inhibition and Loss of Diacylglycerol Production—

The transient production of DAG observed (Fig. 2A, control) may be explained in part by the metabolism of DAG and also by the PKC-mediated negative feedback control of agonist-induced phosphoinositide hydrolysis that has been observed in many neuronal preparations (40, 41). Thus, the contribution of receptor desensitization to the decay in DAG formation was estimated in the presence of the protein kinase inhibitor staurosporine, which largely prevented the drop in DAG formation that follows receptor activation (Fig. 2A, + staurosporine). These results indicate that the activation of PKC is involved in the desensitization of the pathway leading to DAG production.

To study the desensitization of the DHGP-sensitive mGluR with respect to further generation of DAG after stimulation, nerve terminals were pre-exposed to 100 μM DHGP for 1 min (first stimulation) and, after washing to remove the agonist, were rechallenged with DHGP (second stimulation), and DAG production was measured at the peak, i.e., 5 s after the addition of the agonist. As shown in Fig. 3A, 5 min after the first stimulation, DHGP failed to activate the receptor in terms of DAG formation. However, a full recovery of the receptor response was observed 30 min after the first stimulation (Fig. 3A), indicating that agonist-induced desensitization of DAG formation is a reversible process.

In experiments parallel to those shown in Fig. 3A, we determined whether the loss of ability to generate DAG altered the modulatory control of glutamate release by DHGP. It is shown in Fig. 3B that parallel to the loss of DAG production by the agonist, a suppression of the facilitatory effect by DHGP on release was observed and that, at the same time, the agonist became able to inhibit the release induced by maximal depolarization with 4-AP (1 mM) (Fig. 3B, second set of bars). Interestingly, 30 min after the first stimulation, the ability of the agonist to potentiate the release was recovered, and the inhibitory action was lost (Fig. 3B). These results indicate that the desensitization of the DHGP-sensitive mGluR to produce DAG parallels a switch from facilitation to inhibition in the control of glutamate release.

The inhibitory effect was further characterized in nerve terminals desensitized to produce DAG. Thus, Fig. 4 shows that the strong inhibition of the Ca^{2+}-dependent release by DHGP (Fig. 4A) exhibited an EC_{50} value of 3.36 ± 0.89 μM (Fig. 4B) and was abolished by the mGluR antagonist MCPG (Fig. 4C). In addition, the agonists of the adenyl cyclase-coupled mGluRs, L-CCG-I and L-AP4, failed to induce release inhibition, a result consistent with the involvement of a PI-coupled mGluR in this action. Although the presence of an inhibitory control of glutamate release by L-AP4-sensitive mGluRs in cerebrocortical nerve terminals has been previously shown (19, 24), this inhibitory pathway is developmentally controlled (19) such that the inhibition of release is observed in nerve terminals from young animals (2–3 weeks), but not in the adult animals (2–3 months) used throughout this study.

Changes in [Ca^{2+}], Account for Release Facilitation and Release Inhibition—To better understand the mechanism by which DHGP-sensitive mGluRs modulate glutamate release, we determined the [Ca^{2+}], with the Ca^{2+} indicator fura-2. It is important to note that the Ca^{2+} indicator does not detect the high local [Ca^{2+}], that triggers glutamate release (42) and therefore that the changes in [Ca^{2+}], could be greater that the average bulk increase obtained with the indicator. In control synaptosomes (first stimulation), the depolarization of the nerve terminals with 50 μM 4-AP induced a rapid increase in the fura-2 signal (Fig. 5A). This depolarization-induced increase in [Ca^{2+}], was potentiated by the prior addition of DHGP and was prevented by staurosporine (Fig. 5A), indicating the involvement of PKC in the facilitation of Ca^{2+} entry in nerve terminals. Fig. 5A also shows that DHGP failed to alter the increase in [Ca^{2+}], caused by maximal stimulation with 1 mM 4-AP. Bar graphs indicating the changes in [Ca^{2+}], from basal levels prior to depolarization to the stable plateau after depolarization, are shown in Fig. 5B, except for the experiment with staurosporine. In Fig. 5B, it is also shown that in nerve terminals pre-exposed to DHGP, followed by washing and a 5-min lag, DHGP failed to significantly potentiate the fura-2 response induced by submaximal stimulation, but strongly reduced the maximal fura-2 response. The facilitatory and inhibitory actions of DHGP-sensitive mGluRs on release can therefore be explained by an enhancement and a reduction of the increase in [Ca^{2+}], induced by depolarization, respectively.

In another set of experiments, we found that DHGP did not alter the cAMP content under conditions in which basal cAMP levels (10.5 ± 4.2 pmol/mg) were enhanced by the β-adrenergic agonist isoproterenol (65.8 ± 5.8 pmol/mg) and the forskolin-
stimulated cAMP content (150.2 ± 5.5 pmol/mg) was reduced by the adenosine A1 agonist R(−)-N6-(2-phenylisopropyl)adenosine (33.0 ± 4.6 pmol/mg). These results indicate that changes in cAMP levels are not involved in the modulatory effects of DHPG on both release and Ca2⁺ entry in nerve terminals.

To study the sensitivity of the inhibitory effects of DHPG to staurosporine, the nerve terminals were treated first with 100 μM DHPG for 1 min to induce the switch from facilitation to inhibition and then, after washing by centrifugation and resuspension to remove the agonist, with 1 μM staurosporine for 15 min. The extent of the Ca2⁺-dependent release of glutamate was calculated after 5 min of depolarization. In the experiments testing for release facilitation, DHPG (100 μM) was added 5 s prior to the depolarization of the nerve terminals with 50 μM 4-AP in the presence of arachidonic acid (2 μM) added 5 s prior to the agonist. The inhibition of release by DHPG (100 μM) was estimated in nerve terminals depolarized with 1 mM 4-AP. The results are means ± S.D. of data from three to five preparations of synaptosomes.

PKC activity is, however, involved in the switch from facilitation to inhibition of PI-coupled mGluRs. Thus, in experiments in which the treatment with DHPG was carried out after the prior inhibition of PKC with staurosporine (Fig. 6C, staurosporine + DHPG), a second stimulation of the receptor with DHPG 5 min after the first stimulation failed to induce release inhibition.

Increase in [Glu]ext Induces the Switch from Facilitation to Inhibition in Release Modulation—To investigate whether phospholipase C-coupled mGluR activation by a tonic elevation of the [Glu]ext promotes the switch from facilitation to inhibition in glutamate release control, we exposed nerve terminals to increasing concentrations of KCl (3–30 mM) to disrupt the electrical component of the Na⁺ electrochemical gradient used by glutamate transporters to maintain a low basal [Glu]ext (43).

After this treatment, the [Glu]ext at each KCl concentration was determined, as well as release modulation by DHPG. In the absence of added KCl, the basal [Glu]ext was 0.82 ± 0.21 μM, at which the facilitation by DHPG was maximal and inhibition was absent (Fig. 7). The increase in [Glu]ext, however, a decrease in release facilitation with the concomitant appearance of release inhibition, so that at 5 μM extracellular glutamate, facilitation was virtually abolished, whereas inhibition was maximal (Fig. 7). The mGluR antagonist MCPG at 2 mM prevented the KCl-induced switch from facilitation to inhibi-
bition, indicating the involvement of mGluR activation by increased \([\text{Glu}]_{\text{ext}}\) in these changes. Fig. 7 also shows that the ionotropic glutamate receptor antagonists D(-)2-amino-5-phosphonopentanoic acid and 6-cyano-7-nitroquinoxaline-2,3-dione (both at 100 \(\mu M\)) were without effect on the KCl-induced switch in the mGluR function.

**DISCUSSION**

The results of this study provide evidence for a dual role in the control of glutamate release by phosphoinositide-linked mGluRs as the agonist DHPG is able to induce either release facilitation or release inhibition depending on the response pathway activated. Release facilitation is the result of agonist-induced production of diacylglycerol and a protein kinase-dependent increase in the entry of Ca\(^{2+}\) into the nerve terminal. In contrast, release inhibition seems to be the result of an activity-dependent receptor switch since it is observed only after a second stimulation with the agonist. In this case, the inhibition of glutamate release parallels a reduction of the entry of Ca\(^{2+}\) into the nerve terminal in which staurosporine-sensitive protein phosphorylation is not involved.

In addition to activating the facilitatory pathway, PKC seems to cause a homologous desensitization of DAG produc-
The presence of arachidonic acid (2 mM) obtained from three preparations of synaptosomes. The results are means ± S.D. of data from three preparations of synaptosomes. div, division.

**FIG. 6.** Stauroporine does not alter the inhibitory effects of DHPG, but prevents the DHPG-induced switch from facilitation to inhibition. Synaptosomes pre-exposed to DHPG (100 μM) for 1 min were washed by centrifugation and resuspension, followed by incubation with 1 μM staurosporine for 15 min. After this time, the Ca²⁺-dependent release (A) and the changes in [Ca²⁺] (B) induced by maximal depolarization (1 mM 4-AP) were determined in the absence (Control) and presence of DHPG (100 μM) added 10 s prior to depolarization. To determine the sensitivity to staurosporine of the DHPG-dependent switch from facilitation to inhibition (C), synaptosomes were first incubated with 1 μM staurosporine for 15 min and then pre-exposed to DHPG (100 μM) for 1 min, followed by washing by centrifugation and resuspension (staurosporine + DHPG). 5 min after the DHPG treatment, the Ca²⁺-dependent release of glutamate induced by maximal depolarization (1 mM 4-AP) was determined in the absence (Control) and presence of DHPG (100 μM) added 10 s prior to depolarization. The results are means ± S.D. of data from three preparations of synaptosomes. div, division.

**FIG. 7.** Increase in the extracellular concentration of glutamate also induces the switch from facilitation to inhibition in the control of glutamate release. Synaptosomes preincubated for 50 min in the presence of EGTA were treated with increasing concentrations of KCl (3–33 mM) for another 10 min in the absence (circles) or presence of 2 mM MCPG (squares) or 100 μM (-)-2-amino-5-phosphonopentanoic acid and 100 μM 6-cyano-7-nitroquinazoline-2,3-dione (triangles). After this time, a set of samples was centrifuged at 12,000 × g for 1 min, and the supernatant was taken to determine the [Glu]o (μM) (see “Experimental Procedures”). Another set of the synaptosomal samples was used, after centrifugation to remove high KCl, to estimate glutamate release modulation. Facilitation (solid symbols) was established as the increase in the release induced by 50 μM 4-AP in the presence of arachidonic acid (2 μM) and DHPG (100 μM) (100% facilitation corresponds to that observed in control synaptosomes, i.e., in the absence of added KCl). Release inhibition (open symbols) was determined as the decrease by DHPG (100 μM) in the 1 mM 4-AP-induced release (100% inhibition corresponds to the inhibition observed at the highest KCl concentration). The results are means ± S.D. of data obtained from three preparations of synaptosomes.

Addition, the inhibitory action of PKC on agonist-induced hydrolysis of PI has also been shown to occur in other neural preparations (40, 41), and these effects are generally thought to reflect a negative feedback action of the kinase in the signal transduction pathway. Although the locus for the suppression of DAG responses is not known, one likely possibility is the receptor since PI-linked mGluRs contain consensus phosphorylation sites (2, 3), and it has been demonstrated that PKC phosphorylates PI-linked mGluRs (44, 45).

The loss of agonist ability to produce DAG and to facilitate glutamate release observed after the prior activation of the receptor does not seem to involve a loss of receptor ability to respond to extracellularly added agonist, but a switch in receptor function. Thus, the receptor is capable of responding to the agonist DHPG in an MCPG-sensitive manner, but in this case, activates a transduction pathway that leads to the inhibition of Ca²⁺ entry and glutamate release. The selective inhibition by PKC of some signal transduction pathways activated by PI-coupled mGlurRs has been found in Chinese hamster ovary cells transfected with mGluR1 (11). In this study, the activation of PKC with phorbol esters reduced the glutamate response on PI hydrolysis, but did not alter cAMP accumulation or the release of arachidonic acid induced by the agonist.

An inhibitory action by presynaptic PI-linked mGlurRs has also been observed in studies of synaptic transmission in hippocampal slices (30, 31). Moreover, the finding in this paper that the DHPG-induced inhibition of glutamate release was not prevented by the protein kinase inhibitor staurosporine makes the involvement of protein phosphorylation in the inhibitory pathway rather unlikely, while suggesting a mechanism for the inhibition of release-coupled Ca²⁺ channels in which diffusible second messengers are not involved (20, 21).

An alternative explanation to the PKC-mediated switch from facilitation to inhibition in receptor function is the parallel activation of both pathways by the agonist. Thus, release facilitation would be the result of a dominant role of the facilitatory pathway over the inhibitory pathway (46), whereas release inhibition would be revealed only after the desensitization of the facilitatory pathway. If this is the case, the inhibition of PKC activity prior to receptor activation would prevent facilitation, but not PKC-independent release inhibition. However, no release inhibition by DHPG was observed in nerve terminals.
in which PKC activation was prevented by staurosporine prior to the induction of the switch (Fig. 6C). These results therefore rule out a parallel activation of the facilitatory and inhibitory pathways and support the involvement of PKC activity in the switch from facilitation to inhibition in receptor function (Fig. 8).

If phosphorylation by PKC is responsible for the switch from facilitation to inhibition, it is likely that the dephosphorylation of the putative target would be responsible for the recovery of facilitation observed after a 30-min lag. In this regard, we have shown that the protein phosphatase 1 and 2A inhibitor okadaic acid prevented the slow recovery of agonist-induced production of DAG (26). Moreover, the inhibition of protein phosphatases 1 and 2A, but not 2B, prolongs the period during which release inhibition by DHPG is observed.2

Isolated nerve terminals under resting conditions maintain an extracellular glutamate concentration of 0.8 ± 0.2 μM, similar to that reported in the mammalian central nervous system (47, 48), whereas the glutamate concentration in the cleft peaks in the range 1–5 mM for a very brief period after stimulation (49). The data given in this paper that the EC50 value for the loss of facilitation is ~3 μM extracellular glutamate indicate that the switch in the presynaptic control of release has been set up at the very low end of the range changes in extracellular glutamate. Thus, it is assumed that release facilitation is going to be very sensitive to changes in the clearance of transmitter and presumably restricted to conditions under which a strict control of [Glu]ext is maintained. Under these latter conditions, release facilitation might contribute to the reinforcement of glutamatergic synapses during synaptic plasticity (50). An increase in the [Glu]ext has also been related to glutamate neurotoxicity (51). However, a contribution of the release facilitation mechanism to the neurotoxic accumulation of glutamate is rather unlikely due to the [Glu]ext-dependent switch to inhibition. The desensitization of excitatory responses by extracellular glutamate has also been observed in the case of ionotropic glutamate N-methyl-d-aspartate receptors (52, 53) and N-methyl-n-aspartate acid receptors (54, 55) to occur at low μM glutamate concentrations.

The fact that very low extracellular concentrations of glutamate promote the switch from facilitation to inhibition in release control may also explain the difficulties in observing presynaptic facilitatory receptors in slices preparations if sufficient glutamate is present to cause desensitization despite the convincing evidence for synaptic transmission potentiation by a presynaptic PKC-dependent pathway (28, 29) in these preparations. In addition, these results may also provide an explanation for the inhibition of synaptic transmission by presynaptic PI-linked mGluRs observed in hippocampal slices (14, 30, 31).

The identity of the facilitatory-inhibitory DHPG-sensitive mGluR is not known, as the presynaptic location of the PI-coupled mGluRs cloned has not yet been detected (56), although positive results have also been reported for mGluR5 (57). However, we have recently found that both the facilitatory and inhibitory effects of DHPG remain in mGluR1-deficient mice,3 indicating that mGluR5 or an unknown DHPG-sensitive receptor is involved in these effects.

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