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NMDA-induced phosphorylation and regulation of mGluR5

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Abstract

Glutamate regulates neuronal function by acting on ionotropic receptors such as the *N*-methyl-D-aspartate (NMDA) receptor and metabotropic receptors (mGluRs). We have previously shown that low concentrations of NMDA are able to significantly potentiate mGluR5 responses via activation of a protein phosphatase and reversal of phosphorylation-induced desensitization. While low concentrations of NMDA are able to potentiate mGluR5 responses, higher concentrations of NMDA are actually inhibitory. In this report, we show that NMDA receptors and mGluR5 are highly colocalized in cortical regions. We also show that in voltage-clamp recordings obtained from *Xenopus* oocytes expressing mGluR5 and NMDA receptors, high concentrations of NMDA (50–100 μM) that elicited large currents (>400 nA) caused an inhibition of mGluR5 currents. Additionally, agonist-induced phosphoinositide hydrolysis presumably mediated by activation of mGluR5, is inhibited by NMDA (30 μM and above). Additional data presented in this report suggest that the inhibitory effect of NMDA is caused by phosphorylation of mGluR5 at protein kinase C (PKC) sites since NMDA induces phosphorylation of the receptor as measured in a back phosphorylation assay.

Keywords: Metabotropic; Glutamate; LTP; Kinase; Phosphatase; Desensitization

1. Introduction

Glutamate is the major excitatory transmitter in the nervous system. Glutamate acts on both ligand-gated ion channels or ionotropic (iGluRs) and G protein-coupled or metabotropic (mGluRs) receptors. The ionotropic receptors include the *N*-methyl-D-aspartate (NMDA), AMPA and kainate receptors. The metabotropic glutamate receptors are subdivided into three groups on the basis of agonist selectivity and effector coupling. group I mGluRs (mGluR1 and mGluR5) are Gq-coupled receptors that cause an increase in IP3 accumulation. group II (mGluR2 and mGluR3) and group III (mGluR4, mGluR6, mGluR7 and mGluR8) mGluRs are Gi/Go-coupled receptors that are negatively coupled to increases in cAMP accumulation.

One of the functional consequences of activating metabotropic receptors in the CNS is the potentiation of NMDA-mediated responses (Aniksztejn et al., 1991; Attucci et al., 2001; Awad et al., 2000; Doherty et al., 2000; Fitzjohn et al., 1996; Harvey and Collingridge, 1993; Skeberdis et al., 2001). Conversely, NMDA can also regulate metabotropic receptor responses, specifically group I mGluR responses (Alagarsamy et al., 1999; Challiss et al., 1994; Luthi et al., 1994). There have been many studies addressing the mechanisms responsible for mGluR modulation of NMDA receptor function. However, fewer studies have addressed the effects of NMDA receptor activation on mGluR function. Previous studies have shown that group I mGluRs desensitize by a protein kinase C (PKC)-dependent process (Alaluf et al., 1995; Desai et al., 1996; Herrero et al., 1994; Thomsen et al., 1993). Examination of mGluR5 has shown that phosphorylation at specific serine/threonine residues is responsible for this desensitization (Gereau and Heinemann, 1998). Our previous studies have shown that 10 μM NMDA

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is able to reverse the PKC-mediated desensitization by activation of a protein phosphatase and subsequent dephosphorylation of mGluR5 (Alagarsamy et al., 1999).

Although we have identified a potential mechanism for the potentiating effects of low concentrations of NMDA on mGluR5 function, larger amounts of NMDA can actually inhibit group I mGluR responses (Alagarsamy et al., 1999; Challiss et al., 1994; Fitzjohn et al., 1996; Romano et al., 1995). In this report, we attempt to identify a potential mechanism for the inhibitory effect of larger doses of NMDA on mGluR5 function. We show that mGluR5 and NMDA are colocalized in the rat cortex and that mGluR5 function in this area is inhibited by concentrations of NMDA 30 μM and above. Furthermore, we provide evidence that this inhibition is due to phosphorylation of mGluR5 at PKC phosphorylation sites.

2. Materials and methods

2.1. Oocyte electrophysiology

Xenopus oocytes were injected and recorded from as previously described (Alagarsamy et al., 1999; Gereau and Heinemann, 1998). Briefly, oocytes were injected with mRNA encoding mGluR5a alone or with the NMDA receptor subunits 1A and 2B in a ratio of 1:3, respectively. Control oocytes were injected with an appropriate volume of water. Dual electrode voltage-clamp recordings were performed in Barth's medium containing 88 mM NaCl, 1 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, .33 mM $CaNO_3$ and 10 mM HEPES.

2.2. Phosphoinositide hydrolysis in cortical slices

Phosphoinositide hydrolysis was measured as previously described (Chung et al., 1997; Desai et al., 1992; Winder and Conn, 1995). Briefly, slices made from the rat cortex were labeled with [^{3}H]inositol and accumulation of radioactive inositol phosphates was measured during a 15-min incubation with agonists in the presence of 10 mM lithium chloride. Samples were extracted with chloroform/methanol and the [^{3}H]inositol monophosphate fraction was separated by anion exchange chromatography (Dowex 1×4400) using increasing amounts of ammonium formate. [^{3}H]IP content was assessed by liquid scintillation spectrometry. Data are represented as fold increase over same-day basal values and statistical significance was determined by Student's *t* test or ANOVA followed by Dunnett's test from appropriate controls and indicated by an asterisk.

2.3. Back phosphorylation

Male Sprague–Dawley rats were decapitated and the brains were removed and placed in ice-cold ACSF containing 124 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1 mM NaH_2PO_4, 2.0 mM $CaCl_2$, 26 mM $NaHCO_3$ and 10 mM glucose: equilibrated with 95% O_2/5% CO_2. The hippocampi were dissected and thick transverse slices (350 μm) were prepared and maintained in ACSF. After 1 h, equilibration at room temperature slices were treated with drugs and homogenized in buffer containing 50 mM Tris–HCl pH 7.4, 1% Triton X-100, 0.1% SDS, 10 mM EDTA, 1 mM PMSF, 1 mM sodium orthovanadate, luepeptin and aprotinin (each 10 pg/ml). The samples were centrifuged at 14,000 rpm at 4 °C for 10 min. The supernatants containing the solubilized membranes were transferred to fresh tubes containing anti-mGluR5 (4 μg/ml, Upstate) and rocked overnight at 4 °C. Protein A-sepharose beads were added to the samples and mixed for 3 h at 4 °C. The beads were washed three times in wash buffer containing 1% Triton X-100 and once in Tris–HCl (50 mM, pH 7.4). Basic phosphorylation buffer containing 50 mM HEPES–NaOH, pH 7.4, 10 mM $MgCl_2$ and 1 mM EGTA was then added to the samples and incubated at room temperature for 30 min. Samples were then boiled for 2 min and spun for 2 min at 4 °C. The supernatant containing the immunoprecipitated mGluR5 was divided into two aliquots. One of which was phosphorylated by PKC by incubation with PKC (0.25 μg), phosphatidylserine (2.5 mg) and $CaCl_2$ (1.5 mM) and 0.2 μM [γ-^{32}P]ATP in a total volume of 50 μl for 1 h at 37 °C. Sample buffer was added to the tubes and the mixture was separated by SDS-PAGE. Gels were incubated at room temperature for 30 min in 1 M salicylic acid and were dried for 30 min at 80 °C. The dried gels were then exposed to a phosphoscreen and appropriate bands were quantitated on a phosphoimager (Molecular Dynamics).

The remaining aliquot from the immunoprecipitation was subject to SDS-PAGE (7.5% gel). The gel was transferred to the immobilon membrane and was incubated with anti-mGluR5 at 4 °C overnight. The membrane was then washed two times with Tris-buffered saline (TBS, pH 7.2) and was incubated for 1.5 h in goat anti-rabbit (1:10,000) IgG–HRP conjugate. Membranes were then washed with TBS and exposed to ECL reagents and to X-ray film. Bands were quantitated by densitometry (Lynx Software). The data are presented as ^{32}P incorporation obtained from the phosphoimager in each band normalized to the amount of protein determined from the densitometry and expressed as percent of same-day no-drug control. Statistical significance was determined by ANOVA followed by Dunnett's test from untreated control and is indicated with an asterisk.

2.4. Microscopy

Two Sprague–Dawley rats, 250–300 g, were deeply anesthetized with 4% chloral hydrate, and perfused transcardially with a phosphate-buffered solution of 3% paraformaldehyde and 0.15% or 0.2% glutaraldehyde for 12–13 min (260–290 ml). The rats were then refrigerated for 1–2 h, after which their brains were removed and 40–50 μm sections of the cortex were obtained on a

vibratome (Oxford). Sections were incubated in sodium borohydride (1% in phosphate buffered saline), and taken through a freeze/thaw protocol involving graded series of cryoprotectants. Sections were subsequently preblocked with 5% normal goat serum (NGS)/5% normal horse serum (NHS) in a phosphate-buffered saline/bovine serum albumin solution (PBS/BSA; 0.5% Tween 20, 5% BSA, 1% gelatin). Sections were then incubated in primary NMDA monoclonal antibody (1:250 from concentrated cell culture medium) (Pharmingen) and mGluR5 polyclonal affinity purified antibody (1 μg/ml) (Upstate) in PBS–BSA containing 1% NGS/1% NHS for at least 48 h. Control sections were treated identically, except they received only one of the two primary antibodies or no primary antibody. After several rinses in PBS, the sections were incubated in the following secondary antibodies for 24 h: biotinylated horse anti-mouse (for monoclonal NMDA) and gold conjugated goat anti-rabbit (for polyclonal mGluR5) in PBS. The NMDA monoclonal immunoreactivity was amplified with avidin–biotin complex (ABC; Vector Elite) and developed in diaminobenzidine (DAB, Sigma) containing 0.5% hydrogen peroxide, rinsed in TBS followed by a phosphate buffer rinse and then postfixed overnight in 1% glutaraldehyde in phosphate buffer. Sections were then rinsed thoroughly in 0.1 M phosphate buffer (pH 7.6), processed with a silver enhancement protocol (HQ; Nanoprobes) and postfixed in 1% osmium tetroxide in phosphate buffer for 10 min. Sections were then dehydrated through a graded ethanol series, followed by propylene oxide, infiltrated and embedded in Durcupan between glass slides coated with a releasing agent. The neocortex was dissected and placed on precured stubs for thin sectioning. Silver and silver–gold sections were obtained using a diamond knife on a Reichert Ultracut S (Leica) ultramicrotome. Sections were retrieved on grids,and were then viewed and photographed on an electron microscope. Other sections were incubated in the following secondary antibodies: biotinylated goat anti-rabbit (for polyclonal mGluR5) and gold conjugated goat anti-mouse (for monoclonal NMDA). In this case, mGluR5 was developed with DAB and NMDA was silver-intensified and the sections were processed as described above. Profiles were considered immunoreactive if they contained the diffuse electron-dense DAB reaction product, and/or 3+ gold particles within the membrane boundaries of that profile.

Sections photographed at the light microscopic level were processed as described above, but were mounted on glass slides coated with gelatin immediately after the silver enhancement step (they were not osmicated, etc).

3. Results

Fig. 1 shows representative traces obtained from voltage-clamp recordings of *Xenopus* oocytes injected with mRNA encoding mGluR5a and the NMDA receptor subunits 1A and 2B. Fig. 1A shows the characteristic response to two sequential applications of the mGluR agonist, 1*S*,3*R*-ACPD. The response to the second application of agonist is significantly reduced or desensitized. As we have previously

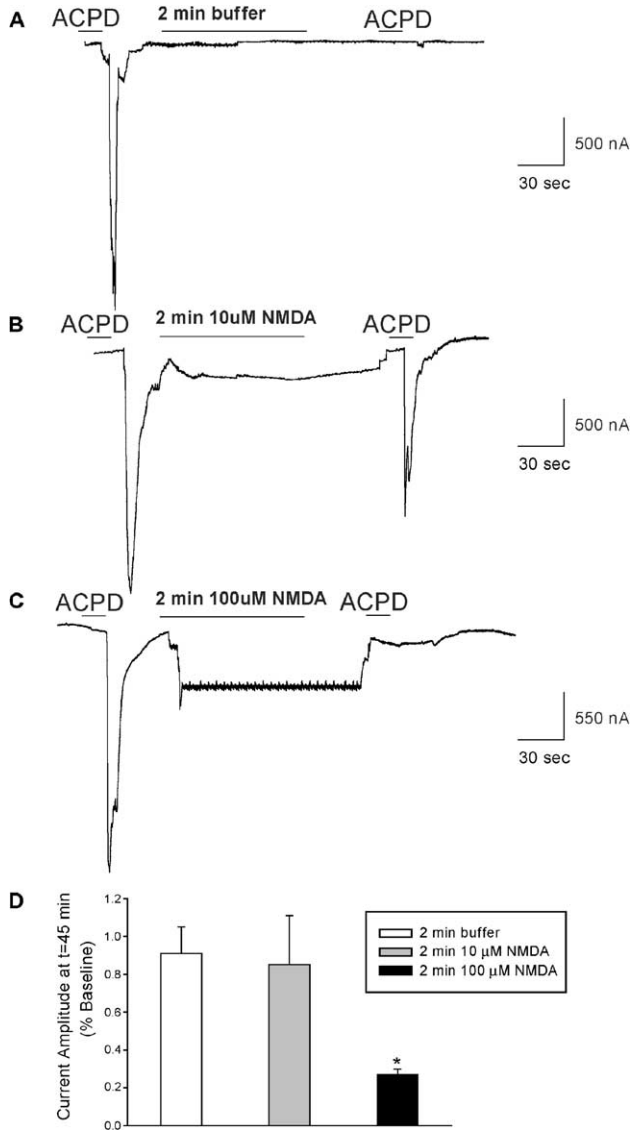


Fig. 1. There is a differential effect of NMDA on mGluR5 currents in *Xenopus* oocytes expressing mGluR5 and NMDA subunits 1A and 2B based on concentration. (A–C) Representative traces of two sequential applications of the mGluR agonist, 1*S*,3*R*-ACPD with a 2-min interval between agonist applications. (A) There is a significant desensitization of response to agonist when buffer is perfused during the interval between agonist applications. (B) Perfusing with 10 μM NMDA produces relatively small currents (50–100 nA) and results in recovery from agonist-induced desensitization. (C) Perfusing with 100 μM NMDA produces larger currents (>400 nA) and fails to induce recovery from desensitization. (D) 100 μM NMDA causes inhibition of mGluR5 currents that have been allowed to recover from desensitization for 45 min after the initial agonist application. Bars represent mean ± S.E.M. of five values for each condition 45 min following initial agonist application normalized to current response at $t = 0$. * Indicates significant difference from buffer control as determined by ANOVA followed by Dunnett's post hoc test ($P < .01$).

described, applying 10 μM NMDA for 2 min prior to the second application of agonist results in a dramatic reversal of desensitization (Fig. 1B). In this system, 10 μM NMDA generally produces currents of 50–100 nA. However, increasing NMDA concentrations (30–100 μM) to produce larger currents (>400 nA) between agonist applications results in a small second mGluR5 response (Fig. 1C).

It is possible that the biphasic effect of NMDA on mGluR5-mediated responses is due to the inhibition of mGluR5 function at higher concentrations. In order to address whether larger doses of NMDA actually inhibit mGluR5 responses or if larger amounts of NMDA are simply unable to reverse desensitization, we allowed for longer intervals between agonist applications. We found that 45 min between agonist applications without NMDA application was sufficient to allow for the natural recovery of agonist-induced desensitization (Fig. 1D). We have previously reported that 10 μM NMDA applied 2 min prior to the second application of 1*S*,3*R*-ACPD at 45 min does not affect the second mGluR5 response. However, if the oocytes are perfused with 100 μM NMDA 2 min prior to the second agonist application at 45 min, there is a significant reduction in current amplitude (Fig. 1D). These data suggest that while 10 μM NMDA is able to reverse mGluR5 desensitization, larger doses of NMDA can inhibit mGluR5 function.

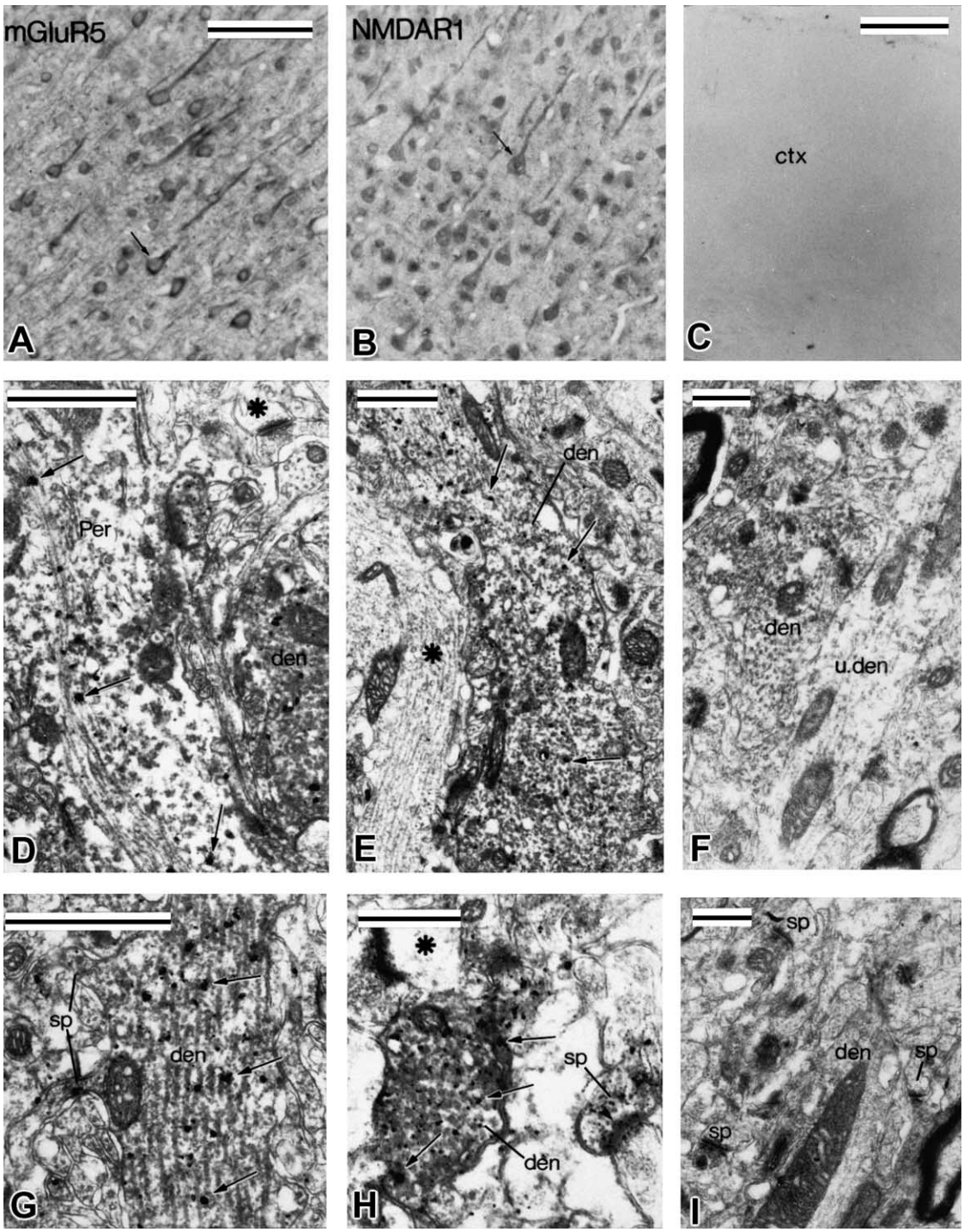
In order to determine whether NMDA receptors and mGluR5 can exhibit similar interactions in the rat brain, we first sought to confirm the subcellular localization of the two receptors. Thus, we performed immunocytochemistry studies at the light and electron microscopic levels using antibodies specific for mGluR5 and NR1 to determine whether these receptors are colocalized at a synaptic level. Consistent with previous studies, analysis at the light microscopic level revealed a very similar pattern of immunoreactivity for mGluR5 and NR1 in the neocortex (Fig. 2A and B). Analysis of at least 10 sections from each of the two animals demonstrated immunoreactivity for both proteins in the cell bodies and neuropil of all cortical areas. Pyramidal cell bodies as well as their apical dendrites were filled with immunoreactivity for both proteins, and it appeared that mGluR5 and NR1 may be expressed in the same population of cortical cells. Analysis of multiple thin sections from at least three areas of the cortex, per animal, at the electron microscopic level, showed immunoreactivity for both the mGluR5 (gold particles) and NR1 (DAB reaction product) in cortical pyramidal cell bodies, as well as specific postsynaptic sites at asymmetric synapses onto proximal and distal dendrites as well as spines (Fig. 2D, E, G, H). Furthermore, the majority of pyramidal cells examined were immunoreactive for both the mGluR5 and NMDA receptor proteins and postsynaptic elements immunoreactive for one receptor were typically immunoreactive for both glutamate receptor subtypes (Fig. 2D, E, G, H). Reliable quantification of the extent of colocalization of these two proteins was precluded by the poor penetration of the gold secondary antibodies. However, these data clearly suggest that mGluR5 and NR1 are colocalized in the same postsynaptic elements. Control experiments in which one of the primary antibodies (NR1 or mGluR5) was omitted verified that no cross-reactivity was seen. For example, no DAB reaction product was seen when the NR1a antibody was omitted and the distribution of gold particles labeling mGluR5 was identical to that seen in the double-labeled condition (control panels, Fig. 2C, F, I). These anatomical experiments indicate that pyramidal cells express both the mGluR5 receptor and the NMDA receptors and transport them to dendrites where they are available to interact and reciprocally modulate receptor function.

Since the NMDA receptor and mGluR5 are colocalized in cortical neurons, it is possible that a direct intracellular mechanism is responsible for the inhibitory effect of NMDA on mGluR5 function. We used phosphoinositide hydrolysis as a measure of mGluR5 function in cortical slices. As in the oocyte system, 10 μM NMDA increased responses to the group I mGluR agonist, DHPG. However, concentrations of 30 μM and above were inhibitory (Fig. 3A). Since, NMDA receptor activation can induce cell toxicity, we tested the effects of 100 μM NMDA on non-mGluR receptor function. Carbamyl chloride (1 mM, carbachol) is known to significantly stimulate phosphoinositide hydrolysis via the activation of muscarinic receptors, and muscarinic receptors have been shown to be colocalized with NMDA receptors (Mar-

Fig. 2. NMDA receptors and mGluR5 are colocalized in cortical neurons. (A–B) Light microscopic localization of the mGluR5 receptor (A) and the NMDA receptor (B) in cortical pyramidal cells (small arrows). Note that both receptors are expressed in numerous pyramidal cells indicating that they may be colocalized. (D, E, G, H) Double-labeling electron microscopic immunocytochemistry demonstrating that the mGluR5 and NMDA receptors are colocalized in cortical cell bodies, dendrites, and dendritic spines. In all panels, the mGluR5 receptor is labeled with silver-enhanced immunogold particles (small arrows in D), and the NR1 subunit of the NMDA receptor is labeled with DAB, a dark diffuse reaction product that fills the profiles. (D) A portion of a cell perikarya (per) immunoreactive for both mGluR5 and NMDA receptors is shown. Note that the gold particles labeling the mGluR5 receptor are lining the endoplasmic reticulum (small arrows), the site of protein processing. Also pictured in this panel are a double-labeled dendrite (den) and a double-labeled spine (sp). Note that the neighboring dendrite (*) is not immunoreactive for either receptor. (E) A double-labeled, large-caliber dendrite (den) and an unlabeled nearby dendrite (*). (G) A double-labeled dendrite (den) that has two spines (sp) emerging that also contain immunoreactivity for both receptors. (H) A small caliber dendrite (den) that contains immunoreactivity for both receptors. Also pictured in this panel is a double-labeled spine (sp) that is making an asymmetric synapse (arrowhead). These data indicate that these two receptors are coexpressed in cortical neurons, transported to the dendritic region of these cells, and are found in synaptically functional compartments (dendritic spines) where they would potentially interact during synaptic transmission. (C) Control section at the light level in which the primary antibody was omitted. (F) Control electron micrograph taken from sections treated with NR1 antibody only. There is only a DAB reaction product in a dendrite (den) and nonspecific gold labeling (arrowhead). A nearby, unlabeled dendrite (u. den) is also shown. (I) Electron micrograph taken from the section in which both primary antibodies were omitted. There is no labeling in spines (sp) or dendrites (den).

ino et al., 1998). NMDA (100 μM) did not significantly inhibit carbachol responses (6.85 ± 2.37, carbachol alone; 5.42 ± 0.9, carbachol + NMDA; fold increase of control), suggesting that the inhibitory effect of NMDA was not due to cytotoxicity. Furthermore, these data suggest that the inhibitory effect of NMDA was specific to mGluRs.

We have previously shown that lower doses of NMDA attenuate agonist-induced phosphorylation of mGluR5 and that dephosphorylation could be a likely mechanism for the potentiating effects of 10 μM NMDA. Therefore, larger amounts of NMDA could induce phosphorylation and subsequent reduction of mGluR5 function. Since PKC activation is known to inhibit mGluR5 function, we used a back phosphorylation assay to test the possibility that 100 μM NMDA causes phosphorylation of mGluR5 at PKC sites. In these experiments, hippocampal slices are treated with agonists and homogenized. mGluR5 is then immunoprecipitated and phosphorylated in vitro by PKC in the presence



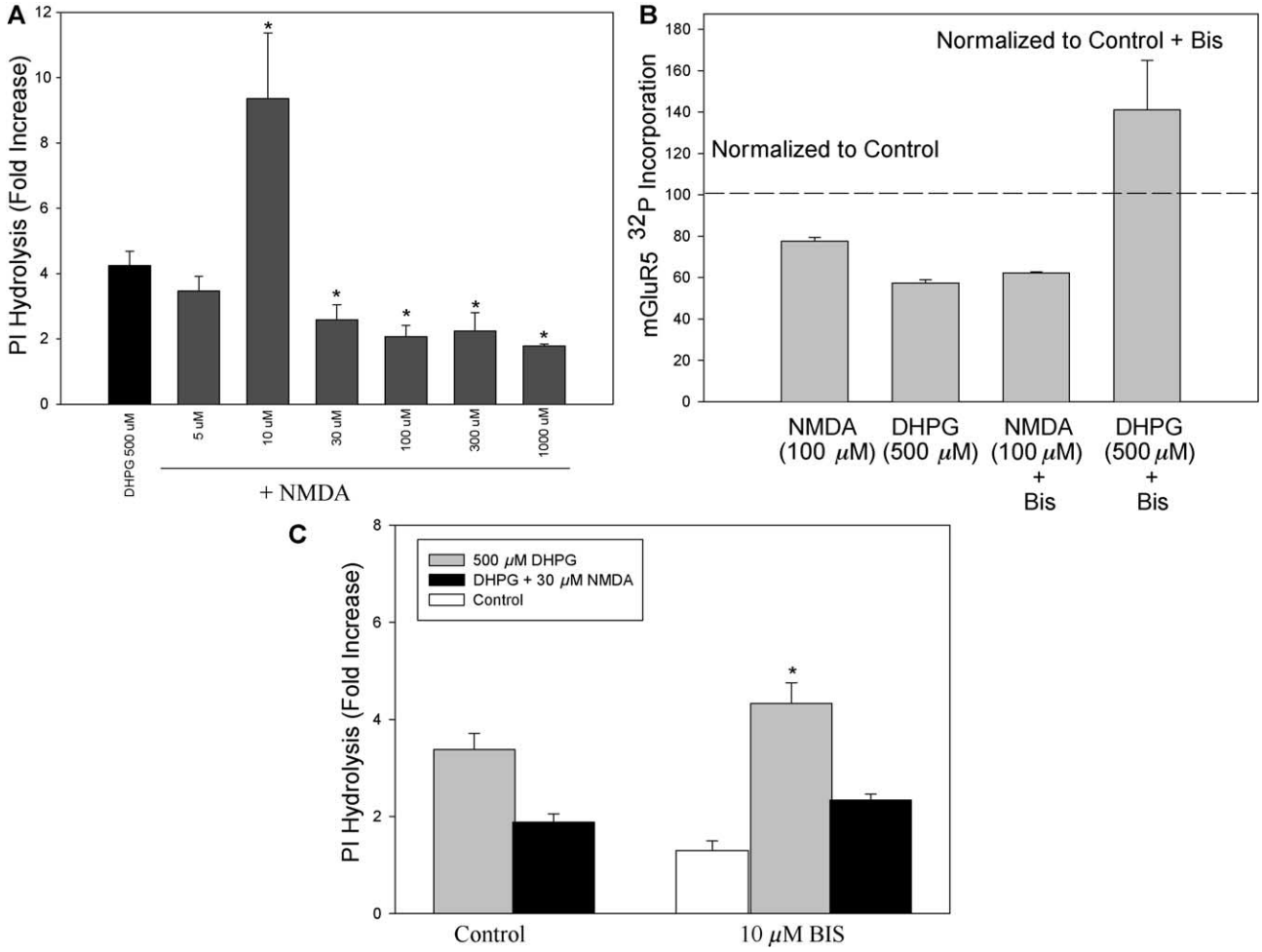


Fig. 3. (A) The group I mGluR agonist, DHPG, produces an increase in phosphoinositide hydrolysis in the cortex. This response is potentiated by 10 μM NMDA and inhibited by concentrations above 30 μM. Bars represent data expressed as fold increase over same-day control and are means ± S.E.M. of three to seven independent experiments done in triplicate. * Indicates significant difference from DHPG control as determined by ANOVA followed by Dunnett's test ($P < .05$). (B) Both NMDA and DHPG induce phosphorylation of mGluR5 in a back phosphorylation assay. The PKC inhibitor, BIS, was able to attenuate the phosphorylation induced by DHPG, but not NMDA. Bars represent means ± S.E.M. of three independent experiments. Data were normalized to protein values estimated from corresponding Western blots and to same-day control conditions. All values were significantly different from untreated control. (C) The PKC inhibitor, BIS, was unable to reverse the inhibitory effect of 30 μM NMDA in phosphoinositide hydrolysis experiments. Bars represent data expressed as fold increase over same-day control and are means ± S.E.M. of four independent experiments done in triplicate. * Indicates significant difference from conditions without BIS as determined by Student's t test ($P < .05$).

of radiolabeled ATP. Therefore, previously unphosphorylated sites on mGluR5 that are subject to PKC phosphorylation are phosphorylated in vitro with ^{32}P-ATP. A large amount of radioactive incorporation suggests little phosphorylation in the intact cells and a reduction in the amount of radioactivity suggests increased phosphorylation. Using this assay, we found that 100 μM NMDA causes phosphorylation of mGluR5 to a similar extent as DHPG, the group I mGluR agonist (Fig. 3B). Interestingly, the PKC inhibitor, bisindolyl maleamide I (BIS), was not able to reverse NMDA-induced phosphorylation of mGluR5 but was able to reverse phosphorylation induced by DHPG (Fig. 3B). These data suggest that 100 μM NMDA causes phosphorylation of mGluR5 at PKC sites, however, this phosphorylation may be independent of PKC activity.

To further test the involvement of PKC in NMDA-induced inhibition of mGluR5 function, we pharmacologically characterized the phosphoinositide hydrolysis response. We found that 10 μM BIS was not able to reverse the inhibitory effect of NMDA (Fig. 3C). Similarly, another inhibitor of PKC, staurosporine (1 μM), was not able to restore mGluR5 responses in the presence of 30 or 100 μM NMDA (data not shown). Inhibitors of other kinases also did not attenuate the inhibitory effect of NMDA. We used inhibitors of PKA (H-89, 30 μM), CAM-KII (Autocetamide, 1 μM), and tyrosine kinase (PD 098059, 100 μM). None of these compounds were effective in blocking the inhibitory effect of NMDA (data not shown). We also confirmed that phosphatase activity was not required for NMDA-induced inhibition of mGluR5 function. The phos-

phatase inhibitors, sodium orthovanadate (1 mM) or cyclosporin A (250 μM), failed to reverse the inhibitory effect of either 30 or 100 μM NMDA (data not shown). These data are consistent with the lack of effect of BIS observed in the back phosphorylation experiments. Therefore, these data would suggest that a kinase other than PKC, PKA, MEK, CAMK-II, or tyrosine kinase is involved in the inhibitory effect of NMDA or that the inhibitory effect of NMDA is not mediated by phosphorylation.

4. Discussion

We have previously shown that 10 μM NMDA is able to potentiate mGluR5 function by reversing desensitization. However, higher concentrations of NMDA have been known to inhibit mGluR function (Alagarsamy et al., 1999; Challiss et al., 1994). While we have identified a mechanism for the potentiating effect of NMDA, the mechanism for the inhibitory effect is not known. PKC is known to inhibit mGluR5 function, likely by phosphorylation at specific serine/threonine residues (Gereau and Heinemann, 1998). Thus, we tested the hypothesis that the inhibitory effect of NMDA was due to PKC-dependent phosphorylation. Interestingly, we found that 100 μM NMDA induced phosphorylation of mGluR5 at sites that are phosphorylated by PKC in a back phosphorylation assay. However, the inhibitory effect did not seem to be dependent on PKC activity. Interestingly, the PKC inhibitor failed to reverse phosphorylation induced by NMDA, but was effective in reducing phosphorylation induced by the mGluR agonist, DHPG. These data suggest that NMDA causes phosphorylation of mGluR5 independent of PKC activity, but the phosphorylation itself may be on a PKC site. However, it is also possible that NMDA-induced phosphorylation is not actually on a PKC site. Since, the PKC reaction was conducted in vitro, the reaction may have been nonspecific.

The data suggesting phosphorylation of mGluR5 at PKC sites may seem inconsistent with the lack of effect of kinase inhibitors on the functional response. However, it is possible that NMDA-induced phosphorylation of mGluR5 is mediated by an unknown kinase that is not inhibited by the protein kinase inhibitors used in this study. Also, it is possible that phosphorylation may not mediate the inhibitory effects of NMDA. Another mechanism may be responsible for the inhibition and the phosphorylation is just coincidental. In fact, there has been evidence for phosphorylation-mediated attenuation of the IP3 pathway of mGluR1 activation while leaving the cAMP pathway intact (Francesconi and Duvoisin, 2000). Similar differential effects of phosphorylation may occur with mGluR5.

Although these data do not completely elucidate the mechanism of NMDA-induced inhibition of mGluR5 function, it is clear that higher levels of NMDA receptor activation lead to an increase in mGluR5 phosphorylation and causes a reduction in mGluR5 function. This may provide a critical brake on NMDA-induced potentiation of mGluR5 at lower levels of NMDA receptor activation. Since mGluR5 also potentiates NMDA-mediated responses (Awad et al., 2000; Mannaioni et al., 2001), this could provide a mechanism for preventing excessive reciprocal potentiation between these two receptor subtypes that could lead to excitotoxicity.

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