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Research report

Metabotropic glutamate receptor modulation of excitotoxicity in the neostriatum: role of calcium channels

Christopher S. Colwell *, Michael S. Levine

Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, University of California-Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024-1759, USA

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Abstract

We have previously shown that metabotropic glutamate receptor (mGluR) activation can attenuate *N*-methyl-D-aspartate (NMDA)-induced excitotoxic injury in the neostriatum both in vivo and in vitro. Our earlier studies made use of the non-subtype selective mGluR agonist 1-amino-cyclopentane-1,3-dicarboxylic acid (tACPD). In the present study, we extended these observations by identifying the subtype of mGluR involved. Using selective mGluR agonists, we provide evidence that the Group II mGluRs are responsible for inhibition of NMDA excitotoxicity in the neostriatum. In addition, we provide evidence that the inhibitory effects of tACPD on excitotoxicity are dependent upon calcium influx as they are blocked by a low calcium solution as well as the broad-spectrum calcium channel blocker cadmium. The tACPD-induced attenuation was also blocked by ω-conotoxin GVIA suggesting participation of N-type calcium channels. Whole cell voltage clamp recordings were made to directly determine the effects of mGluRs on voltage-gated calcium channels in neostriatal neurons. As predicted, both tACPD and the Group II agonist 3C4HPG inhibited calcium currents in neostriatal neurons. Again this effect was blocked by ω-conotoxin GVIA. Overall the results suggest that mGluR regulation of voltage-gated calcium channels can limit NMDA toxicity in the neostriatum.

Keywords: Calcium channel; Excitotoxity; Metabotropic glutamate receptor; Neostriatum; *N*-Methyl-D-aspartate; Toxicity

1. Introduction

Glutamate, the primary excitatory neurotransmitter in the vertebrate central nervous system, acts on two general types of receptors: ion channel-linked ionotropic receptors (iGluRs) and G protein-coupled metabotropic receptors (mGluRs). Although these two classes of GluRs can exert membrane effects independently, they can also interact and we have been exploring these interactions in neurons in the neostriatum. Activation of mGluRs can affect fast glutamatergic synaptic transmission directly by modulating GluR-gated ion channels and indirectly by modulating voltage-gated ion channels including those permeable to calcium (Ca^{2+}) and potassium (K^{+}). Thus, the post-synaptic cell's response to the pre-synaptic release of glutamate is determined by the integration of responses from multiple glutamate receptors.

Neostriatal cells are innervated by a major glutamate-containing projection from the cortex and thalamus [32]. These projections form the main excitatory drive into the basal ganglia. Both iGluRs and mGluRs are present in the neostriatum [1,43]. The mGluRs are generally divided into three groups based on amino acid sequence homology, pharmacology and effector coupling: Groups I (mGluR1 and 5), II (mGluR 2, 3) and III (mGluR4, 6, 7, and 8). All three subtypes of mGluRs are expressed in the neostriatum with mGluRs 3 and 5 being the most abundant [28–30,33,38,43]. Previous studies have shown that activation of mGluRs in the neostriatum inhibits excitatory synaptic input [23] as well as NMDA-mediated responses [14]. Collectively, these studies provide electrophysiological evidence that mGluR activation can modulate iGluR-evoked responses and suggest that activation of mGluRs may protect neostriatal cells against excitotoxicity.

The aim of the present study was to examine the mechanisms by which mGluRs modulate NMDA receptor-evoked responses in neostriatal neurons. In order to investigate this issue, infrared differential interference contrast (IR DIC) videomicroscopy [20] was utilized to assess the excitotoxic response of neostriatal neurons to GluR stimulation. This technique takes advantage of the

* Corresponding author. Fax: +1-310-206-5060; E-mail: ccolwell@mednet.ucla.edu

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finding that GluR activation induces cell swelling, an early event in an excitotoxic cascade that can produce cell death [10]. We have previously shown that NMDA-induced swelling is associated with cell death measured by the dye trypan blue and have used this technique to follow the dynamics of GluR-evoked responses in single cells in neostriatal brain slices [11,12,16]. The present study makes use of the cell swelling assay to characterize the effects of selective mGluR agonists on NMDA-induced swelling of cells in the neostriatal brain slice and to determine the possible role of Ca^{2+} channels in mediating this modulation. In addition, whole cell patch clamp recordings were made to directly determine the effects of mGluRs on voltage-sensitive Ca^{2+} currents (VSCC) in neostriatal neurons.

2. Materials and methods

2.1. Brain slice preparation for cell swelling assay and electrophysiology

Brain slices were prepared using standard techniques. Briefly, Sprague–Dawley rats (12–14 days of postnatal age) were killed by decapitation, brains dissected and placed in cold oxygenated ACSF containing (in mM) NaCl 130, $NaHCO_3$ 26, KCl 3, $MgCl_2$ 5, NaH_2PO_4 1.25, $CaCl_2$ 1.0, glucose 10 (pH 7.2–7.4). After cutting slices, transverse sections (350 μm) were placed in ACSF (25–27°C) for at least 1 h (in this solution $CaCl_2$ was increased to 2 mM, $MgCl_2$ was decreased to 2 mM and 4 mM lactate was added). Slices were constantly oxygenated with 95%O_2–5% CO_2 (pH 7.2–7.4, osmolality 290-300 mosm). Slices were placed in a custom designed chamber attached to the stage of the fixed-stage upright microscope. The slice was held down with thin nylon threads glued to a platinum wire and submerged in continuously flowing, oxygenated ACSF (25°C) at 2 ml/min. Solution exchanges within the slice were achieved by a rapid gravity feed delivery system. In our system, the effects of bath applied drugs begin within 15 s and are typically complete by 1–2 min.

2.2. IR DIC videomicroscopy

Slices were viewed with an upright compound microscope (Zeiss Axioskop, Milpitas, CA) using a water immersion lens (40×) and dic optics. They were illuminated with near IR light by placing an IR bandpass filter (750–1050 nm) in the light path. The image was detected with an IR-sensitive CCD camera (Hamamatsu C2400, Bridgewater, NJ) and displayed on a video monitor. A camera controller allowed analog contrast enhancement and gain control. Digital images were stored on computer/optical disk for subsequent analysis and additional digital contrast adjustment when necessary. Cells were typically visualized from 30–100 μm below the surface of the slice. In order to quantify changes in response to activation of glurs, image analysis software (Optimas, Edmonds, WA) was used to measure cross-sectional somatic area (the perimeter, maximal length and width were also measured in some cells) prior to, during and after experimental treatments. Each measurement was made twice and the average value recorded. Measurements were only taken from cells that exhibited clear borders, convex shapes, and phase brightness.

2.3. Whole cell patch clamp electrophysiology

Methods were similar to those described previously [9]. Briefly, electrodes were pulled on a multistage puller (Sutter P-97, Novato, CA). Electrode resistance in the bath was typically 2–4 MΩ. The standard solution in the patch pipette contained (in mM): Cs-methanesulfonate, 125; Phosphocreatine, 10; EGTA, 9; Hepes, 8; MgATP, 5; NaCl, 4; KCl, 3; GTP, 1; $MgCl_2$, 1; leupeptin 0.1. The pH was between 7.25 and 7.3 and the osmolality was between 280 and 290 mosm. With this internal solution, stable Ca^{2+} currents were recorded for up to 30 min. Whole cell recordings were obtained with an Axon Instruments 1D amplifier and monitored on-line with pCLAMP (Axon Instruments, Foster City, CA). To minimize changes in offset potentials with changing ionic conditions, the ground path used an ACSF agar bridge. Cells were approached with slight positive pressure (2–3 cm H_2O) and offset potentials were corrected. The pipette was lowered to the vicinity of the membrane keeping a positive pressure. After forming a high-resistance seal (2–10 GΩ) by applying negative pressure, a second pulse of negative pressure was used to break the membrane. While entering the whole-cell mode, a repetitive test pulse of 10 mV was delivered in a passive potential range (≈ -60 to -70 mV). Whole-cell capacitance and electrode resistance were neutralized and compensated (50–80%) using the test pulse. Data acquisition was then initiated. Series and input resistance was monitored throughout the experiment by checking the response to small pulses in a passive potential range.

A common concern in voltage clamp electrophysiological experiments is the adequacy of the space clamp in cells in slices, i.e., the ability to maintain voltage control of the membrane at sites distant to the recording electrode. In this study, our ability to maintain voltage control was improved by the blockade of most voltage-gated currents and the use of cells from younger tissue which do not have large dendritic trees. Nevertheless, it is likely that we were unable to fully clamp more distal processes. For all cells, smooth transitions in the current–voltage relationships were used as indicators of good voltage control. In addition, any cell which showed a high series resistance (> 20 MΩ) or tail currents which did not decay rapidly with a single exponential were not used.

2.4. Statistical analyses

Differences between experimental and control groups were evaluated using *t*-tests or Mann–Whitney rank sum tests when appropriate. Values were considered significantly different if $p < 0.05$. All tests were performed using SigmaStat (Jandel, San Rafael, CA). In the text, values are shown as mean ± S.E.M.

3. Results

3.1. mGluR modulation of NMDA-induced swelling

A total of 546 neostriatal cells were visualized using IR DIC videomicroscopy in brain slices prepared from 12–14 day old Sprague–Dawley rats. All groups contained data from at least four animals. Large interneurons were easily identified and were not included in the data set. The effects of experimental and control manipulations were determined by measuring cell area before and after treatments that were 10 min in duration. The results are presented in the text and figures as percent change in area. With this assay, measurements of cell area under control conditions were stable through time. In addition, the NMDA receptor antagonist AP5, but not the AMPA/KA antagonist CNQX, blocked NMDA-induced swelling. Finally, neostriatal neurons did not appear to recover from the NMDA-induced swelling [12,16].

Bath application of NMDA (100 μM, 10 min) caused swelling of neostriatal cells (26 ± 1% swelling, $n = 150$; Fig. 1). This swelling was inhibited by the application of the broad-spectrum mGluR agonist tACPD (100 μM; Fig. 1). While this concentration of tACPD is rather high and does not distinguish between mGluR subtypes, we have previously shown that this concentration of tACPD alters both electrophysiological [14] and excitotoxic [11] responses in neostriatal neurons. Furthermore, the inhibitory effect of tACPD is stereoselective [11] and is blocked by the mGluR antagonist MCPG (1 mM; Fig. 1). These results suggest that the effects of tACPD are mediated by a mGluR but do not help identify which one. Thus, in order to define the subtype of mGluR involved, three additional mGluR agonists were also tested (Fig. 1). The Group II mGluR agonist (*S*)-3-carboxy-4-hydroxyphenylglycine (3C4HPG) significantly inhibited NMDA-induced swelling (100 μM 3C4HPG + NMDA: 13 ± 2% swelling, $n = 30$, $p < 0.01$). However, treatment with the Group I mGluR agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) or the Group III agonist L-2-amino-4-phosphonopentanoic acid (L-AP4) had no effect on NMDA-induced swelling (100 μM DHPG + NMDA: 29 ± 4% swelling, $n = 23$; 100 μM L-AP4 + NMDA: 24 ± 3% swelling, $n = 33$). When applied alone, none of these agonists had any effect (tACPD 2 ± 1% swelling, $n = 46$; 3C4HPG 2 ± 2% swelling, $n = 11$; DHPG −2 ± 2% swelling, $n = 12$; L-AP4 1 ± 2% swelling, $n = 37$). Thus, the Group II subtype of mGluR appears to mediate the inhibitory mGluR effect on cell swelling in the neostriatum.

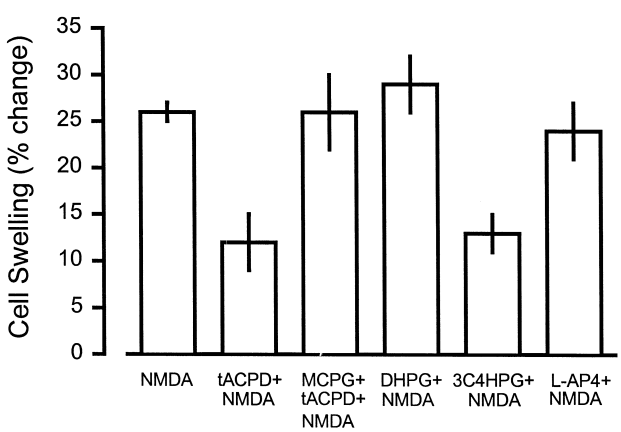

Fig. 1. The mGluR agonists tACPD and 3C4HPG inhibited NMDA-induced swelling. Swelling induced by NMDA (100 μM, 10 min) alone was compared to swelling induced by mGluR agonists plus NMDA. The mGluR agonists tACPD and 3C4HPG significantly ($p < 0.01$) inhibited swelling caused by NMDA while DHPG and L-AP4 did not. The mGluR antagonist MCPG blocked tACPD's inhibitory effect. In this figure, the cross-sectional area of cells was determined before and after 10 min treatments and is expressed as percent change. Error bars indicate standard error. Data were collected from tissue from 12–14 day old rats.

Table 1
In order to test the role of HVA currents Ca^{2+} currents in the mGluR modulation of NMDA responses, we examined the effects of a low Ca^{2+} (1 mM Ca^{2+}/10 mM EGTA) solution as well as Ca^{2+} channel blockers cadmium (Cd^{2+}, 100 μM) and ω-conotoxin GVIA (ω-CnTx, 5 μM). Each of these treatments (low Ca^{2+}, Cd^{2+}, ω-CnTx) produced a significant ($p < 0.05$) inhibition of NMDA-induced swelling alone as well as preventing further inhibition of swelling by mGluR agonists

Treatment	Swelling (%)	*N*
NMDA	26 ± 1	150
NMDA + low Ca^{2+}	17 ± 2	87
NMDA + low Ca^{2+} + tACPD	18 ± 2	29
NMDA + low Cd^{2+}	18 ± 2	22
NMDA + low Cd^{2+} + tACPD	19 ± 2	23
NMDA + low Cd^{2+} + 3C4HPG	19 ± 2	30
NMDA + ω-CnTx	18 ± 2	22
NMDA + ω-CnTx + tACPD	17 ± 3	30
NMDA + ω-CnTx + 3C4HPG	18 ± 4	20

3.2. Role of Ca^{2+} channels in mediating the mGluR modulation

One of the better known consequences of mGluR activation is the inhibition of Ca^{2+} currents. Specifically, activation of mGluRs by tACPD inhibits high voltage activating (HVA) currents in neostriatal cells [41]. In order to test the role of these currents in the mGluR modulation of NMDA responses, we examined the effects of a low Ca^{2+} (1 mM Ca^{2+}/10 mM EGTA) solution as well as Ca^{2+} channel blockers cadmium (Cd^{2+}, 100 μM) and

ω-conotoxin GVIA (ω-CnTx, 5 μM). The results are shown in Table 1. Each of these treatments (low Ca^{2+}, Cd^{2+}, ω-CnTx) produced a significant ($p < 0.05$) inhibition of NMDA-induced swelling alone as well as preventing further inhibition of swelling by mGluR agonists. These finding suggest that a mGluR-mediated inhibition of Ca^{2+} currents is responsible for the modulation of the NMDA-evoked swelling in the neostriatum.

3.3. mGluR inhibition of Barium (Ba^{2+}) currents in the neostriatum

If Ca^{2+} currents mediate the effects of mGluR activation, then we should be able to directly measure the inhibitory effects of mGluR activation on Ca^{2+} currents. In order to test this prediction, a total of 45 neurons were examined with whole-cell patch clamp recording techniques. In order to isolate Ca^{2+} currents, these studies were carried out in an external solution that contained tetrodotoxin (1 μM), tetraethylammonium (10 mM), and Ba^{2+} (2 mM) in place of Ca^{2+}. Cesium (125 mM) was also present inside the patch electrode. Ba^{2+} currents were evoked by a series of voltage-steps before, during, and after exposure to a mGluR agonist. Ba^{2+} currents recorded under these conditions were stable over time and after 30 min, peak current was still $95 \pm 5\%$ of controls ($n = 5$). Peak current averaged 786 ± 59 pA ($n = 45$) and was typically recorded at -10 mV. Cd^{2+} (100 μM, 5 min) inhibited the peak Ba^{2+} current to $14 \pm 3\%$ of control values ($n = 6$).

The mGluR agonist tACPD (100 μM, 10 min) caused the peak current to decline $21 \pm 6\%$ ($n = 9$, $p < 0.01$) from control values (Figs. 2 and 3). Since the Group II mGluR agonist 3C4HPG inhibited NMDA-induced swelling, the effect of this agonist on Ba^{2+} currents was also investigated. Application of 3C4HPG inhibited peak

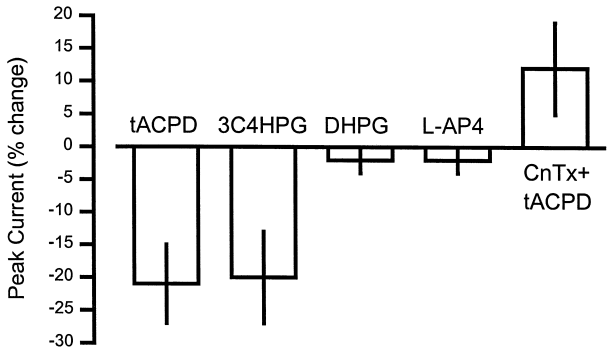

Fig. 3. The mGluR agonist 3C4HPG inhibited Ba^{2+} currents in neostriatal neurons. The mGluR agonists tACPD and 3C4HPG significantly ($p < 0.01$) inhibited peak Ba^{2+} currents while DHPG and L-AP4 did not. Error bars indicate standard error. Data were collected from 12–14 day old rats.

Ca^{2+} currents ($20 \pm 7\%$; $n = 7$, $p < 0.01$). There was some variability in this inhibitory response as only four out of the seven cells showed a clear inhibition in response to 3C4HPG. Overall, the inhibitions caused by tACPD and 3C4HPG were not significantly different. The other two mGluR agonists tested did not significantly inhibit the peak Ba^{2+} currents (100 μM DHPG: $2 \pm 2\%$, $n = 8$; 100 μM L-AP4: $2 \pm 2\%$, $n = 5$). Thus, the Group II subtype of mGluR appears to mediate the inhibitory effect on Ba^{2+} currents in the neostriatum.

Finally, in order to investigate the subtype of Ca^{2+} channel involved, we examined whether the inhibitory effect of tACPD was blocked by application of ω-CnTx (Fig. 4). By itself, ω-CnTx caused an inhibition of the peak Ba^{2+} current ($26 \pm 7\%$, $n = 4$) that was not reversible within the time-course of these experiments. When tACPD was applied in the presence of CnTx, no further inhibition was found. In fact, three of six cells tested in the presence of CnTx showed an enhancement in peak current

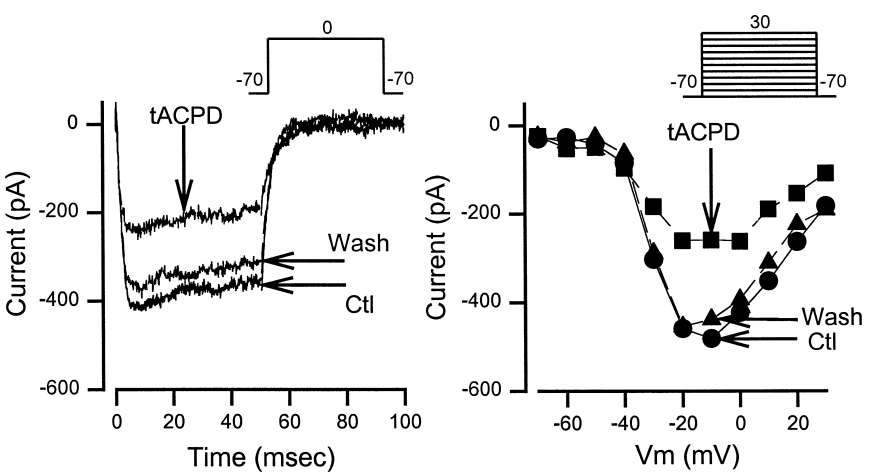

Fig. 2. Bath application of tACPD inhibits Ba^{2+} currents in a neostriatal neuron. Left: Inward currents obtained by stepping for 50 ms to 0 mV from a holding potential of -70 mV before, during, and after treatment with tACPD (100 μm, 5 min). Right: Current–voltage relationships for peak Ba^{2+} current in the same neuron. Circles = control; square = tACPD-treated; triangle = wash. Data obtained from a 14-day old rat. Inserts show voltage commands used to evoke responses.

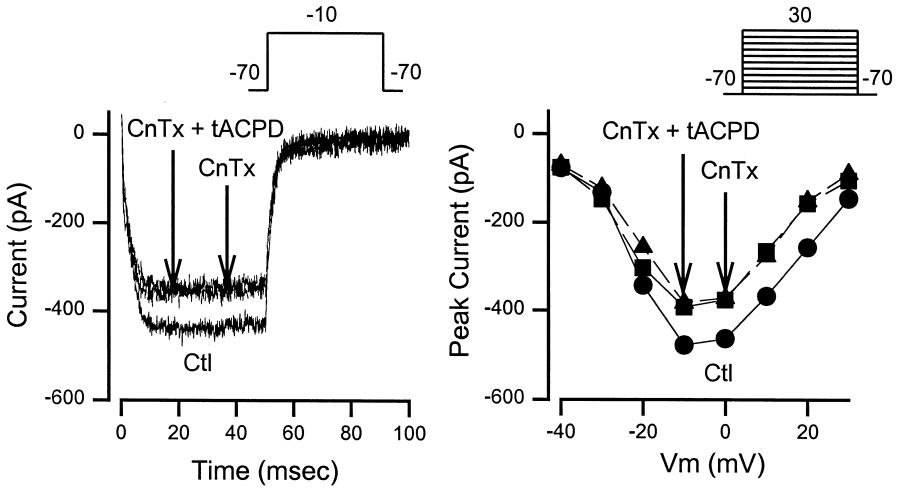

Fig. 4. Bath application of tACPD did not inhibit Ba^{2+} currents in the presence of ω-CnTx in a neostriatal neuron. Left: Inward currents obtained by stepping for 50 ms to −10 mV from a holding potential of −70 mV under control conditions, in the presence of ω-CnTx (5 μM), and after tACPD (100 μm, 5 min) was applied in presence of ω-CnTx. Right: Current–voltage relationships for peak Ba^{2+} current in each of these conditions. Circles = control; square = ω-CnTx treated; triangle = tACPD + ω-CnTx-treated. Inserts show voltage commands used to evoke responses.

in response to tACPD, although the overall effect was not significant (ω-CnTx + tACPD: 12 ± 7%, $n = 6$). Thus, application of ω-CnTx mimicked the effects of tACPD on Ba^{2+} currents as well as prevented further inhibition by tACPD. These results suggest the involvement of an N-type Ca^{2+} channel in mediating the effects of mGluR activation.

4. Discussion

We have previously shown that mGluR activation can attenuate NMDA-induced excitotoxic injury in the neostriatum both in vivo and in vitro [11,12]. Electrophysiologically, mGluR activation also inhibits NMDA-evoked currents recorded in current clamped neostriatal neurons [14]. These earlier studies made use of the non-subtype selective mGluR agonist tACPD. In the present study, we extended these observations by identifying the subtype of mGluR involved. Using selective mGluR agonists, we provide evidence that the Group II mGluRs are responsible for inhibition of NMDA excitotoxicity in the neostriatum. These results add to a body of evidence that Group II mGluRs (proteins and mRNAs) are expressed and are functional in the neostriatal region of mammals [28–30,33,38,39,43]. In addition, our previous studies did not determine whether tACPD's protective effects were the result of a direct action on ligand-gated currents or modulation of intrinsic voltage-gated membrane currents. We favored this latter possibility as a large number of studies provided evidence for mGluR modulation of voltage-gated currents, especially VSCC [17,41]. In the present study, we show evidence that tACPD's inhibitory effects on cell swelling are dependent upon Ca^{2+} influx as they can be blocked by a low Ca^{2+} solution as well as the broad spectrum Ca^{2+} channel blocker Cd^{2+}. The tACPD-induced attenuation was also blocked by ω-CnTx suggesting a role for the N-type Ca^{2+} channel. Whole cell voltage clamp recordings were made to directly determine the effects of mGluRs on VSCC in neostriatal neurons. As predicted, both tACPD and the Group II agonist 3C4HPG inhibited HVA Ca^{2+} currents in neostriatal neurons. Again this effect was blocked by ω-CnTx suggesting that N-type HVA currents were involved. Overall the results suggest that mGluR regulation of VSCC can limit NMDA toxicity in the neostriatum.

There is considerable evidence that mGluRs can modulate NMDA-induced excitotoxicity in neural tissue. For example, application of tACPD attenuates excitotoxic neuronal cell death in a variety of preparations [11,12,22,35,40]. The present observation that 4C3HPG is protective against NMDA-induced toxicity is also consistent with prior reports [6,7,31]. Furthermore, Group II mGluR agonists have been reported to have anticonvulsive and neuroprotective effects (e.g., Refs. [19,26,42]). In contrast, central administration of tACPD has been found to induce injury and seizures [25,36,45]. These differences may well reflect developmental changes and regional differences in the subtypes of mGluRs expressed (e.g., Ref. [13]). Certainly, the use of a broad-spectrum mGluR agonist, like tACPD, that activates multiple mGluR subtypes might contribute to the variability in the reported effects of mGluR activation. Although it is probably premature to generalize, the weight of the evidence suggests that activation of mGluR Group II/III subtypes more frequently inhibits excitotoxic damage.

Metabotropic receptors, including mGluRs, are coupled via G-proteins to membrane-delimited and second-messenger mediated signaling pathways that have a wide-ranging impact on a cell's physiology and function. Accord-

ingly, there are a number of possible mechanisms by which mGluRs may be modulating NMDA-induced toxicity. One possibility is that these mGluR regulated second messenger cascades may inhibit pathways activated by NMDA and thus limit excitotoxic injury. For example, it has been suggested that NMDA receptor-mediated toxicity may be due in part to an increase in cAMP (see Refs. [8,37]). If this is true, then the Group II/III mGluRs inhibition of the adenylyl cyclase/protein kinase A (AC/PKA) cascade may directly limit NMDA toxicity. There are a number of potential target sites for this type of regulation. One possibility is that mGluRs act via the AC/PKA cascade to regulate NMDA receptor activity. NMDA receptors contain multiple consensus sites for phosphorylation by several protein kinases (e.g., Refs. [27,44]). In the rat neostriatum, NMDA-induced responses are enhanced by both PKA activators and activation of dopamine receptors positively coupled to AC [9,15]. More recently, in oocytes expressing neostriatal mRNA, activation of PKA enhanced NMDA responses through regulation of the protein phosphatase inhibitor DARPP-32 [5]. Thus, the Group II/III mGluRs are in a good position to attenuate NMDA responses through their inhibition of the AC/PKA cascade whereas the Group I mGluRs would be expected to enhance NMDA responses through their positive coupling to phospholipase C.

Given the expectation that different mGluR subtypes would have opposite modulatory effects on NMDA channels, it is perhaps not surprising that electrophysiological studies using the broad-spectrum agonist tACPD have produced, often contradictory, evidence for mGluR modulation of NMDA responses. For example, while a number of studies have found that tACPD enhances NMDA-mediated currents [17], there are also several reports of mGluR inhibition of NMDA-mediated currents and EPSCs [2–4,47]. This variation is observed in studies of basal ganglia neurons. In these regions, tACPD and mGluR activation has been found to have inhibitory effects on NMDA-mediated EPSCs and evoked responses [14,21,24]. However, a recent study reported that tACPD and Group I mGluR agonists enhanced NMDA responses in the neostriatum [34]. Again, we expect that much of this variation is the result of heterogeneity in both NMDA receptors and mGluRs as well as the use of a non-subtype selective agonist tACPD. It is also possible that more subtle procedural differences between the studies contribute to this variation. Regardless, there is ample evidence in support of the hypothesis that mGluR-induced modulation of NMDA currents is one mechanism by which mGluRs can act to modulate excitotoxicity.

Besides a direct action on NMDA currents, mGluRs could also alter NMDA responses through regulation of intrinsic, voltage-sensitive, currents. An mGluR-induced reduction of N-type HVA Ca^{2+} currents has been previously reported in neurons from a variety of brain regions (e.g., Refs. [17,41]). The mGluR-mediated inhibition of HVA Ca^{2+} currents would limit both the presynaptic release of glutamate as well as the postsynaptic cell's response to glutamate receptor activation. Since the excitotoxic response generated in neurons in response to NMDA is thought to be critically dependent upon a Ca^{2+} influx, the mGluR modulation of Ca^{2+} currents may well play a role in altering the NMDA-induced changes in intracellular Ca^{2+} and by this mechanism attenuate NMDA-induced toxicity. Although this possibility has not been previously explored, earlier studies have reported that Group II/III mGluR agonists as well as inorganic Ca^{2+} channel blockers attenuate β-amyloid induced toxicity in cultured neurons [18,46].

The present study provides the first evidence for Ca^{2+} channel involvement in the mGluR modulation of NMDA-induced toxicity. Blockade of Ca^{2+} influx by removal of extracellular Ca^{2+} and blockade of Ca^{2+} channels with Cd^{2+} eliminated mGluR attenuation of NMDA-induced swelling. Application of the N-type Ca^{2+} channel blocker ω-CnTx also significantly reduced the mGluR modulation. In addition, mGluR activation inhibited Ba^{2+} currents in neostriatal neurons in slices. This effect was also prevented by ω-CnTx. These results suggest that mGluR modulation of NMDA toxicity may be mediated by regulation of Ca^{2+} currents. These findings highlight the importance of voltage-gated currents in determining the response of a neuron to glutamatergic stimulation and add support to the suggestion that manipulations of voltage-gated currents may have therapeutic utility as treatments to protect against excitotoxic damage.

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