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Regulation of *N*-methyl-D-aspartate-induced toxicity in the neostriatum: A role for metabotropic glutamate receptors?

(excitotoxicity/infrared videomicroscopy/metabotropic glutamate receptors/quinolinic acid/1-aminocyclopentane-1,3-dicarboxylic acid)

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ABSTRACT **Glutamate release activates multiple receptors that interact with each other and thus determine the response of the cell. Exploring these interactions is critical to developing an understanding of the functional consequences of synaptic transmission. Activation of metabotropic glutamate receptors (mGluRs) inhibits *N*-methyl-D-aspartate (NMDA)-evoked responses measured electrophysiologically in neostriatal slices. The present study examines the functional consequences of this regulation using infrared differential interference contrast videomicroscopy to measure and characterize glutamate receptor-induced cell swelling in a neostriatal brain slice preparation. This swelling is, in many cases, a prelude to necrotic cell death and the dye trypan blue was used to confirm that swelling can result in the death of neostriatal cells. Activation of mGluRs by the agonist 1-aminocyclopentane-1,3-dicarboxylic acid (tACPD) inhibited NMDA but not amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate-induced swelling. This regulation was cell-type specific as tACPD did not alter NMDA-induced swelling in pyramidal cells of the hippocampus. Importantly, these findings could be extended to *in vivo* preparations. Pretreatment with tACPD limited the size of lesions and associated behavioral deficits induced by intrastriatal administration of the NMDA receptor agonist quinolinic acid.**

Glutamate receptors are classified into two general forms: (*i*) metabotropic receptors (mGluRs), which are coupled to various signal transduction processes through GTP-binding proteins; and (*ii*) ionotropic receptors (iGluRs), which are ligand-gated cation channels (1, 2). The iGluRs are further subdivided according to their preferential agonists as *N*-methyl-D-aspartate (NMDA), amino-3-hydroxy-5-methyl-4-isoazole (AMPA), and kainate (KA) receptors. It has become increasingly clear that the response of a neuron to the release of glutamate is determined by multiple, interacting receptors. This cellular integration permits considerable plasticity in responses to synaptic inputs and could form the basis for therapeutic intervention in diseases associated with GluR dysfunctions.

Neostriatal cells are innervated by a major glutamate-containing projection from the neocortex (3–5). Like many glutaminergic synaptic connections, both AMPA/KA and NMDA GluRs contribute to excitatory postsynaptic potentials (EPSPs) recorded in neostriatal neurons (6, 7). In addition to the iGluRs, several mGluR subtypes are localized within the neostriatum (8). Although little is known about their function, one possibility is that mGluRs inhibit excitatory input and thus can limit excitotoxic damage to these cells (9). This hypothesis is supported by the findings that activation of mGluRs in the neostriatum inhibits both EPSPs (10, 11) as well as NMDA-evoked responses (9). The implications of this interaction among GluRs on excitotoxicity have not been adequately investigated *in vitro* or *in vivo*. Given the prominent role played by GluRs in diseases affecting the neostriatum (12, 13), understanding such interactions is clinically important.

The present studies were designed to determine (*i*) whether activation of mGluRs by 1-aminocyclopentane-1,3-dicarboxylic acid (tACPD) limits the NMDA- or AMPA/KA-induced swelling in a neostriatal brain slice as measured by infrared differential interference contrast (IR DIC) videomicroscopy; (*ii*) whether tACPD limits NMDA-induced swelling in hippocampal pyramidal cells; and (*iii*) whether tACPD limits quinolinic acid (QA)-induced neostriatal lesions as well as apomorphine-induced rotational behavior *in vivo*.

MATERIALS AND METHODS

Preparation of Neostriatal Slices. Male Sprague–Dawley rats 12–16 days old were used. After animals were killed by decapitation, brains were dissected and placed in cold oxygenated artificial cerebrospinal fluid (ACSF) containing 130 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 2 mM $MgCl_2$, 1.25 mM NaH_2PO_4, 1.0 mM $CaCl_2$, 10 mM glucose (pH 7.2–7.4). After cutting with a Dosaka microslicer (Ted Pella, Redding, CA), transverse sections (350 μm) were placed in ACSF at 25–27°C for at least 1 hr (in this solution $CaCl_2$ was increased to 2 mM, and 4 mM lactate was added). The osmotic pressures of the ACSF and all experimental solutions were between 290 and 300 mosm. Individual tissue sections were then transferred to the perfusion chamber in which the slice was held down with thin nylon threads. The slice was submerged in continuously flowing (2 ml/min) oxygenated ACSF (25°C).

IR DIC Videomicroscopy. An upright, fixed stage compound microscope (Zeiss ×40 water immersion lens; achroplan; numerical aperture, 0.75) equipped with differential interference contrast optics was used to view slices. Slices were illuminated with near infrared light by placing a bandpass filter (790 nm; bandpass, 750–1050 nm; Ealing Optics, Holliston, MA) in the light path and images were detected with an infrared-sensitive CCD camera [Hamamatsu (Middlesex, NJ) model C2400]. Digitized images were stored for subsequent analysis and additional digital contrast adjustment when necessary. Image analysis software (OPTIMAS, BioScan, Edmonds, WA) was used to quantify changes in cell area (perimeter, maximal length, and width were also measured in some cells) before and after experimental treatments. Each measurement was made twice and the average value was recorded. Measurements were taken only from cells that exhibited clear borders, concave shapes, and phase brightness.

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Abbreviations: mGluR, metabotropic glutamate receptor; iGluR, ionotropic glutamate receptor; NMDA, *N*-methyl-D-aspartate; IR DIC, infrared differential interference contrast; tACPD, 1-aminocyclopentane-1,3-dicarboxylic acid; AMPA, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; KA, kainate; QA, quinolinic acid; ACSF, artificial cerebrospinal fluid; AP5, DL-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; MCPG, α-methyl-4-carboxyphenylglycine; TTX, tetrodotoxin.

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Excitotoxic Lesions. Adult male Sprague–Dawley rats (200–300 g) were anesthetized with pentobarbital (50–60 mg/kg; intraperitoneal). Experimental methods for induction of neostriatal lesions with the NMDA GluR agonist QA are well established (14). Briefly, intrastriatal injections were made in the left neostriatum (coordinates relative to bregma: anterior, 0.2 mm; lateral, 3.0 mm; ventral, −5.0 mm). Drugs were delivered through a Hamilton syringe in a volume of 1 μl over 10 min. The syringe was left in place for an additional 5 min before being slowly withdrawn. Before and after surgery, rats were housed in group cages of three or four animals with free access to food and water.

For histological evaluation of lesions, animals were deeply anesthetized 2 weeks after treatment and perfused with phosphate-buffered saline, followed by 10% formalin in phosphate-buffered saline. Brain sections (50 μm thick) were stained for Nissl substance with cresyl violet. Surface area measurements of damaged zones within the neostriatum were made with image analysis software. Briefly, a digitized image was displayed on a monitor and a mouse was used to outline the lesion area (defined by loss of Nissl stained neurons). Because the borders of the lesion are somewhat arbitrary, measurements were made by an observer who was blind to the treatment group. All sections from the neostriatum from 2.0 mm anterior to 0.5 mm posterior to bregma were measured. Volume of the lesion was then estimated as the sum of the surface area (mm^2) × section thickness (0.05 mm). In addition, estimates of cell density were made at several locations in the lesioned areas and in comparable areas from the contralateral neostriatum. To start, a judgement was made as to the location of the center of the lesion and counts were made in a random location within the center of the neostriatum in sections 200, 400, and 600 μm anterior and posterior to the center of the lesion. For this analysis, neurons in a fixed area (2×10^6 μm^3) were counted using an eyepiece graticule by two observers who were blind to the treatment group and the values from each observer were averaged. The counts in each of these seven locations were then averaged to provide a single estimate of cell density per animal.

Behavioral Analysis. It has been shown that unilateral neostriatal lesions induce rotation in the direction of the lesion (ipsilaterally) when apomorphine is administered (15, 16). Tests for rotation began 2 weeks after surgery. Experimental and control rats were placed in a circular arena for 30 min. Apomorphine (1 mg/kg in saline containing 0.1% sodium metabisulfate) was then administered subcutaneously in the neck. Rotational behavior (full 360° turns) was determined by direct observation for 2 min every 5 min for 60 min following apomorphine administration.

Chemicals. Pharmacological agents (unless otherwise noted, obtained from Research Biochemicals, Natick, MA) were AMPA, tACPD, DL-2-amino-5-phosphonopentanoic acid (AP5), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), glutamic acid (glutamate; Sigma), KA (Sigma), α-methyl-4-carboxyphenylglycine (MCPG; Tocris Neuramin, Bristol, U.K.), NMDA (Sigma), tetrodotoxin (TTX; Sigma). The AMPA/KA GluR antagonist CNQX was dissolved in dimethyl sulfoxide (10 mM stock solution) prior to final dilution. Antagonist concentrations were chosen based on our experience in blocking GluR-evoked responses measured electrophysiologically in neostriatal slices.

Statistical Analysis. Differences between average values for experimental and control groups were evaluated using *t* tests or Mann–Whitney rank sum tests. Differences among concentration–response curves were evaluated using appropriate ANOVAs. Values were considered significantly different if $P < 0.05$. In the text, values are shown as means ± SEM.

RESULTS

A total of 1320 cells from the neostriata of 29 rats (average age was postnatal day 14) were visualized using IR DIC videomicroscopy (Fig. 1). Typically, 5–10 cells could be visualized at one time. Images were usually obtained from 50 to 100 μm below the slice surface. Cells had average areas of 70.3 ± 0.7 μm^2, perimeters of 35 ± 0.3 μm, maximal lengths of 13.7 ± 0.1 μm, and maximal widths of 8.5 ± 0.1 μm. The effects of experimental and control manipulations were determined by measuring cell area before and after treatments (results are presented as percentage change in area). Under control conditions in which the cells were perfused continuously with ACSF, these measurements were stable. No significant changes in area occurred up to 30 min (1% ± 1% at 10 min; $n = 65$; 1% ± 2% at 30 min; $n = 33$).

GluR Agonists Caused Cells to Swell. Bath application of the GluR agonists AMPA, glutamate, KA, and NMDA all caused concentration-dependent swelling of neostriatal cells in the slice (Fig. 1*A*). NMDA (50 μM; 10 min)-induced swelling was blocked by treatment with the competitive NMDA GluR antagonist AP5 (50 μM AP5 + NMDA, −1% ± 3%; $n = 21$ vs. NMDA, 28% ± 1%; $n = 138$; $P < 0.001$) but not by the AMPA/KA GluR antagonist CNQX or the mGluR antagonist MCPG (5 μM CNQX + NMDA, 27% ± 4%; $n = 21$; 1 mM MCPG + NMDA, 28% ± 3%; $n = 15$). In contrast, KA (100 μM; 10 min)-induced swelling was prevented by CNQX (5 μM CNQX + KA, −0% ± 2%; $n = 21$ vs. KA, 28% ± 3%; $n = 46$; $P < 0.001$) but not by AP5 or MCPG (50 μM AP5 + KA, 41% ± 7%; $n = 17$; 1 mM MCPG + KA, 24% ± 5%; $n = 16$). Finally, neither NMDA- nor KA-induced swelling was affected by the addition of TTX, a blocker of voltage sensitive Na^+ channels (TTX + NMDA, 23% ± 3%; $n = 33$; TTX + KA,

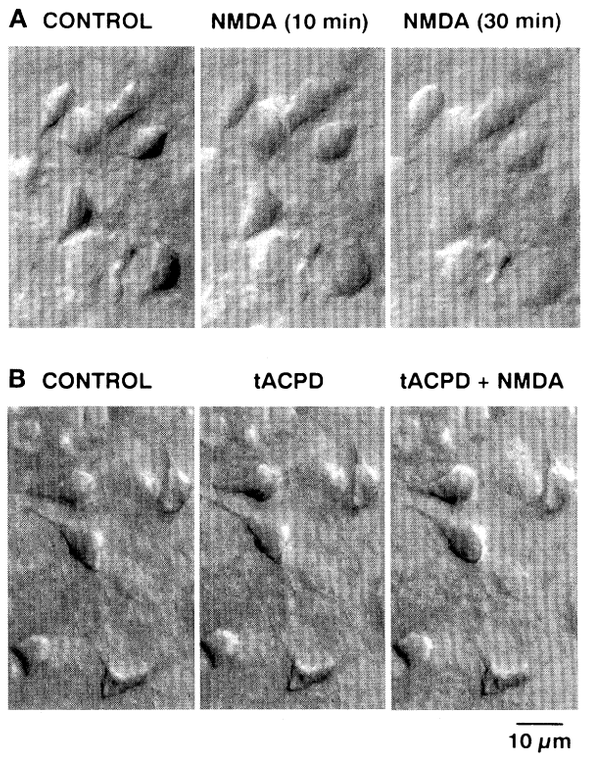

FIG. 1. IR DIC image of neurons in a neostriatal brain slice from a 14-day-old rat. (*A*) Five cells under control conditions (*Left*). Exposure to NMDA (50 μM) causes cells to swell (*Center* and *Right*). (*B*) tACPD limits NMDA-induced swelling. tACPD alone (100 μM; 5 min) had little effect on cell area (*Center*). When NMDA (50 μM; 10 min) was applied in the presence of tACPD, swelling was reduced (*Right*).

30% ± 5%; n = 12). Alone, AP5 (50 μM), CNQX (5 μM), MCPG (1 mM), or TTX (1 μM) did not alter cell area (data not shown). These results suggest that if tonic glutamate release is occurring, it is not sufficient to cause swelling. Thus, activation of NMDA or AMPA/KA GluRs can cause the swelling of neostriatal neurons.

Swelling Is Associated with Cell Death. Some cells swelled to such an extent that their borders became indistinct and could no longer be visualized. More cells "disappeared" in this manner when treated with higher concentrations or longer durations of NMDA. For example, proportionately more cells were lost (could no longer be visualized) after 10 and 30 min with 500 μM NMDA (17%, 8/47 cells, and 78%, 36/46 cells, respectively) than with 50 μM NMDA (9%, 12/138 cells, and 49%, 20/41 cells, respectively). The dye trypan blue was used to examine whether the loss in the ability to visualize cells is related to cell death. This dye is normally excluded from healthy cells but is taken up by dead cells and is widely used in cell culture as a marker of cell death (17). Measurements were taken from cells that exhibited clear borders, concave shape, and phase brightness. Cells with these characteristics never took up the dye when slices were incubated in trypan blue (0.4%). In contrast, other cells exhibiting indistinct borders, convex shape, and low-phase brightness with the nucleus visible became stained when exposed to trypan blue and were likely to have been damaged or dead. Exposure to NMDA (500 μM; 30 min) dramatically increased the number of cells that took up the trypan blue (Fig. 2). In five cases, it was possible to follow a single identified cell and demonstrate that a cell that excluded trypan blue initially took up the dye after exposure to NMDA. However, some cells that showed NMDA-induced swelling still retained the ability to exclude trypan blue. Overall, these results are consistent with the hypothesis that extensive swelling is an initial step in a cascade of events that can, but does not always, lead to cell death.

tACPD Limits NMDA- but Not KA-Induced Swelling. In neostriatal neurons, the mGluR agonist tACPD inhibits NMDA- but not AMPA/KA-mediated responses measured electrophysiologically (9). One prediction from this result is that tACPD should limit NMDA- but not AMPA/KA-induced swelling. Pretreatment with tACPD (100 μM; 10 min) inhibited NMDA (50 μM; 10 min)-evoked swelling (Fig. 1*B*; tACPD + NMDA, 4% ± 3%; n = 38 vs. NMDA, 28% ± 1%; n = 138; $P < 0.0001$). When NMDA concentration was increased to 500 μM, the protective effect of tACPD was overcome (tACPD + NMDA, 26% ± 4%; n = 25 vs. NMDA, 33% ± 6%; n = 47). Activation of the mGluRs must precede NMDA application in order to reduce the effects of NMDA. When NMDA (50 μM) was applied first and then tACPD was added, no protective effect was observed (30% ± 5%; n = 14). The inhibitory effect of tACPD was prevented by the mGluR antagonist MCPG (26% ± 4%; n = 24). The protective effect did not occur when the less active isomer 1*R*,3*S*-ACPD was applied with NMDA (27% ± 4%; n = 21). In contrast, tACPD did not protect against KA-induced cell swelling. The application of tACPD (100 μM) prior to KA (100 μM) resulted in swelling that was not distinguishable from that induced by KA alone (tACPD + KA, 31% ± 4%; n = 26 vs. KA, 28% ± 3%; n = 46). By itself, tACPD (100 μM) did not alter cell area (data not shown). Thus, activation of mGluRs by tACPD selectively limits NMDA-induced swelling in neostriatal cells.

tACPD Does Not Alter NMDA-Induced Swelling in Hippocampal Cells. It has been shown that tACPD enhances, rather than reduces, NMDA-evoked electrophysiological responses in hippocampal pyramidal cells (18). Thus, hippocampal cells were examined to determine whether tACPD enhances NMDA-induced swelling. Alone, NMDA (10 min) caused small but significant increases in pyramidal cell area (100 μM NMDA, 12% ± 2%; n = 63; 500 μM NMDA, 20% ± 3%; n = 20; $P < 0.05$). In contrast to neostriatal cells, pretreatment with tACPD (100 μM; 10 min) did not significantly alter NMDA-induced swelling in hippocampal cells (tACPD + 100 μM NMDA, 12% ± 3%; n = 23; tACPD + 500 μM NMDA, 16% ± 4%; n = 20). By itself, tACPD (100 μM;

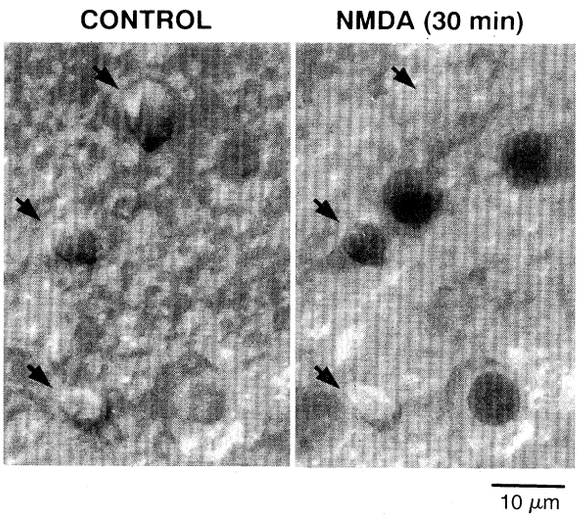

FIG. 2. NMDA increases uptake of trypan blue in neostriatal slices. Neostriatal slices (16-day-old rat) were exposed to trypan blue (0.4%) for 10 min. The dye is excluded from healthy cells but taken up by dead cells in which a blue precipitant forms (shown here as black). (*Left*) Image from untreated control. (*Right*) Image from the same slice after exposure to NMDA (500 μM; 30 min). Arrows indicate three cells that showed the full range of responses. Top arrow indicates a cell that swelled to such an extent that it became phase invisible but did not take up the dye. Middle arrow indicates a cell that became permeable to the dye after NMDA treatment. Bottom arrow indicates a cell that did not show a marked response to NMDA.

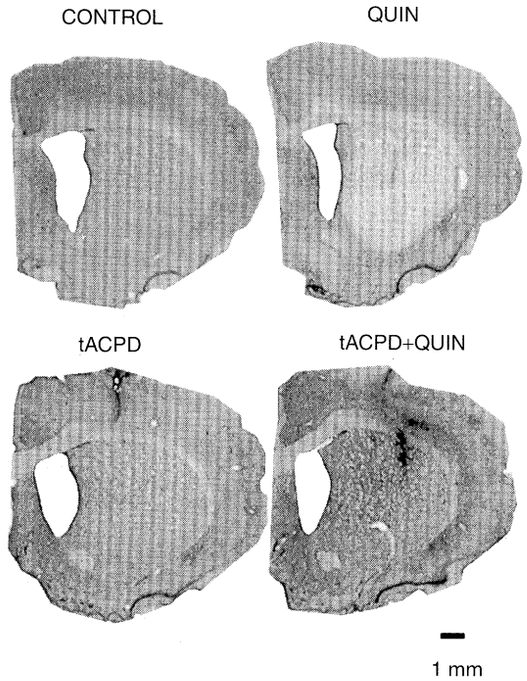

FIG. 3. tACPD limits extent of QA-induced lesion. All sections were stained with cresyl violet. Intrastriatal injection of QA (QUIN) caused large and consistent lesions in the neostriatum, which were marked by the dramatic loss of Nissl staining in tissue. tACPD applied prior to QA limited the extent of the lesion. tACPD, by itself, did not cause a lesion.

10 min) did not cause any measurable change in hippocampal cell area (4% ± 2%; $n = 19$). These results demonstrate that mGluR-mediated inhibition of NMDA-induced swelling is cell-type specific.

tACPD Limits QA-Induced Lesion Size and Behavioral Effects *in Vivo*. The intrastriatal injection of the NMDA agonist QA caused an average lesion of 5 ± 1 mm^3 ($n = 5$) and reduced the neuronal density from 114 ± 3 neurons per 2×10^6 μm^3 ($n = 7$) in the untreated neostriatum to 37 ± 4 neurons per 2×10^6 μm^3 ($P < 0.01$) (Fig. 3). Animals treated with tACPD (100 μM) 5–10 min prior to injection of QA had a lesion size of 1 ± 0.2 mm^3 and a neuronal density of 84 ± 13 neurons per 2×10^6 μm^3 ($n = 5$). These animals still had an obvious lesion near the injection site but the excitotoxic effects of QA as measured by lesion volume and neuronal density were significantly reduced ($P < 0.05$). Alone, injection of tACPD ($n = 5$) or saline ($n = 5$) did not produce obvious damage to the neostriatum except for the cannula tract.

To determine whether the protective effects of tACPD extended to QA-induced behavioral deficits, animals were treated with the dopamine agonist apomorphine. QA-induced unilateral neostriatal lesions caused ipsilateral rotation after apomorphine (peak rotation, 4 ± 1 turns per min; total rotations, 20 ± 4 turns per 60 min; $n = 5$). Apomorphine-induced rotations were significantly reduced in the animals pretreated with tACPD before QA administration (peak rotation, 1 ± 1 turns per min; total rotations, 1 ± 1 turns per 60 min; $n = 6$; $P < 0.001$). Thus, tACPD also limits NMDA-mediated toxicity in the neostriatum and associated behavioral deficits *in vivo*.

DISCUSSION

Previous work, primarily on cultured cells, has shown that an initial consequence of the application of GluR agonists is cell swelling and that this swelling is an early step in a cascade of events that can eventually lead to cell death (19, 20). In the present study, IR DIC videomicroscopy was used to examine these dynamic physical changes as a rapid measure of toxicity in a brain slice preparation (21). The results with trypan blue provide further support for the view that NMDA and AMPA/KA GluR-induced cell swelling is an early marker of excitotoxic damage. Although we do not know whether cell swelling always results in cell death, the events appear to be closely associated. The effects of GluR agonists on neostriatal cell area were reproducible and quantifiable, and the dynamics of this swelling could be followed in single cells with IR DIC videomicroscopy. As many as 95% of the neostriatal neurons are medium spiny projection neurons (22) and it is likely that most of the data in the present study were collected from this cell type. When biocytin or Lucifer yellow was injected through a patch pipette, all cells labeled ($n = 10$) were medium-sized neurons (unpublished data). Large interneurons were systematically excluded from the present data set. IR DIC videomicroscopy appears to offer great promise as a tool to study the excitotoxic responses of single neurons in a brain slice preparation in which many of the local synaptic connections remain intact.

The present study demonstrates that activation of mGluRs can limit NMDA-induced cell swelling and presumably cell death *in vitro*. Similar results (i.e., tACPD protects against NMDA-induced cell death) have been reported in cultured neocortical neurons (23–25) as well as in the retina (26). Activation of mGluRs is thought to act through two main transduction mechanisms: the activation of phospholipase C and the inhibition of adenylate cyclase (27–29). Evidence for both types of regulation has been found in neostriatal tissue (30, 31) and thus they are potential mechanisms for the mGluR-mediated protection. In cortical cells, there is pharmacological evidence that mGluRs negatively coupled to cAMP formation may be responsible (25). The consequences of these changes in second messenger cascades and how they may be related to toxicity are not well understood. Likely effectors of second messenger-induced changes include voltage-gated ion channels and mGluR-mediated regulation of these channels is well documented (29). However, it is fair to say that the intracellular mechanisms by which tACPD provides such protection are currently unclear. In the neostriatum, activation of mGluRs inhibits both excitatory synaptic transmission through a presynaptic mechanism (32) as well as NMDA-induced responses postsynaptically (9). Perhaps this combination of inhibitory regulatory mechanisms will be a common feature in cell types/tissues in which activation of mGluRs offers protection against excitotoxic damage.

There is evidence that tACPD potentiates rather than inhibits NMDA-induced responses in other brain regions including the CA1 area of the hippocampus (18, 33, 34). As had been previously reported (19, 21), NMDA caused swelling of hippocampal cells, although the effect was reduced in magnitude compared to that observed in neostriatal cells. Surprisingly, in the present experiments, activation of mGluRs by tACPD did not have any impact on NMDA-induced swelling in the hippocampus. At least one other study that utilized cultured cerebellar neurons also found that tACPD did not alter NMDA-induced toxicity (35). These results suggest that the modulatory effect of mGluR activation will vary with the cell type involved. Part of the explanation for these regional differences may involve heterogeneity of mGluRs (27, 28). Subtypes of mGluRs show distinct distributions and it is likely that more than one subtype of mGluR can mediate the effects of tACPD in a particular brain region or during a specific developmental period. For example, in the neostriatum, mGluRs 1, 2, 4, and 7 are expressed at low levels while mGluRs 3 and 5 are more abundant (e.g., see refs. 8 and 36). Other regions appear to have different compositions of mGluR subtypes (27, 28).

Previous studies have established that intrastriatal injections of the NMDA receptor agonist QA in rats caused large lesions, including loss of medium-sized spiny neurons, but relative sparing of interneurons containing somatostatin, neuropeptide Y, and/or acetylcholine (14). These results are similar to postmortem findings in Huntington disease and have led to the use of QA-lesioned animals as a model for this disorder (e.g., see ref. 37). In the present study, two approaches were used to assess modulation of QA-induced neostriatal damage *in vivo*. In the first, the sizes of lesions induced by the intrastriatal injection of QA with and without tACPD pretreatment were compared. In the second, the dopamine agonist apomorphine was systemically administered and the number of rotations was compared between groups receiving QA injections with and without tACPD. It is well established that unilateral neostriatal lesions cause ipsilateral rotation when apomorphine is administered and that the number of rotations is related to the extent of the lesion (15, 16). With both approaches, pretreatment with tACPD inhibited QA-induced effects. Thus, these results extend the initial observations from a brain slice preparation to the intact neostriatum and the behaving animal. Furthermore, these findings suggest that mGluRs may modulate excitotoxic damage to the neostriatum and perhaps other brain regions.

The present findings are in some ways inconsistent with previous reports indicating that intrastriatal injections of 1*S*,3*R*-ACPD in perinatal rats are both toxic (ref. 38, but also see ref. 39) and potentiate NMDA- but not AMPA-mediated brain injury (39). In this previous work, younger animals (7 days old) were examined during a developmental period in which mGluR expression is transiently enhanced (40). Thus, one possibility to account for differences among studies is age. However, unpublished preliminary studies from our laboratory indicate that tACPD also inhibits NMDA-induced swell-

ing in 7-day-old rats. Other methodological variables may provide an explanation for the differences between the studies (e.g., use of 1*S*,3*R*-ACPD in previous work vs. 1*SR*,3*RS*-ACPD in the present study).

In conclusion, the present findings demonstrate that tACPD limits NMDA- but not AMPA/KA-induced swelling in the neostriatal slice. The finding that this regulation did not occur in the hippocampus shows that such modulation is cell-type specific and suggests that electrophysiological results cannot always be directly applied to predict the results of toxicity studies. Activation of mGluRs by tACPD also limited excitotoxic damage induced by administration of the NMDA agonist QA into the neostriatum. These results demonstrate that activation of mGluRs can limit excitotoxic damage in the intact central nervous system. Finding novel mechanisms to limit excitotoxicity is important in the central nervous system in general, and in the neostriatum in particular, as a number of diseases are thought to involve dysfunction in excitatory synaptic transmission in this brain region.

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1. Monaghan, D. T., Bridges, R. J. & Cotman, C. W. (1989) *Annu. Rev. Pharmacol. Toxicol.* **29,** 365–402.
2. Hollmann, M. & Heinemann, S. (1994) *Annu. Rev. Neurosci.* **17,** 31–108.
3. McGeer, P. L., McGeer, E. G., Shorer, V. & Singh, K. (1977) *Brain Res.* **128,** 369–373.
4. Fonnum, F., Storm-Mathisen, J. & Divac, I. (1981) *Neuroscience* **6,** 863–873.
5. Meshul, C. K., Stallbaumer, R. K., Taylor, B. & Janowsky, A. (1994) *Brain Res.* **648,** 181–195.
6. Cherubini, E., Herrling, P. L., Lanfumey, L. & Stanzione, P. (1988) *J. Physiol. (London)* **400,** 677–690.
7. Jiang, Z. G. & North, R. A. (1991) *J. Physiol. (London)* **443,** 533–553.
8. Testa, C. M., Standaert, D. B., Young, A. B. & Penney, J. B. (1994) *J. Neurosci.* **14,** 3005–3018.
9. Colwell, C. S. & Levine, M. S. (1994) *Neuroscience* **61,** 497–507.
10. Lovinger, D. M. (1991) *Neurosci. Lett.* **129,** 17–21.
11. Calabresi, P., Mercuri, N. B. & Bernardi, G. (1992) *Neurosci. Lett.* **139,** 41–44.
12. DiFiglia, M. (1990) *Trends Neurosci.* **13,** 286–289.
13. Ulas, J., Weihmuller, F. B., Brunner, L. C., Joyce, J. N., Marshall, J. F. & Cotman, C. W. (1994) *J. Neurosci.* **14,** 6317–6324.
14. Beal, M. F., Kowall, N. W., Ellison, D. W., Mazurek, M. F., Swartz, K. J. & Martin, J. B. (1986) *Nature (London)* **321,** 168–171.
15. Schwarcz, R., Fuxe, K., Agnati, L. F., Hokfelt, T. & Coyle, J. T. (1979) *Brain Res.* **170,** 485–495.
16. Pycock, C. J. (1980) *Neuroscience* **5,** 461–514.
17. Phillips, H. J. (1973) in *Tissue Culture: Methods and Applications*, eds. Kruse, P. K. & Patterson, M. K. (Academic, New York), pp. 406–408.
18. Aniksztejn, L., Otani, S. & Ben-Ari, Y. (1992) *Eur. J. Neurosci.* **4,** 500–505.
19. Rothman, S. M. (1992) *Ann. N.Y. Acad. Sci.* **648,** 132–139.
20. Choi, D. W. (1988) *Neuron* **1,** 623–634.
21. Dodt, H. U., Hager, G. & Zieglgansberger, W. (1993) *J. Neurosci. Methods* **50,** 165–171.
22. Gerfen, C. R. (1992) *Annu. Rev. Neurosci.* **15,** 285–320.
23. Koh, J. Y., Palmer, E. & Cotman, C. W. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9431–9434.
24. Birrell, G. J., Gordon, M. P. & Marcoux, F. W. (1993) *Neuropharmacology* **32,** 1351–1358.
25. Bruno, V., Copani, A., Battaglia, G., Raffaele, R., Shinozaki, H. & Nicoletti, F. (1994) *Eur. J. Pharmacol.* **256,** 109–112.
26. Siliprandi, R., Lipartiti, M., Fadda, E., Sautter, J. & Manev, H. (1992) *Eur. J. Pharmacol.* **219,** 173–174.
27. Nakanishi, S. (1994) *Neuron* **13,** 1031–1037.
28. Pin, J. P. & Duvoisin, R. (1995) *Neuropharmacology* **34,** 1–26.
29. Schoepp, D. & Conn, P. J. (1993) *Trends Pharmacol. Sci.* **14,** 13–20.
30. Manzoni, O. J. J., Poulat, F., Do, E., Sanuquet, A., Sassetti, I., Bockaert, J. & Sladeczek, F. (1991) *Eur. J. Pharmacol.* **207,** 231–241.
31. Prezeau, L., Manzoni, O., Homburger, V., Sladeczek, F., Curry, K. & Bockaert, J. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8040–8044.
32. Lovinger, D. M., Tyler, E., Fidler, S. & Merritt, A. (1993) *J. Neurophysiol.* **69,** 1236–1244.
33. Bleakman, D., Rusin, K. I., Chard, P. S., Glaum, S. R. & Miller, R. J. (1992) *Mol. Pharmacol.* **42,** 192–196.
34. Kinney, G. A. & Slater, N. T. (1993) *J. Neurophysiol.* **69,** 585–594.
35. Thomsen, C., Frandsen, A., Suzdak, P. D., Andersen, C. F. & Schousboe, A. (1993) *NeuroReport* **4,** 1255–1258.
36. Saugstad, J. A., Kinzie, J. M., Mulvihill, E. R., Segerson, T. P. & Westbrook, G. L. (1994) *Mol. Pharmacol.* **45,** 367–372.
37. Ferrante, R. J., Kowall, N. W., Cipolloni, P. B., Storey, E. & Beal, M. F. (1993) *Exp. Neurol.* **119,** 46–71.
38. McDonald, J. W., Fix, A. S., Tizzano, J. P. & Schoepp, D. D. (1993) *J. Neurosci.* **13,** 4445–4455.
39. McDonald, J. W. & Schoepp, D. D. (1992) *Eur. J. Pharmacol.* **215,** 353–354.
40. McDonald, J. W. & Johnston, M. V. (1990) *Brain Res. Rev.* **15,** 41–70.