Calpain Activation Contributes to Dendritic Remodeling after Brief Excitotoxic Injury In Vitro

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The calcium-dependent protease calpain may contribute to neuronal death in acute neurological insults and may be activated very early in the neuronal injury cascade. We assessed the role of calpain in a model of rapid, reversible dendritic injury in murine cortical cultures. Brief sublethal NMDA exposure (10–30 μM for 10 min) resulted in focal swellings, or varicosities, along the length of neuronal dendrites as visualized with the lipophilic membrane tracer DiI or with immunostaining using antibodies to the somatodendritic protein MAP2. These varicosities appeared within minutes of NMDA exposure and recovered spontaneously within 2 hr after NMDA removal. Addition of the calpain inhibitors MDL28,170, calpain inhibitors I and II, and leupeptin (all 1–100 μM) had little effect on the development of NMDA-induced dendrite injury. However, the resolution of varicosities was substantially delayed by addition of calpain inhibitors after sublethal excitotoxic exposure. Using Western blots and immunocytochemistry, we observed reactivity for a calpain-specific spectrin proteolytic fragment during the period of recovery from dendritic swelling, but not during its formation. Spectrin breakdown product immunoreactivity could be blocked by the calpain inhibitor MDL28,170 and appeared in neuronal cell bodies and neurites in a time course that paralleled dendritic recovery. These observations suggest that calcium-dependent proteolysis contributes to recovery of dendritic structure after NMDA exposure. Calpain activation is not necessarily detrimental and may play a role in dendritic remodeling after neuronal injury.

Key words: excitotoxicity; calpain; neuronal injury; cell culture; cytoskeleton; glutamate; spectrin

The calcium-activated cysteine proteases, or calpains, have been implicated as a major component in a cascade of events leading to neuronal death in the setting of excitotoxic, hypoxic, and traumatic insults (Wang and Yuen, 1994; Bartus et al., 1995). Calpain-mediated proteolysis is selective for a subset of cellular proteins that include cytoskeletal and membrane proteins, kinases, phosphatases, and transcription factors (Goll et al., 1992; Saito et al., 1994). Although calpain isoforms are present in all vertebrate cells, the role of calpain under physiological conditions remains poorly understood. When intracellular calcium levels are markedly elevated under pathological conditions, however, the resulting calpain activation can be a destructive force within the cell.

There is substantial evidence that calpain-mediated proteolysis occurs in many settings of acute neuronal injury (Wang and Yuen, 1994). Pharmacological inhibition of calpain is neuroprotective in many, though not all, models. The effects of calpain inhibitors have been variable in in vitro systems and are influenced, perhaps, by cell type and mode of injury (Di Stasi et al., 1991; Manev et al., 1991; Caner et al., 1993; Rami and Kriegstein, 1993; Broson et al., 1995; Chard et al., 1995; Wang et al., 1996). Calpain inhibitors reduce proteolysis and cell death in several in vivo models of cerebral ischemia and brain trauma (Lee et al., 1991; Bartus et al., 1994; Saatman et al., 1996). Accordingly, substantial interest has been generated in assessing the therapeutic potential of calpain inhibitors in a variety of neurological disorders (Siman, 1992; Wang and Yuen, 1994; Bartus, 1995; Linnik, 1996).

We examined the role of calpain in dendritic injury after glutamate receptor activation. A common manifestation of many forms of neuronal injury is the formation of focal swellings or varicosities along the length of the dendritic arbor. This pattern of dendritic injury, illustrated by Ramón y Cajal a century ago (Ramón y Cajal, 1909, 1995), has been observed in neuronal injury models both in vivo (Olney, 1971; Hsu and Buzsaki, 1993; Kwei et al., 1993; Hori and Carpenter, 1994; Matesic and Lin, 1994) and in vitro (Stewart et al., 1991; Bateman and Goldberg, 1992; Bindokas and Miller, 1995; Emery and Lucas, 1995). In cultured mouse cortical neurons, NMDA receptor-dependent dendritic varicosity formation occurs during exposure to oxygen and glucose deprivation and can be reproduced within minutes of exposure to glutamate or NMDA (Bateman and Goldberg, 1992; Park et al., 1996). Interestingly, dendritic varicosities form even after brief sublethal excitotoxic exposure, and they resolve spontaneously within 1–2 hr (Park et al., 1996). Because increased intracellular calcium is a critical step of the excitotoxic injury cascade, we considered the hypothesis that calpain-mediated cytoskeletal proteolysis might be a central event leading to dendritic varicosity formation. Here we present observations that suggest calpain activation does not have a major role in formation of rapid dendritic injury. In contrast, calpain seems to be critical for spontaneous recovery after sublethal neuronal injury.
Preliminary reports have appeared in abstract form (Faddis and Goldberg, 1995; Meschia et al., 1995).

MATERIALS AND METHODS

Mouse cortical cell culture. Mouse neocortical neurons from gestational day 15 embryos were dissociated and plated on confluent astrocyte cultures at 2 weeks in vitro as described previously (Rose et al., 1993). Culture substrates included glass coverslips glued to the bottom of cutout 35 mm culture dishes (MatTek, Ashland, MA), which were coated with poly-d-lysine (5%, room temperature for 2 hr) and laminin (0.01 mg/ml, room temperature for 2 hr). Tissue culture-treated polystyrene 24-well plates (Nunc, Inc., Rochester, NY) were used also. Cells were plated at a density of 2–3 neocortex hemispheres per 10 cm plating media, which contained 5% horse serum, 5% fetal bovine serum, 200 mg/ml glutamine, 12.9 mm NaHCO₃, and 10 mm D-glucose in MEM. Cultures were maintained at 37°C with 5% CO₂. After 7 d in vitro (DIV), proliferation of non-neuronal cells was halted by treatment with 10 μM cytosine arabinoside for 1–3 d. Experimental procedures were conducted on cultures at 14–17 DIV, when the density of synaptic contacts was sufficient to produce an excitotoxic response to NMDA exposure.

NMDA exposure. All experimental pretreatments and treatments were conducted in a HEPES- and bicarbonate-buffered balanced salt solution (HBBSS), with the following components (in mM): NaCl (116), KCl (0.40), MgSO₄·7H₂O (0.80), NaH₂PO₄ (1.01), NaHCO₃ (25), HEPES (12), D-glucose (5.5), CaCl₂ (1.8), and phenol red, pH 7.4. After thorough mechanical shaking to remove serum-containing culture media, cells were exposed to wash conditions or to 5–50 μM NMDA (dissolved in HBBSS) for 10 min at room temperature. In some experiments, cultures were transferred to DMEM and returned to the 37°C culture incubator for 1–24 hr. Cultures were additionally exposed to the calpain inhibitors leupeptin (Sigma, St. Louis, MO), calpain inhibitors I and II (Sigma), or MDL28,170 (100 μM), which was prepared in DMSO. The final concentration of each vehicle was corrected for total protein content. Proteins samples (10 μg) were run on prestained 12.5% SDS-PAGE gels and transferred to nitrocellulose membranes, which were incubated in 5% nonfat milk for 1 hr and then incubated in 1% DMSO for 2 hr at room temperature or overnight at 4°C. After exposure to 30 μM NMDA for 10 min and fixed immediately, neurons showed the appearance of focal swellings or beads along the length of the dendrite (Fig. 1B). Distal portions of the dendrite more frequently exhibited varicosities than proximal portions, and varicosities occurred with a regular periodicity. Axons showed focal swelling along the length of the dendrite (Fig. 1A). Therefore, NMDA reactivity represents a useful, selective marker for active calpain during neuronal injury. Primary antibodies were washed off several times after being incubated with compressed PBS and replated with Cy3-conjugated goat antimouse or goat anti-rabbit IgG (Jackson Immunoresearch Labs, West Grove, PA) at 1:50–1:100 for 30–60 min.

RESULTS

NMDA-induced dendritic injury and recovery

In fixed cortical cultures, immunofluorescence using antibodies to the somatodendritic microtubule-associated protein MAP2 revealed neurons with round somata and an intricately branched dendritic arbor with smooth dendrites (Fig. 1A). Fluorescent labeling using a suspension of the membrane tracer DiI(C₁₈₃) in living or fixed cultures demonstrated a similar morphology, except that dendritic spines were readily visualized along the contour of the otherwise smooth dendrites, and axons could be observed arising from the soma or proximal dendrite. When cultures were exposed to 30 μM NMDA for 10 min and fixed immediately, neurons showed the appearance of focal swellings or beads along the length of the dendrite (Fig. 1B). Distal portions of the dendrite more frequently exhibited varicosities than proximal portions, and varicosities occurred with a regular periodicity. Axons did not show focal swelling. Although MAP2 immunofluorescence suggested the appearance of discrete fragmented dendrites, DiI labeling indicated that dendritic membranes remained contiguous. NMDA exposure was associated with a change in distribution of MAP2 immunoreactivity, which was decreased in distal dendrites and increased in neuronal somata and proximal dendrites. NMDA-induced varicosity formation of mouse cortical neurons occurred in a concentration-dependent manner (Fig. 3). A small background level of variance formation was evident even in control cultures exposed only to wash conditions. A 10 min exposure to NMDA at concentrations of 5 and 10 μM caused a
minimal increase in the amount of varicosity formation, whereas 30 and 50 \( \mu M \) NMDA resulted in near-maximal varicosity formation. NMDA-induced dendritic injury was blocked by addition of the selective NMDA receptor antagonist, 10 \( \mu M \) MK-801.

Dendritic varicosity formation was reversible if NMDA was removed after brief (10 min) exposure (Fig. 1C). NMDA exposure at these concentrations was not lethal: phase-contrast microscopy and assay for LDH efflux performed 24 hr after NMDA exposure showed no evidence of increased toxicity, as compared with wash control sister cultures. Cultured cortical neurons exposed to 30 \( \mu M \) NMDA for 10 min and washed back into normal balanced salt solution showed significant time-dependent recovery of dendritic shape (Fig. 5). The most significant portion of recovery occurred during the first 30–60 min, when the percentage of neurons with varicosities decreased from 90 to 20%. Recovery progressed almost to wash levels by the end of a 120 min postexposure period.

**Effects of calpain inhibitors on dendritic injury and recovery**

Calpain is expressed ubiquitously in central neurons and glia *in vivo* (Ivy et al., 1988; Perlmutter et al., 1988), but its distribution has not been characterized in murine cortical neuronal cultures. Immunofluorescence using antibodies to calpain I (\( \mu \)-calpain) revealed diffuse labeling of neuronal somata, excluding nuclei, with some reactivity in neurites (Fig. 2A,B). Antibodies to calpain II (m-calpain) also demonstrated fluorescence in neuronal cytoplasm and emphasized the presence of this isoform in neurites (Fig. 2C,D).

We used pharmacological inhibition of calpain to examine the hypothesis that NMDA-induced varicosity formation was mediated by calcium-dependent proteolysis. Dendritic injury was not reduced when sister cultures were treated during NMDA exposure with calpain inhibitors, including leupeptin and calpain inhibitors I and II (all 1–100 \( \mu M \); data not shown). A 2 hr pretreatment and cotreatment with the cell-permeant calpain inhibitor MDL28,170 (Mehdi, 1993) (1–100 \( \mu M \)) also had little effect on dendritic varicosity formation at most concentrations of NMDA (Fig. 3). MDL28,170 pre/cotreatment did result in a small, but statistically significant, reduction in varicosity formation in the 20 \( \mu M \) NMDA group (Fig. 3).

Surprisingly, addition of MDL28,170 during the postexposure period significantly reduced the rate of recovery from NMDA-induced varicosity formation. This effect was especially evident in cultures immunostained with antibodies to MAP2, because all dendrites were visualized (Fig. 4). In quantitative studies, the percentage of DIL-labeled neurons with varicosities in the MDL28,170 post-treatment group was approximately twice that of the non-MDL28,170 group at 30 and 120 min postexposure (Fig. 5). Additional studies showed that other inhibitors of calpain, including leupeptin and calpain inhibitors I and II (1–100 \( \mu M \)), also attenuated recovery from NMDA-induced varicosity forma-

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**Figure 1.** NMDA-induced varicosity formation and recovery in mouse cortical neurons. MAP2 immunofluorescence is shown for sister cortical cultures demonstrating the effects of the following treatments: A, wash control; B, exposure to 30 \( \mu M \) NMDA for 10 min, followed by immediate fixation; and C, 10 min exposure to NMDA, followed by recovery in normal culture medium for 90 min. NMDA exposure results in widespread appearance of dendritic varicosities, which resolve over the subsequent 30–120 min.

**Figure 2.** Calpain I and II immunoreactivity in cortical cultures. The distribution of calpain I (A, B) and II (C, D) was assessed by using antibodies specific for these two isoforms. Videomicrographs show phase-contrast (A, C) and fluorescence (B, D) images of the same fields in sister cultures. Immunofluorescence was detected in both neuronal somata and processes, although the calpain II fluorescence (D) was much weaker than that for calpain I (B). Camera exposure settings were not the same for these two images. Scale bar, 50 \( \mu m \).
The effect of the tested calpain inhibitors was concentration-dependent between 1 and 100 μM (Fig. 6). Some varicosity recovery remained attenuated even 48 hr (10 μM MDL28,170) after NMDA exposure; however, the varicosities that persisted were small and usually limited to the most distal portions of the dendritic arbor.

Although addition of calpain inhibitors delayed recovery of dendritic shape, they did not increase the amount of neuronal death even when incubated for 24–48 hr after NMDA exposure. There was no evidence that delayed recovery of dendritic shape reflected direct neurotoxicity of the calpain inhibitors. Two hour exposure to MDL28,170 alone did not cause varicosity formation or other morphological change in control sister cultures over the course of these experiments and did not produce neuronal death by the next day. Prolonged exposure to high concentrations (100 μM for 24–48 hr) of MDL28,170 caused a small number of varicosities and slightly increased neuronal death (<10%; data not shown).

Spectrin proteolysis during NMDA-induced varicosity formation and recovery

Because pharmacological inhibition of calpain was effective in blocking dendritic injury, but not initial dendritic injury, we sought specific evidence for calpain activity during and after NMDA exposure. Siman and colleagues recently have developed antibodies (Ab38 and Ab39) that recognize spectrin fragments specific for calpain-mediated proteolysis (Roberts-Lewis et al., 1994). Immunocytochemistry with Ab38 showed no increase over background signal with 10 min of NMDA exposure (Fig. 7A,B). Calpain-mediated spectrin proteolysis became evident in neuronal cell bodies and neurites during the subsequent 90 min recovery period (Fig. 7D–F). Western blot analysis with Ab38 revealed the appearance of a ~150 kDa band, consistent with calpain-specific spectrin breakdown product, between 30 and 90 min after 10 min NMDA exposure (Fig. 7G). This was not present in control cultures or in cultures harvested immediately after NMDA exposure (Fig. 7G). Post-treatment with 100 μM MDL28,170 blocked appearance of the spectrin breakdown product demonstrated by Western blots (Fig. 7G) and immunocytochemistry (data not shown). The delayed time course of calpain.
activation observed in these studies suggested that protein synthesis might be required for reversal of dendritic varicosities; however, pretreatment with the protein synthesis inhibitor cycloheximide did not delay dendritic recovery (our unpublished results).

DISCUSSION

Role of calpain in dendritic injury and recovery

Acute swelling of the neuronal soma and dendrites is a pathological hallmark of excessive glutamate receptor activation, or excitotoxicity (Olney, 1971). This process can be examined directly in neuronal cell cultures, with visualization of dendrites by DiI labeling or MAP2 immunofluorescence. In cultured cortical neurons, NMDA receptor activation produces rapid focal swelling, or varicosities, along the length of the dendritic arbor. These alterations in dendritic shape do not indicate lethal or irreversible neuronal damage. Although prolonged NMDA exposure is neurotoxic, brief sublethal exposure results in extensive dendritic beading that resolves spontaneously over 2 hr after agonist removal (Fig. 1; Park et al., 1996).

Intracellular calcium concentrations in neurons may be markedly elevated during intense glutamate receptor stimulation (MacDermott et al., 1986; Rajdev and Reynolds, 1993; Petrozzino et al., 1995; Hyrc et al., 1996). One consequence is activation of calcium-dependent protease, or calpain. Calpain is involved in cell death and in the formation of membrane blebs of several cell types, including hepatic and renal cells (Elliget et al., 1994; Miyoshi et al., 1996). Considering the wide distribution of calpain in the CNS, its activation by elevated intracellular calcium, and its ability to degrade cytoskeletal proteins, we felt that calpain activation in neurons might contribute to observed excitotoxic changes in dendritic structure. However, our results suggest that calpain activation does not play a significant role in formation of dendritic varicosities. Surprisingly, calpain activation seems to be required for restoration of dendritic structure.

Our study has two major observations. First, application of calpain inhibitors failed to alter dendritic swelling substantially during NMDA receptor activation. Some calpain inhibitors, such as leupeptin, are known to have relatively low potency and poor cell permeability, and it is possible that these agents did not reach sufficient intracellular concentrations. However, other inhibitors, including MDL28,170, have considerably greater cell permeability (Mehdi, 1991). Under our loading conditions, calpain inhibitors including MDL28,170 effectively blocked NMDA-induced proteolysis of calpain substrates, including MAP2 (Meschia et al., 1995) and spectrin (Fig. 7G). Therefore, the cell permeability and concentrations of these agents were sufficient to produce effective inhibition of calpain. In addition, during the 10 min period of dendritic varicosity formation, we did not observe a significant increase in immunoreactivity for the spectrin proteolytic fragment recognized by antibody Ab38. Because MDL28,170 did produce a small but statistically significant reduction of varicosity formation after application of 20 μM NMDA (Fig. 4), we cannot exclude a small contribution of calpain activation in this process. However, it seems likely that calpain activation does not play a large role in the formation of dendritic varicosities.

This study did not establish the mechanisms of dendritic swelling after NMDA receptor activation. Rapid dendritic shape changes may not require intracellular proteolysis. For example, NMDA application triggers depolymerization of neuronal microtubules, and pretreatment with microtubule-stabilizing compounds such as taxol prevent varicosity formation (Goldberg et al., 1994). These observations suggest a mechanism for dendritic varicosities involving calpain-independent disruption of the neuronal cytoskeleton.

Our second observation is that calpain inhibitors impeded spontaneous reversal of dendritic injury after NMDA exposure. Although the available inhibitors are not fully selective for calpain, there is evidence that calpain was specifically responsible for the observed effects on dendritic recovery. First, several structurally disparate calpain inhibitors yielded similar results. Leupeptin and calpain inhibitors I and II are relatively less specific, showing strong inhibition of cathepsins B and L, trypsin, and plasmin as well as calpain (Sasaki et al., 1990; Mehdi, 1991). Of the enzyme inhibitors used in this study, MDL28,170 demonstrates improved selectivity for calpain, although it too has approximately similar Ki values for calpain I (10 nm) and the lysosomal protease cathepsin B (25 nm) (Mehdi, 1991). The activity of cathepsin B is optimal at such acidic pH that it is unlikely to play a major role in our model. Second, a calpain-specific spectrin breakdown product appeared during the recovery period, but not during the immediate period of NMDA-mediated dendritic injury. The appearance of Ab38 immunoreactivity closely paralleled the process of dendritic recovery in several respects: (1) Ab38 reactivity occurred in a delayed time course (30–90 min) after NMDA application, (2) it appeared in a segmental pattern in dendrites (Fig. 7E–F), and (3) it was blocked by application of a calpain inhibitor (MDL28,170), which also blocked dendritic recovery (Fig. 7G). Together, these observations support the hypothesis that activation of intracellular calpain has a major role in remodeling neuronal structure after NMDA-induced focal dendritic injury.

How does calpain contribute to dendritic remodeling?

Calpain is unique in its capacity to cleave a large number of substrates involved in cellular physiology, including cell-surface
ion channels and receptors and intracellular mediators, including kinases, phosphatases, and transcription factors (see Saido et al., 1994). Among the most frequently considered targets of calpain degradation in neuronal injury are cytoskeletal proteins, suggesting that calpain contributes to dendritic shape changes by a process of cytoskeletal remodeling or disassembly. Although calcium-dependent cytoskeletal degradation frequently is considered detrimental to neurons, it also has been proposed as a mechanism leading to synaptic plasticity (Lynch and Baudry, 1984) and may contribute to membrane resealing after neurite transection (Schlaepfer and Bunge, 1973; Xie and Barrett, 1991). Calpain-mediated proteolysis might serve to remove aberrant cytoskeletal elements before structural repair.

The actin-binding protein brain spectrin, or fodrin, is a well-established cytoskeletal target for calpain proteolysis (Siman et al., 1984). Spectrin is a prominent component of the postsynaptic dendritic membrane (Ivy et al., 1988) that serves to anchor the cytoskeleton to integral cell membrane proteins. Extensive early observations by Siman, Baudry, Lynch, and coworkers established that glutamate receptor stimulation causes calpain activation and spectrin cleavage (Seubert et al., 1988; Siman and Noszek, 1988), leading to the proposal that calpain-mediated spectrin proteolysis might underlie structural changes leading to synaptic plasticity and long-term potentiation (Lynch and Baudry, 1984). Given the appearance of spectrin breakdown products in neurites during the time of dendritic remodeling, it is possible that a similar process occurs in our experiments. However, spectrin is only one of many cytoskeletal targets for calpain-mediated proteolysis. Other potential substrates include actin-binding proteins (such as α-actinin and talin), MAP2, tubulin, tau, and neurofilaments (see Goll et al., 1992; Saido et al., 1994). Therefore, the appearance of spectrin breakdown fragments should be viewed as a marker of calpain activity, rather than as a necessary component of dendritic remodeling. Calpain activity also can result in limited proteolysis of several noncytoskeletal proteins, including membrane proteins such as voltage-gated calcium channels (Hell et al., 1996), protein kinases such as protein kinase C and calcium–calmodulin-dependent kinase II (Kwiatkowski and King, 1989; Saido et al., 1994), and protein phosphatases, including calcineurin (Saido et al., 1994); calpain might mediate varicosity recovery via any of these substrates.

Calpain activation was first evident at 30 min after NMDA administration and is therefore a relatively late occurrence in these experiments. MAP2 or spectrin proteolysis is not required for varicosity formation, which can be observed as early as 3–5 min after NMDA application. The time course of calpain activation in our cell culture model may be slower than that sometimes observed during hypoxic–ischemic (Saido et al., 1993; Matesic and Lin, 1994; Roberts-Lewis et al., 1994; Blomgren et al., 1995) or traumatic (Taft et al., 1992; Kampfl et al., 1996) brain injury in vivo. It is possible that calpain expression in our embryonic cortical culture system differs from that of the adult rodent brain. Although the presence of calpain I and II isoforms in cortical cultures was established by immunocytochemistry (Fig. 2) and

![Figure 6](image_url)

Figure 6. Several calpain inhibitors effectively block varicosity recovery. Sister cultures were exposed to wash conditions (A), 30 μM NMDA for 10 min (B), or NMDA, followed by 90 min recovery (C). Other sister cultures were exposed to NMDA and then washed into medium containing various calpain inhibitors, including MDL28,170 (D), calpain inhibitor I (E), or leupeptin (F) for 90 min. All inhibitors were present at a final concentration of 10 μM. MAP2 immunofluorescence shows that all inhibitors attenuated recovery of dendritic shape, as compared with untreated cultures (C). Scale bar, 50 μm.
Cytoskeletal proteolysis was profound once initiated (Meschia et al., 1995), we cannot exclude the possibility that the remodeling role of calpain is more robust in embryonic than in adult tissue. Another explanation is that the present experiments used a low NMDA concentration ($30 \mu M$), which produces a low-grade insult and slower onset of calpain activation. Alternatively, calpain activation may depend on a delayed change in the balance of intracellular substances known to alter the threshold for calpain autolysis, including the endogenous inhibitor calpastatin, or positive modulators such as phospholipids (Goll et al., 1992; Saito et al., 1994).

### Significance of calpain-mediated dendritic remodeling

Our studies demonstrate that calpain activation in cultured neurons does not contribute significantly to rapid development of dendritic injury after sublethal glutamate receptor activation but plays a critical role in subsequent restoration of dendritic structure. In impeding dendritic recovery, calpain inhibitors might have lasting effects on the function of neurons that survive excitotoxic insults. These results do not conflict with studies demonstrating a protective action of calpain inhibitors in the setting of *lethal* neuronal injury from cerebral ischemia or trauma. However, they

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**Figure 7.** Calpain-specific spectrin breakdown during recovery. Sister cultures were exposed to wash conditions (A) or to $30 \mu M$ of NMDA for 10 min, followed by recovery periods of 0 (B), 30 (C), 60 (D), 90 (E), and 120 (F) min. Immunofluorescence of the calpain-specific spectrin breakdown product Ab38 is readily detected at 90 and 120 min but is not appreciable at time points earlier than 60 min. In similar experiments, immunoblot analysis (G) using the same antibody demonstrates a similar time course of spectrin breakdown. Spectrin breakdown product was not detected in sister cultures treated with the calpain inhibitor MDL28,170 ($100 \mu M$) for 90 min after NMDA exposure. Scale bar, 50 $\mu M$. 
suggest that it may be wise to consider beneficial as well as detrimental effects of calpain activation in neuronal injury. It will be interesting to explore possible roles of calpain in nervous system development and aging, other settings in which dendritic remodelling shapes neuronal function.

REFERENCES


