Reduced Voltage-Dependent Ca\textsuperscript{2+} Signaling in CA1 Neurons After Brief Ischemia in Gerbils

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Connor, J. A., S. Razani-Boroujerdi, A. C. Greenwood, R. J. Cormier, J. J. Petrozzino, and R.C.S. Lin. Reduced voltage-dependent Ca\textsuperscript{2+} signaling in CA1 neurons after brief ischemia in gerbils. J. Neurophysiol. 81: 299–306, 1999. An initial overload of intracellular Ca\textsuperscript{2+} plays a critical role in the delayed death of hippocampal CA1 neurons that die a few days after transient ischemia. Without direct evidence, the prevailing hypothesis has been that Ca\textsuperscript{2+} overload may recur until cell death. Here, we report the first measurements of intracellular Ca\textsuperscript{2+} in living CA1 neurons within brain slices prepared 1, 2, and 3 days after transient (5 min) ischemia. With no sign of ongoing Ca\textsuperscript{2+} overload, voltage-dependent Ca\textsuperscript{2+} transients were actually reduced after 2–3 days of reperfusion. Resting Ca\textsuperscript{2+} levels and recovery rate after loading were similar to neurons receiving no ischemic insult. The tetradotoxin-insensitive Ca spike, normally generated by these neurons, was absent at 2 days posts ischemia, as was a large fraction of Ca\textsuperscript{2+} dependent spike train adaptation. These surprising findings may lead to a new perspective on delayed neuronal death and intervention.

INTRODUCTION

The degeneration and death of CA1 pyramidal neurons a few days after a transient insult in human stroke (Horn and Schlote 1992; Petito et al. 1987) and in rodent ischemia models (Crain et al. 1988; Kirino 1982; Kirino and Sano 1984; Pulzinski et al. 1982) were hypothesized to follow from Ca\textsuperscript{2+} overload that persists from the time of the insult until the time of death (Siesjo and Bengtsson 1989). However, this hypothesis has received its best support from evidence limited to the period of ischemia and the following day. For example, Ca\textsuperscript{2+} influxes (Andine et al. 1988, 1992) and cytoplasmic Ca\textsuperscript{2+} (Silver and Erecinska 1990, 1992) were elevated during transient ischemia and immediately after reperfusion. A variety of drugs that would be expected to limit damage from a Ca\textsuperscript{2+} overload were neuroprotective when present during ischemia (Gill and Lodge 1997; Hunter 1997; Small and Buchan 1997). Also, when CA1 neurons in vivo were briefly aided by an agent likely to reduce Ca\textsuperscript{2+} entry by blocking a subset of glutamate receptors or Ca\textsuperscript{2+} channels during day 1 after ischemia, many cells were able to overcome whatever might otherwise kill them (Sheardown et al. 1993; Tsuda et al. 1991; Valentino et al. 1993).

In contrast, little is known about more delayed effects of ischemia on Ca\textsuperscript{2+} homeostasis, except by inference from electrophysiological measurements from neurons that showed depolarized and unstable resting potentials in post-ischemic slice experiments (Kirino et al. 1992; Tsukobawa et al. 1992, 1994). Autoradiographic evidence of Ca\textsuperscript{2+} accumulation in postischemic CA1 neurons (Dienel 1984) was confirmed in single cells with Ca\textsuperscript{2+} staining that correlated so closely with morphological death markers as to imply that Ca\textsuperscript{2+} accumulation did not appreciably precede death (Bonnekoh et al. 1992). Also, not until day 3 after bilateral carotid occlusion in gerbils was high-resolution imaging of the Ca\textsuperscript{2+}-indicator fura-2 able to detect enhanced Ca\textsuperscript{2+} influx through α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors that were believed to lack the GluR2 subunit (Gorter et al. 1997).

Here we report a striking reduction in the magnitude of Ca\textsuperscript{2+} transients mediated by voltage-gated Ca\textsuperscript{2+} entry in CA1 neurons 2 and 3 days after a 5-min ischemic insult in gerbils. Basal Ca\textsuperscript{2+} levels were found to be normal to subnormal. Concurring results were obtained with micro-electrode-injected fura-2, noninvasively loaded fura-2/AM, and electrophysiology without fura-2. Our data are most consistent with a direct reduction of voltage-gated Ca\textsuperscript{2+} channel (VGCC) activity, although they cannot rule out the contribution of a possible increase in Ca\textsuperscript{2+} buffering. Preliminary reports were published as abstracts (Connor et al. 1996; Razani-Boroujerdi et al. 1997).

METHODS

Adult male mongolian gerbils were anesthetized with 1.5–2% halothane, kept at 37–38°C rectal temperature with a heating pad and lamp, and subjected to 5 min of either unilateral or bilateral carotid occlusion with aneurysm clamps (Lin et al. 1990). In seven of the animals with bilateral occlusion that were studied, temperature in the temporalis muscle was also monitored and shown not to drop below 36°C. In addition to the populations used for physiological studies, the brains of an additional 10 postischemic gerbils (bilateral occlusions) were removed and fixed 10 days after occlusion, and sections (80–100 μm) were stained with cresyl violet to assess neuronal loss.

The unilateral occlusion protocol made it possible to use slices from the contralateral hemisphere (nonischemic) as within experiment controls and was employed where the time course of postischemic changes was analyzed. No differences in the parameters measured in this study were observed in neurons from the nonischemic, contralateral, hemisphere, and neurons from controls or sham-operated animals. After the experiments, each slice from the ipsilateral hemisphere that was studied was fixed and examined, after MAP-2 immuno-labeling, for the presence of beaded dendritic morphology, as an indicator of whether effective ischemic conditions had
been established (Matesic and Lin 1994). Only cells from slices where this beading occurred or where injected biocytin showed a dendritic beading pattern were included in the analysis. We observed MAP-2 dendritic beading in ~50% of slices 2 or 3 days after the surgery consistent with the ~60% efficacy of a 10 min unilateral protocol (Gill et al. 1987).

For Ca\(^{2+}\) and electrophysiological measurements, gerbils from the control population and 1–3 days after ischemia were anesthetized by ketamine/xylazine injection (40/5 mg/Kg), decapitated, and coronal hemispheric slices (400 μm thick) were cut with the use of a vibrotome. Slices were incubated for ≥1.5 h at 25°C in the following solution (in mM): 126 NaCl, 3 KCl, 1.25 NaHPO\(_4\), 1 MgSO\(_4\), 26 NaHCO\(_3\), 2 CaCl\(_2\), and 10 dextrose, and then they were gassed with a mixture of 95% O\(_2\)/5% CO\(_2\). Experiments were run at both 23–25°C and at 32°C, with no differences in outcomes. Micropipettes were tip-filled with 10 μM fura-2 in 0.5 mM KAcetate and sometimes 2% biocytin. They were then back-filled with 3 M KCl/1 M KAcetate. To limit impalement quality as a confounding variable, we selected cells with input resistance >50 MΩ and steady resting membrane potentials more negative than ~60 mV. Holding current was adjusted to maintain the potential between ~65 and ~75 mV. After experiments in which biocytin was injected, slices were fixed in 3.5% paraformaldehyde in 0.1 M phosphate buffer for 3–6 h, cryoprotected by addition of 20% sucrose for 3–6 h, and resectioned at 40–60 μm with a freezing microtome. Sections were processed with the avidin:biotinylated enzyme complex (ABC) method (Vector kit) for photomicroscopy.

Ester loading of fura-2 followed the basic procedure of Regehr and Tank (1991). A large bore micropipette filled with fura/2-AM:dimethyl sulfoxide (DMSO) (10 μM/0.3%) was positioned in the alveolus and pressure pulses (1 Hz, 0.5 duty cycle) applied for ≥15 min. Basal dendrites of CA1 neurons steadily accumulated fura-2, which diffused to cell bodies and apical dendrites. This positioning of the pipette allowed measurements to be made in the cell body layer distant from the loading site. Calcium measurements were made by ratio imaging of fura-2 (Gryniewicz et al. 1985), with the use of 350/380 nm excitation, an upright microscope, and a cooled frame-transfer CCD-camera system (Petrozzi et al. 1995).

**RESULTS**

Ischemia was induced in vivo with the use of either bilateral and unilateral carotid occlusion protocols to exploit different advantages. After 5 min of bilateral ischemia, the extent and time course of cell death was well established (Crain et al. 1982; Kirino 1982; Kirino and Sano 1984; Pulsinelli et al. 1982). Consistent with these studies, we observed pyramidal cell loss, exceeding 90%, in area CA1 of both hemispheres in 8 of 10 brains fixed 10 days postischemia, with two animals showing near total loss in only one hemisphere. At 2 days postischemia however, cell loss, as detected by toluidine blue staining, was very low (see also Gorter et al. 1997). Also, the degree of difficulty in making and maintaining microelectrode penetrations was judged to be similar in slices from control and postischemic brains.

Figure 1A shows Ca\(^{2+}\) increases during spike trains driven by 1-s injections of current into the somata of CA1 neurons in slices from control gerbils and postischemic gerbils (2 days after bilateral ischemia). Stimulus current levels were adjusted between 0.5 and 0.6 nA to fire 15–18 spikes in control cells (n = 6) and 16–20 spikes in the postischemic cells (n = 6). For each cell, the time course of the Ca\(^{2+}\) change was determined in the most responsive region of primary dendrite, which in this stimulus protocol exhibits the largest change in the cell (Jaffe et al. 1994). As can be seen, the Ca\(^{2+}\) increase elicited by the spike trains was much smaller in the postischemic neurons as compared with the controls, ~8-fold smaller peak on average, even though the postischemic neurons fired more spikes. Correspondingly smaller Ca\(^{2+}\) signals were seen in all parts of the dendritic tree and in the soma. Instead of being elevated, resting Ca\(^{2+}\) levels in postischemic cells were about the same as those in normal neurons (see also Table 1). Exemplar spike trains (300 ms) from control and postischemic neurons are shown in Fig. 1A, right. A stimulus current of 0.5 nA was applied to both neurons. It generally required slightly less stimulus current to fire equal numbers of spikes in postischemic neurons than in controls because of lessened adaptation in the postischemic neurons.

We hypothesize that the Ca\(^{2+}\) transients in Fig. 1A arose primarily from VGCCs opened during the depolarization-induced spike trains. This interpretation is supported by reports that internal Ca\(^{2+}\) release does not add significantly to Ca\(^{2+}\) signals from single spikes (Markram et al. 1995) or to Ca\(^{2+}\) signals up to 500 nM evoked by hundreds of milliseconds of depolarization (Garaschuk et al. 1997; Petrozzino and Connor unpublished observations).

The above data leave open the possibility that CA1 neurons could achieve normal Ca\(^{2+}\) increases on day 2 after ischemia, if sufficient current were injected. Figure 1B argues against this possibility with a time course summary of near-maximal dendritic Ca\(^{2+}\) signals that we induced by injecting current (3 nA for 4.5 s) into contra- and ipsilateral CA1 cells on days 1, 2, and 3 after unilateral ischemia. In each case, Ca\(^{2+}\) increased during an initial train of spikes and then settled back while the potential hovered at approximately ~20 mV. It is important to note that there was no significant difference between pooled contralateral controls and day 1 postischemic cells (n = 11 and 4, respectively). Therefore, a progressive decline in peak dendritic Ca\(^{2+}\) signals on postischemic days 2 and 3 (P < 0.02, n = 6 and 6, respectively) was observed only in the slices that showed dendritic beading as assessed by MAP-2 or biocytin staining.

On day 3, the mean Ca\(^{2+}\) transient peaked at only ~45% of the control peak (Fig. 1B). In the soma, the progressive decline in the size of Ca\(^{2+}\) transients on days 2 and 3 was similar. At day 2, the maximum soma increase was 51% of control in this same group of neurons (P < 0.02, n = 6 and 6, respectively).

To extend this result to bilateral ischemia, we made a similar comparison between six CA1 neurons from four unoperated gerbils and six cells from five gerbils 2 days after bilateral ischemia. At 2 days, the peak value of the mean dendritic Ca\(^{2+}\) transient elicited by a 2-s 2-nA current pulse was 57% of the control value (P < 0.05, data not shown). With either occlusion operation, the greatest contrast in Ca\(^{2+}\) signaling between controls and ischemics was observed using action potential trains as the stimulus rather than maintained depolarization.

Two zinc-related aspects of these data are briefly reported here, whereas the important role of zinc in ischemia (Koh et al. 1996) is discussed later. First, it is unlikely that zinc impeded Ca\(^{2+}\) flux through VGCCs as in rat heart myocytes (Winagar and Lansman 1990) because Ca\(^{2+}\) signals were unaffected on day 1 when the extracellular zinc should have
FIG. 1. **A:** comparison showing the strong decrement of maximum dendritic Ca\(^{2+}\) transients elicited by action potential trains fired by 1-s current injections in normal CA1 neurons (control) and neurons studied 2 days after bilateral ischemia. Measurements were made in a small region of the apical dendrite showing maximal excursions (Fig. 2 illustrates typical region with white rectangle). Mean resting Ca\(^{2+}\) levels were nearly the same (see also Table 1). Action potential trains were very similar. Right: initial 300 ms of such spike trains in each group. For each time point, the mean [Ca\(^{2+}\)] ± SE is plotted for each group (n = 6). Horizontal bar denotes time of stimulus. R denotes recovery level, measured at ~3nts s.

**B:** Ca\(^{2+}\) responses to a "maximal" current pulse (3 nA, 4.5 s, denoted by horizontal bar) showing the evolution of the Ca\(^{2+}\) response decrement, 1–3 days following unilateral occlusions. Depolarizing current evoked a short train of high-frequency spikes followed by sustained depolarization to ~−20 mV. Response at day 1 postischemia was nearly the same as in contralateral (no ischemia) neurons (n = 6, each group). C: Ca\(^{2+}\) measurements emphasizing recovery time course after loading. Depolarizing (1 s) current injections were given to each group, maximum amplitude for the postischemic neurons, with amplitude reduced for the controls to give a matched peak Ca\(^{2+}\) excursion (n = 6).

been most concentrated. Second, in our data the dependence of fura-2’s excitation spectrum on zinc concentration (Atar et al. 1995) limits free cytosolic zinc to <1 nM, as is consistent with intracellular binding and compartmentalization of zinc (Hirayama 1990; Palmiter et al. 1996).

We saw no effect of ischemic insult on Ca\(^{2+}\) recovery time courses after stimulation. To make this point explicit, Fig. 1C compares the average time course of recovery from similar dendritic Ca\(^{2+}\) loads, 3 days after effective unilateral occlusions and in contralateral control cells (n = 6 and 6, respectively). Enough current was injected for 1 s to bring the peak Ca\(^{2+}\) signal to 350–400 nM, which required more current (depolarization) after ischemia. It is clear that the recovery time courses were similar. Thus Fig. 1 shows that putatively VGCC-mediated Ca\(^{2+}\) transients were markedly reduced after ischemia with no detectable impairment of recovery mechanisms.

One might worry that these unexpected results came perhaps from the small minority of postischemic cells (5–10%) that survived the ischemic insult and that could therefore withstand implalement. We addressed this concern in two ways. First, we coinjected biocytin along with fura-2 into 19 cells, and after Ca\(^{2+}\) imaging experiments examined their morphology (see METHODS). Figure 2 shows images of a CA1 neuron made from fura-2 fluorescence in the live cell (left) and biocytin staining (middle) in the fixed cell. Two
a typical fluorescent image from a control slice. The somata clear spikes in TTX that resembled reported Ca$^{2+}$ transient patterns. The biocytin picture at higher magnification to better show beading, characteristic of early stages of degeneration, was nonetheless significant (Fig. 2C).

Ratio signals were not converted to [Ca$^{2+}$] because of two limitations of the fura-2/AM method. First, nonspecific loading leads to background from out-of-focus cells, which may include damaged high Ca$^{2+}$ cells near the slice surface. Second, the fluorescent signal includes an unquantifiable component from the unconverted ester form of fura-2/AM, which does not bind Ca$^{2+}$. These two limitations of the method would tend to lessen the observed difference between Ca$^{2+}$ transients in postischemics and controls, which was nonetheless significant (Fig. 2C).

To corroborate these Ca$^{2+}$ concentration measurements, we examined neurons for changes in Ca$^{2+}$-dependent electrogensis. It is well established that CA1 neurons are capable of generating modified action potentials after block of Na channel by tetrodotoxin (TTX), and that the inward current carrier for the action potentials is Ca$^{2+}$ (Schwartzkroin and Slawsky 1977; Wong et al. 1979).

Figure 4A compares firing patterns before (left) and after (right) the addition of TTX (2 μM) to solution superfusing control (top) and a postischemic slice (bottom). Note that in normal saline the same stimulus current generates similar firing in the control and postischemic neuron. In eight of eight control neurons, increasing the stimulus current elicited clear spikes in TTX that resembled reported Ca$^{2+}$ spikes (36.14 ± 4.4 mV amplitude and 13.4 ± 3.8 ms duration, mean ± SE). However, in the seven of seven neurons from four gerbils studied 2 days after bilateral ischemia, we could not elicit recognizable spike activity in TTX, despite increasing the stimulus current to very large values (e.g., Fig. 4A, bottom right).

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**TABLE 1. Comparison of electrical properties in control and post-ischemic neurons**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 7)</th>
<th>3 days after unilateral occlusion (n = 7)</th>
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<tbody>
<tr>
<td>sAHP</td>
<td>9.3 ± 0.7 mV</td>
<td>6.5 ± 1.0 mV (P &lt; 0.05)</td>
</tr>
<tr>
<td>Rest Potential</td>
<td>−65.2 ± 1.7 mV</td>
<td>−67.9 ± 2.9 mV (P &lt; 0.05)</td>
</tr>
<tr>
<td>Dendritic Ca$^{2+}$</td>
<td>84 ± 10 nM</td>
<td>70 ± 13 nM (n = 11)</td>
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sAHPs are reduced in affected CA1 neurons 3 days after unilateral occlusion despite normal resting levels of dendritic Ca$^{2+}$ and membrane potential. The sAHP after a 1-sec 0.5 nA current pulse was significantly reduced on day 3 compared to controls (P < 0.05, Student’s unpaired t-test). The trend toward low resting Ca$^{2+}$ levels after ischemia was not significant (P = 0.41). Measurements of resting potential and Ca$^{2+}$ outnumbers show regions of bleb formation and the addition of TTX (2 μM) to solution superfusing a control (top) and a postischemic slice (bottom). Note that in normal saline the same stimulus current generates similar firing in the control and postischemic neuron. In eight of eight control neurons, increasing the stimulus current elicited clear spikes in TTX that resembled reported Ca$^{2+}$ spikes (36.14 ± 4.4 mV amplitude and 13.4 ± 3.8 ms duration, mean ± SE). However, in the seven of seven neurons from four gerbils studied 2 days after bilateral ischemia, we could not elicit recognizable spike activity in TTX, despite increasing the stimulus current to very large values (e.g., Fig. 4A, bottom right).
This result supports the idea that the spike/depolarization-mediated Ca\(^2+\) transients were reduced after ischemia by a direct effect on VGCCs. First, it is implausible that enhanced K\(^+\) currents could prevent normal VGCCs from generating Ca\(^2+\) spikes given the large depolarizations achieved and the fact that the repetitive firing pattern in normal saline suggested normal or subnormal K currents. Second, Ca\(^2+\) spikes would be minimally affected by putative changes in postischemic Ca\(^2+\) buffering or sequestration that might be invoked to explain, albeit implausibly given the normal recovery shown in Fig. 1C, the reduced fura-2 signals. We used the Ca\(^2+\) spike as a measure of Ca\(^2+\) influx because it is impossible in intact CA1 neurons to obtain voltage-clamp conditions necessary for accurate Ca\(^2+\) current measurements.

There were also other changes in Ca\(^2+\)-related electrophysiology of postischemic CA1 cells. Spike train adaptation, which progressively lowers firing rate during depolarization, and the slow afterhyperpolarization (sAHP) that follows depolarization were markedly reduced. The sAHP is attributed to the Ca-dependent K\(^+\) current, I\(_{\text{Ca}}\), whereas adaptation derives from a mixture of K\(^+\) currents with and without Ca-dependence (Hotson and Prince 1980). Table 1 summarizes data showing the reduction in sAHP on days 2 and 3 after unilateral ischemia, whereas Fig. 4B shows reduced spike-frequency adaptation on day 3 after unilateral ischemia compared with controls. The population data of Fig. 4C were generated by measuring the first five interspike intervals in trains elicited by 0.5 nA of injected current. Intervals two through five were expressed as percentages of the first spike interval for each cell before the group mean for each interval was calculated and plotted, the day 2 cells showing much less adaptation (\(P < 0.05\)). Because adaptation was reduced, the postischemic cells fired more spikes for a given current (as in Fig. 1, A and B) and were more excitable in this sense. However, these more numerous spikes in postischemic cells produced much smaller Ca\(^2+\) increases. We also observed a marked reduction in the broadening of successive spikes during the trains evoked on day 2 after bilateral ischemia, relative to controls (data not shown).

DISCUSSION

We report the first measurements of intracellular Ca\(^2+\) homeostasis in living CA1 cells prepared 1–3 days after brief ischemia in vivo. In electrophysiologically viable cells and in cells loaded noninvasively with fura-2/AM, our data give no support to the prevailing idea of chronic postischemic Ca\(^2+\) overload during this extended period. However, we do not question at all that large, acute loads of Ca occur during the ischemic episode and perhaps for a short period thereafter (Silver and Erecinska 1990, 1992). The time course and extent of these acute loads in response to in vitro models of ischemia (Lobner and Lipton 1993) or to prolonged glutamate exposure in vitro (Connor et al. 1988; Randall and Thayer 1992; Wadman and Connor 1992; Weiss et al. 1993) also were studied extensively.

We surmise that earlier ex-vivo electrophysiological support for the Ca\(^2+\) overload hypothesis may have stemmed, at least in part, from the inclusion of acutely compromised cells in the analysis (Kirino et al. 1992; Tsubokawa et al. 1992, 1994). These studies deliberately avoided viability criteria to study pathophysiology. There has also been a strong tendency to extrapolate from neuronal tissue culture studies where, in most cases, death occurs within a few hours (not a few days) of ischemic or excitotoxic insult and is well correlated with increased intracellular Ca\(^2+\) (Choi 1994; Dubinsky 1993a; Randall and Thayer 1992). In contrast, our experiments were designed to examine neurons well before this final, probably irreversible stage. The observation of reduced VGCC-mediated Ca\(^2+\) signaling in the noninvasively loaded cells implies that the viability criteria in our microelectrode experiments served only to eliminate phenomena stemming from impalement and did not select for an unhealthy healthy subpopulation of cells. In addition to the fura-2/AM results, two other considerations argue against the possibility that reduced Ca\(^2+\) signaling might be preferentially associated with destined survival instead of death. First, before selection by impalement and viability criteria, cells had a 90–95% probability of dying in a few days (Bonnehok et al. 1990; Crain et al. 1988). Second, impaled cells showed morphological indications of degeneration, dendritic beading (Fig. 2).

Thus our results are consistent with the view that accumulation of intracellular Ca\(^2+\) does not appreciably precede cell death in CA1 cells that are dying after brief ischemia (Bonnehok et al. 1992), leaving aside the initial Ca\(^2+\) transient associated with the ischemic insult. Also consistent with this view was the observation of prolonged normal resting levels of Ca\(^2+\) before delayed excitotoxic death in neuronal/glial cocultures (Dubinsky 1993b), despite the
nearly constant frequency, leading to a 110 nM peak Ca$^{2+}$ bottom after the pulse. The postischemic cell (top) leading to a 850 nM peak Ca$^{2+}$.

In loading intracellular IP3-sensitive Ca$^{2+}$ stores in whole cell, patched, CA1 cells (Garaschuk et al. 1997; Jaffe and Andine 1996) have been shown to play a key role in the second insufficiency that would normally generate Ca$^{2+}$ levels. In light of our results, GluR2 down-regulation may be a compensatory mechanism that does not work after an insult as severe as 5 min of total ischemia. In any event, the new results reported here stand alone in suggesting that experiments elevating Ca$^{2+}$ levels in CA1 neurons during a wide postischemic window could lead eventually to the development of a late clinical intervention in delayed neuronal death after ischemia.

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Chalmers-Redman, R. M., Fraser, A. D., Ju, W. Y., Wadda, J., Tatton, Brown 1994). These IP3-sensitive stores include the rough endoplasmic reticulum and nuclear envelope (Malviya et al. 1990; Parys et al. 1992; Stehno-Bittel et al. 1995a). Second, the chronic, near-complete impairment of protein synthesis observed after ischemia (Thilmann et al. 1986) may be maintained by depressed cytoplasmic and nuclear Ca$^{2+}$ levels, which could dysregulate mRNA transcription (Ghosh and Greenberg 1995; Hardingham et al. 1997) and reduce traffic through nuclear pores (Greber and Gerace 1995; Perez-Terzic et al. 1996; Stehno-Bittel et al. 1995b). Finally, there is evidence that reduced Ca$^{2+}$ influx can lead to apoptotic mechanisms (Franklin and Johnson Em 1992; Gallo et al. 1987; Koh and Cotman 1992), which may contribute to postischemic cell death in a manner complicated by the dependence of apoptosis on protein synthesis (Chalmers-Redman et al. 1997; Choi 1995).

Of the postischemic changes in gene expression in which VGCC activity may play a role (Kogure and Kato 1993), one especially warrants discussion in relation to our results. mRNA for the GluR2 glutamate-receptor subunit was progressively down-regulated after ischemia (Pellegrini-Giampietro et al. 1992; Pollard et al. 1993), leading to increased Ca$^{2+}$ influxes through AMPA-receptor channels on and near the soma on day 3 and not before (Gorter et al. 1997). In light of our results, GluR2 down-regulation may be a compensatory mechanism that does not work after an insult as severe as 5 min of total ischemia. In any event, the new results reported here stand alone in suggesting that experiments elevating Ca$^{2+}$ levels in CA1 neurons during a wide postischemic window could lead eventually to the development of a late clinical intervention in delayed neuronal death after ischemia.


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