Imaging Anoxic Depolarization During Ischemia-Like Conditions in the Mouse Hemi-Brain Slice

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Joshi, I. and R. D. Andrew. Imaging anoxic depolarization during ischemia-like conditions in the mouse hemi-brain slice. J Neurophysiol 85: 414–424, 2001. Focal ischemia evokes a sudden loss of membrane potential in neurons and glia of the ischemic core termed the anoxic depolarization (AD). In metabolically compromised regions with partial blood flow, peri-infarct depolarizations (PIDs) further drain energy reserves, promoting acute and delayed neuronal damage. Visualizing and quantifying the AD and PIDs and their acute deleterious effects are difficult in the intact animal. In the present study, we imaged intrinsic optical signals to measure changes in light transmittance in the mouse coronal hemi-brain slice during AD generation. The AD was induced by oxygen/glucose deprivation (OGD) or by ouabain exposure. Potential neuroprotective strategies using glutamate receptor antagonists or reduced temperature were tested. Eight minutes of OGD (n = 18 slices) or 4 min of 100 μM ouabain (n = 14) induced a focal increase of increased light transmittance (LT) in neocortical layers II/III that expanded concentrically to form a wave front coursing through neocortex and independently through striatum. The front was coincident with a negative voltage shift in extracellular potential. Wherever the LT front (denoting cell swelling) propagated, a decrease in LT (denoting dendritic beading) followed in its wake. In addition the evoked field potential was permanently lost, indicating neuronal damage. Glutamate receptor antagonists did not block the onset and propagation of AD or the extent of irreversible damage post-AD. Lowering temperature to 25–30°C protected the tissue from OGD damage by inhibiting AD onset. This study shows that anoxic depolarization evoked by global ischemia-like conditions is a spreading process that is focally initiated at multiple sites in cortical and subcortical gray. The combined energy demands of O2/glucose deprivation and the AD greatly exacerbate neuronal damage. Glutamate receptor antagonists neither block the AD in the ischemic core nor, we propose, block recurrent PID arising close to the core.

INTRODUCTION

Classic spreading depression (SD) is a migrating inactivation of gray matter first described by Leao (1944) that can be focally induced by mechanical, electrical, or chemical stimulation under normal metabolic conditions. The propagating wave of depolarization and associated electrical silence traverses the cerebral cortex at 2–5 mm/min. SD is responsible for migraine aura, commonly a marching visual or somatosensory deficit that may precede migraine pain (Lauritzen 1994). Although SD involves the mass depolarization of neurons and glia for a minute or longer, no neuronal damage results (Nedergaard and Hansen 1988).

Leao (1947) first observed that a propagating depolarization similar to normoxic SD arises in the cerebral cortex following 2–5 min of global ischemia, the depolarization lasting as long as the ischemic period. Electrophysiologists commonly use the term “anoxic” (Bures et al. 1974, Ch. 4) or “hypoxic” depolarization, although this downplays what may be initially a spreading event. In the hippocampal slice preparation globally deprived of O2 and glucose, we have imaged a SD-like wave of depolarization which we previously termed “ischemic” SD (Obeidat and Andrew 1998; Obeidat et al. 2000) but might also be considered as representing anoxic depolarization (AD).

With focal ischemia, arterial occlusion induces a depolarization that is maintained in what becomes the ischemic core. Recurring peri-infarct depolarization (PID) arises at the border of the ischemic core during the first 3–4 h poststroke (Back et al. 1994; Nallet et al. 1999; Nedergaard and Astrup 1986; Rother et al. 1996; Strong et al. 1996). PID propagation into regions adjacent to the core expands the damage (Back et al. 1994; Dietrich et al. 1994; Dijkstra et al. 1999; Hossman et al. 1996; Nedergaard 1996). A PID may continue into uncompromised gray matter as SD where repolarization is swift and metabolism is normal, so no damage arises. The gradient from a region of high to low ischemia undoubtedly alters the physiochemical properties of the spreading signal. Thus N-methyl-o-aspartate (NMDA) receptor antagonists can block SD in normoxic tissue but not the AD in ischemic tissue as shown in several intact animal studies (Hernandez-Caceres et al. 1987; Koroleva et al. 1998; Lauritzen and Hansen 1992; Marrannes et al. 1988; Nedergaard and Hansen 1988; Nelligan and Wiechlo 1992). Likewise in rat brain slices, NMDA receptor antagonists block normoxic SD (Anderson et al. 1999) and hypoxic SD (Radek and Giardina 1992, although see Aitken et al. 1988) but not the AD evoked by lowering both O2 and glucose (Obeidat et al. 2000).

Intrinsic optical signals (IOSs) represent change in the way directed light is absorbed or scattered by biological tissue. IOSs can be detected by either collecting the light transmitted through a brain slice or by measuring the light reflected from brain tissue (Aitken et al. 1998, 1999; Andrew et al. 1999; Villringer and Chance 1997). In the present study, IOSs were imaged by analyzing light transmittance (LT) through submerged tissue slices. Elevated LT is associated with cell swelling as shown by osmotic studies (Andrew and MacVicar 1994; Kreisman et al. 1995), measurement of increased tissue resis-

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tance (Andrew et al. 1996, 1997), and measurement of decreased extracellular volume (Holthoff and Witte 1996). Brain cell swelling at the spreading depression front (Jing et al. 1993) can be imaged as it propagates through the hippocampal slice (Aitken et al. 1998; Basarsky et al. 1998; Kreisman et al. 2000; Obeidat and Andrew 1998). In addition, acute neuronal damage can be assessed in the submerged brain slice by measuring a delayed reduction in light transmittance caused by dendritic beading (Jarvis et al. 1999; Obeidat et al. 2000; Polischuk et al. 1998).

The neocortex and the striatum in the intact animal are highly susceptible to damage following stroke and both regions generate recurrent PIDs (Amemori and Bures 1990; Bures et al. 1974; Ch. 6). Mass cellular depolarization in the form of AD and PIDs promotes stroke damage but detailed study is difficult in the intact animal immediately following stroke onset. IOSs imaged in the mouse striatum and the overlying neocortex in the present study permitted a direct analysis. There were three objectives to the following study: first to study the characteristics of the signal induced under the metabolically compromised conditions of oxygen/glucose deprivation (Taylor et al. 1999) or ouabain exposure (Balestrino et al. 1999) by analyzing intrinsic optical signals and the extracellular field potential; second, to examine the role of glutamate receptors in the generation of this signal; and third, to study if lowering temperature may be neuroprotective by inhibiting AD onset during ischemia-like conditions (Morris et al. 1991).

METHODS

Mouse slice preparation

Male C57 black mice (21–30 days) were decapitated using a guillotine. The brain was quickly removed and placed in cold, oxygenated (95% O2-5% CO2) artificial cerebrospinal fluid (ACSF). The ACSF was prepared by dissolving in double-distilled water (in mM) 120 NaCl, 3.3 KCl, 26 NaHCO3, 1.3 MgSO4, 1.23 NaH2PO4, 11 d-glucose, and 1.8 CaCl2. The ACSF osmolality was 292 ± 2 mOsm and pH was 7.3–7.4. In oxygen/glucose deprivation (OGD) studies, Ni replaced O2 and the glucose concentration was reduced from 11 to 1 mM. NaCl was added to the ACSF to balance slice 5-mOsm and pH was 7.3–7.4. In oxygen/glucose deprivation (OGD) studies, Ni replaced O2 and the glucose concentration was reduced from 11 to 1 mM. NaCl was added to the ACSF to balance osmolality. NMDA, AMPA, quinolinic acid, and ouabain were ob-

Electrophysiological recordings

To measure the evoked field potential or the spontaneous negative shift, a micropipette (5–10 MΩ) was pulled from a thin-walled capillary glass, filled with 2 M NaCl, and mounted on a three-dimensional (3-D) micromanipulator. It was connected by a silver wire coated with AgCl to an amplifier probe whose output was monitored using an on-line oscilloscope. The amplified signals were digitized (Neuro Data Instruments) and stored on video cassette. This extracellular recording electrode was placed in layers II/III of the neocortex and a concentric bipolar electrode (Rhodes Electronics) was placed in layer VI to stimulate the immediately overlying layers. A current pulse (0.1–1.5 mA; 0.1-ms duration; 0.25 Hz) was applied to produce a population spike of near-maximal amplitude. Digitized data were signal-averaged (6 sweeps/trace), displayed, and plotted using pCLAMP software (Axon Instruments).

Note that the ACSF superfusion rate was approximately 1 mm/min. A higher rate of 3–4 mm/min permitted better recovery from OGD but was not examined here.

RESULTS

AD induced by OGD

In metabolically compromised hemi-brain slices at 35°C, a large optical signal consistently initiated within 5 min of OGD onset (Fig. 1A). There was a dramatic focal increase in LT in layers II/III of the neocortex (n = 18) that expanded as a concentric front of elevated LT. The LT peak (Fig. 1B) was followed by a marked decrease in LT, resulting in a wave-like propagation of the front across the brain slice. The route of propagation was usually inferiorly through the neocortex and inferiorly through the striatum toward the anterior commissure, averaging 1.8 mm/min. The signal first ignited in the neocortex, followed 2–3 min later by independent initiation in the striatum. There was some variation. In 6 of 18 slices, a single focus spread in opposite directions along the neocortex. In three slices, it arose near the anterior commissure and propagated laterally in neocortex. In two slices, it initiated in the striatum before initiation in the neocortex. In all areas where it

Imaging intrinsic optical signals

Individual slices were transferred to a recording chamber with ACSF flowing at a rate of 1.3 ml/min. The temperature of the flowing ACSF was raised by 2°C every 5 min to 35°C in most experiments. A slice was held down at its edges using small pieces of silver wire and ACSF was raised by 2°C every 5 min to 35°C in most experiments. A halogen lamp with a voltage-regulated DC power supply was used to transilluminate the slice. Video images were obtained using a charged coupled device (CCD camera) set at maximum gain and medium black level. With the CCD gamma set at 1.0, output was linear with respect to change in light intensity. Images were averaged and digitized using an image processor board (DT 2867, Data Translation) in a pentium computer controlled with Axon Imaging Workbench software (Axon Instruments). Images were archived on record-

An experiment required a series of images, each image consisting of 128 averaged frames acquired at 30 Hz. The first image of the series was the control (Tcontrol), which was subtracted from each of the subsequent images (Texp) in the series. Each subtracted image, which demonstrated the change in light transmittance (ΔLT) over time, was divided by the gain set by adjusting the software program. The gain was usually set at one, but could be increased so that smaller signal changes could be detected in an image series. The difference signal is normalized by dividing by Tcontrol, which varies across the slice depending on the zone sampled. For example, Tcontrol is lower in white matter than gray matter. Note then that the plotted data are normalized but the images are not. This value was then presented as a percentage of the digital intensity of the control image of that series. That is, ΔLT = {[(Texp - Tcontrol)/Tcontrol] x 100 = ΔLT%}. The change in light transmittance was displayed using a pseudocolor intensity scale. The black-and-white image in bright field was displayed using a gray intensity scale. Zones of interest (ZOIs) in the striatum and the neocortex were selected using the Axon Imaging Workbench software. The values in these areas were averaged, saved as Excel (Microsoft) files and plotted using Excel software. A graphics program (CorelDRAW) was used to import and label figures.
A  Anoxic depolarization induced by O$_2$/glucose deprivation in neocortex/striatum (35°C)

B  Time course of LT changes

C  Evoked field potential

D  Negative shift

E  Optical signal
had just passed, the LT front returned to baseline within 5 min followed by a progressive decrease in LT.

The LT decrease developed exclusively in regions that supported the LT front. To confirm that the front was indeed a spreading depolarization, the negative shift was recorded. First the evoked extracellular field potential was recorded in layers II/III of the neocortex (n = 7). Stimulation evoked a mixed antidromic/orthodromic response, confirming that the electrode was in a viable region (Fig. 1C, top). Occasionally one recording electrode was placed in layers II/III of the neocortex and another in the striatum. A negative shift of 10 mV recorded in the neocortex was followed by a negative shift of 15 mV in the striatum 1.5 min later (Fig. 1D). Similarly, Fig. 1E shows initiation, propagation, and negative transmissance change first in neocortex and then in striatum. At 20 min and thereafter, no evoked response could be recorded in layers II/III, indicating that the tissue was functionally compromised (Fig. 1C, bottom).

In more caudal slices that included hippocampus (n = 12), the signal initiated in the neocortex prior to a second independent focus arising in the CA1 region (Fig. 2A). The propagation rate was similar to more rostral neocortex (i.e., 2 mm/min). Again where the LT increase propagated, a negative LT change developed over the ensuing minutes (Fig. 2B). In another slice, the negative shift was recorded at the time of the LT increase (Fig. 2C). The pre-OGD field potential in layers II/III was consistently and permanently lost following OGD (Fig. 2D), indicating functional damage.

**AD induced by ouabain exposure**

Brief bath application of 100 μM ouabain, a Na+/K+ ATPase inhibitor, elicited a sequence that was remarkably similar to that induced by OGD. In all 14 slices tested at 35°C, the signal ignited in layers II/III of the neocortex after approximately 4 min of ouabain exposure. Again, a focal LT increase expanded and migrated as a front across the neocortex (and later in the striatum) at about 2.5 mm/min (Fig. 3A). In some cases, there were two sites of ignition in the neocortex that propagated and collided (not shown). As with OGD, propagation was immediately followed by an irreversible decrease in LT only in regions where the AD front had passed.

The change in peak light transmittance induced by ouabain exposure was dependent on dose (Fig. 3B). At 10–25 μM (n = 9), there was a small general increase in LT in the neocortex that returned to baseline. An increase to 50 μM (n = 4) gave a higher elevation peak in LT, and then AD was elicited but only in the neocortex. At 100 μM (n = 14), SD initiated in the neocortex and then independently in striatum as described in the preceding text.

As with OGD, the post-AD development of slice opacity (indicated by magenta pseudocoloring) was associated with permanent loss of the evoked field potential. Both observations implicate serious neuronal damage.

**Glutamate receptor antagonists and the AD**

Excitotoxicity theory states that ischemic insult elicits an accumulation of glutamate extracellularly that results from the mass depolarization of neurons and the failure of re-uptake mechanisms. It is plausible that glutamate receptor activation is involved in the signal sequence induced by OGD or ouabain. To test this, slices were pretreated with 2 mM kynurenate (a nonspecific glutamate receptor antagonist) for 20 min at 35°C before exposure to either OGD (n = 10) or ouabain (n = 8). Three regions of interest were plotted across neocortex. The front of elevated LT passed each region in sequence during OGD (Fig. 4A) or ouabain exposure (Fig. 4B). In all 18 slices tested, kynurenate failed to block signal initiation and propagation in neocortex or striatum. Kynurenate also had no significant effect on the onset time of signal (Fig. 5A), its propagation rate (Fig. 5B), or the maximum LT increase comprising the signal front (Fig. 5C). Most importantly, there was no significant decrease in the extent of irreversible damage (based on the maximum LT reduction) as compared with control slices (Fig. 5C).

Some slices were pretreated with the selective NMDA receptor antagonist AP-5 (25 μM of the D-isomer) for 20 min before exposure to OGD (n = 5). Others were pretreated with 50 μM of the selective non-NMDA receptor antagonist CNQX (n = 5). In both cases, there was no significant difference in signal onset (Fig. 5A) propagation rate (Fig. 5B) or posts ischemic damage (Fig. 5C) when compared with control slices.

**Glutamate agonists**

To further investigate the role of glutamate receptors in the ischemic signal, glutamate agonists were briefly bath-applied under normal oxygen and glucose conditions. A very brief application allowed testing for signal induction while avoiding slice damage. Slices exposed to 50 μM AMPA (n = 8 of 8 slices) or to 100 μM NMDA (n = 12 of 23 slices) showed a generalized, nonpropagating LT increase in the gray matter that peaked and then slowly dissipated (Fig. 6A). The lack of negative LT values indicated that there was no subsequent damage. In these respects, the response contrasted with the ischemic event evoked by OGD (compare Figs. 1A and 6A). Likewise with 0.7 (n = 4) or 2.0 mM (n = 6) quinolinate (an NMDA receptor agonist), swelling was generalized and no damage resulted (not shown). At 3 mM quinolinate (n = 14) and in the other 11 of 23 slices treated with 100 μM NMDA, a generalized LT increase was followed by a sudden reduction in signal at several sites that appeared to migrate along the gray.
FIG. 2. The AD induced by O$_2$/glucose deprivation (8 min, 35°C) in a slice of neocortex/hippocampus. A: AD initiates 1st in neocortex (6:21) and propagates bidirectionally along the gray matter (6:35). AD independently initiates in hippocampus (6:54) and propagates along CA1 (†), finally invading the dentate gyrus (*). By 20:05, dendritic regions display a pronounced opacity (magenta pseudocoloring) due to dendritic beading while hippocampal cell body regions (lacking dendrites) display elevated LT (yellow-red). B: time course of the optical signal in the three neocortical zones (1–3) is shown in A. The sequential peaks in LT represent the moving AD front across zones 1–3. By 20 min, each region displays a reduced LT (damage) due to dendritic beading. C: in another slice, the negative shift recorded in layers II/III is activated approximately 6 min after the start of OGD, corresponding to the peak LT of the AD front. D: the evoked field potential in layers II/III is lost following OGD. It does not recover following a 30 min return to control artificial cerebrospinal fluid (ACSF).
matter. However, given the extremely high concentrations of the glutamate agonists required to elicit this “spreading” signal, we did not pursue it further.

To test that the preceding responses were indeed glutamate receptor mediated, slices were pretreated with glutamate receptor antagonists. The majority of the NMDA response was blocked by 25 μM AP-5 (Fig. 6, B and D) as was the quinoline response (Fig. 6, C and D).

Temperature dependence of SD

At lower temperatures, metabolic processes slow, possibly providing protection from the energy demands of ischemia. OGD experiments were repeated at 31–32, 30, and 24–28°C and compared with slices at 35°C. Experiments performed at 31–32°C (n = 9) displayed similar latency to signal onset times (Fig. 7A) and propagation rates (Fig. 7B). The extent of irreversible damage (based on decreased LT values, Fig. 7C) was similar to those values measured at 35°C.

At 30°C, however, the signal was induced by OGD in only four of eight slices. In two of these slices, it arose in neocortex but not striatum. Accordingly, the ensuing LT decrease developed only in the cortex. In the other two slices, the response was typical of those elicited at higher temperature and the irreversible damage was observed in both the striatum and neocortex where AD had passed. There was a 2- to 3-min delay in the ignition time and a slightly slower propagation rate. In the four slices that did not respond to OGD, there was a general increase in LT followed by a gradual decrease toward baseline with no negative LT change (Fig. 7C). Again, negative LT values (dendritic beading) were associated only with a prior spreading AD signal.

**DISCUSSION**

Measuring IOSs provides a means of monitoring cell swelling and the ensuing acute neuronal damage induced by stroke-like conditions (Obeidat and Andrew 1998; Obeidat et al. 2000). Light transmittance *increases* in submerged brain slices when cells swell (reviewed by Aitken et al. 1999; Andrew et al. 1999; Jarvis et al. 1999). Hippocampal slices acutely damaged by excitotoxic stress (Jarvis et al. 1999; Polischuk et al. 1998) or by OGD (Obeidat et al. 2000) develop a reduced light transmittance (LT) in dendritic regions caused by dendritic “beading.” These varicosities form along dendrites in response to brief but acute metabolic stress. The beading of hundreds of dendrites across the thickness of the slice is of an ideal configuration to scatter light, reducing LT even as the tissue continues to swell (Jarvis et al. 1999). In hippocampal slices, an acutely damaged cell body layer displays permanently increased LT because dendrites are sparse. In neocortex, dendrites are distributed across all layers so early neuronal cell body and glial swelling (which increases LT) is eventually overwhelmed by beading (which decreases LT). As observed in vivo (Nedergaard and Hanson 1988), repeated SD in neocortical slices under normoxic conditions (Footit and Newberry 1998) causes no damage in terms of reduced LT or lost evoked field potential (Anderson et al. 1999). The current study demonstrates that acute neuronal damage in neocortex and striatum is caused by the high metabolic demand of OGD and the spreading signal, which we argue represents AD.

**Imaging the AD**

Classic SD in normoxic tissue represents a profound increase in membrane permeability. K⁺ and hydrogen ions immediately leave the neurons while Na⁺, Ca²⁺, and Cl⁻ enter along with water, thereby decreasing the extracellular space (Hansen 1985; Nicholson and Kraig 1981). Ion concentrations return to near normal within a minute or so where there is no decreased energy supply or neuronal damage (Lauritzen 1994). Following focal ischemia, a more prolonged event, the PID, can arise at the border of the ischemic core and spread into the penumbra. Here neurons are at risk and recurring PIDs exacerbate damage during the 3–4 h following stroke onset (Back...
et al. 1996; Dijkhuizen et al. 1999; Mies 1997; Nedergaard and Hansen 1993).

We found that depriving the hemi-brain slice of oxygen/glucose or inhibiting Na⁺/K⁺ ATPase with ouabain induced a spreading AD in neocortex and independently in striatum. A single electrode within cortical gray records the AD but misses the spreading nature of the signal. The rate of AD propagation observed in the present study (∼2 mm/min) is consistent with that described for SD in the intact cortex (reviewed by Nedergaard 1996). Only in areas where AD passed did an irreversible decrease in LT develop within 10 min of the insult. Several lines of evidence indicate that this represents cellular damage.

FIG. 4. A: glutamate receptor antagonism does not affect AD during metabolic compromise nor ensuing acute damage. AD induced by OGD at 35°C in neocortex (and later in striatum, not shown) during exposure to 2 mM kynurenate. Plot shows time course of LT changes in the zones (denoted 1–3), in the neocortex. Each peak represents the propagation of the AD front (cell swelling). Each is followed by an irreversible decrease in LT (damage). B: AD induced by 100 µM ouabain at 35°C in the neocortex (and later in the striatum, not shown) during exposure to 2 mM kynurenate. Plot shows time course of LT changes in zones (denoted 1–3) in the neocortex. The peaks show AD propagation across each zone, followed by an irreversible decrease in LT, indicating neuronal damage.

Obeidat et al. (2000) have shown a similar LT reduction associated with damage to CA1 dendrites in the wake of AD. Filling single CA1 neurons with the fluorescent dye Lucifer yellow revealed extensive dendritic beading not observed in control tissue. Likewise in the present study, irreversible de-
creases in LT occurred only where AD propagated; the evoked field potential was lost in these areas. Our results indicate that acute neuronal swelling and beading is not due to metabolic compromise of OGD alone but is contingent on the AD occurring concurrently, increasing energy demand and thereby accelerating neuronal damage.

The correlation in time and space between the negative shift and the LT front confirms that the optical signal represents AD. The negative shift may return to near baseline after several minutes, even in the face of maintained OGD but the irreversible LT reduction and loss of the evoked field potential argue against neuronal recovery. It is possible that in the brain slice, the glia can partly repolarize to account for the voltage return because released extracellular K$^+$ diffuses into superfusate rather than accumulating and maintaining the depolarization as would occur in vivo.

AD onset was usually within layers II/III of the neocortex and was not related to the orientation of the slice in the chamber, so it was not dependent on where the superusing ACSF first contacted the slice. Histological analysis of the neocortical layers show that layers II/III are the most densely packed cell body layer (Cormack 1987). The high density of neurons and the reduced number of glia may promote a mutual promotion of depolarization and K$^+$ release/accumulation, making layers II/III more susceptible to AD onset.

Lack of a role for glutamate in AD onset and damage

We found no significant difference in the peak LT values at the AD front (representing cell swelling) between control slices and those pretreated with GluR antagonists. In addition, there was no significant difference in the time of signal onset, propagation rate, or the extent of LT reversal (representing neuronal damage). Kynurenate (2 mM) abolished the evoked field potential when bath applied to the cortical slice, indicating that this dose was effectively antagonizing NMDA receptors. These results are consistent with several electrophysiologic studies of intact animals. Competitive and noncompetitive NMDAR antagonists (but not non-NMDAR antagonists) block normoxic SD (Hernandez-Caceres et al. 1987; Lauritzen and Hansen 1992; Marrannes et al. 1988; Nedergaard and Hansen 1988; Nellgard and Wieloch 1992). These same studies show that NMDA antagonists are ineffective against the AD. MK-801 reduces the number of PIDs and infarct size (Iijima et al. 1992).
1992; but see Koroleva et al. 1998) probably by inhibiting PID propagation in tissue away from the core where conditions are less ischemic. The implication is that accumulating extracellular glutamate is not an important factor that contributes to AD genesis and early ischemic damage. Recent modeling of the reversal of glutamate uptake mechanisms during ischemia (Rossi et al. 1999) agrees with data reviewed by Obrenovitch and Urenjak (1997) that glutamate starts to accumulate only after the anoxic depolarization. Such accumulation does not drive the AD and PIDs (Andrew et al. 2000) or represent a major cause of ischemic damage (Obrenovitch 1999).

We found that 100 μM NMDA or 3 mM (but not 2 mM) quinolinate (QUIN) caused a generalized swelling followed by a diffuse spreading event. These concentrations were highly unphysiological and so do not argue that glutamate evokes the AD. The induced swelling was indeed glutamate receptor mediated because it was inhibited in slices pretreated with the selective NMDA antagonist AP-5 prior to QUIN or NMDA application. In those slices that did not undergo a diffuse spreading event when exposed to NMDA, there was a general increase in LT, followed by a return to baseline over the next 10 min. The extent of damage in these cases was significantly less than those slices in which the spreading event had passed. So again a spreading signal, even in these unphysiological conditions, was a prerequisite for significant acute damage to result.

Anoxic depolarization is temperature dependent

We found that lowering temperature was much more effective in blocking the AD and its damaging effects than GluR antagonists. Between 32 and 34°C, there was no delay in AD onset and no significant difference in the rate of propagation. The extent of damage (decreased LT) was slightly less when compared with control slices that were exposed to OGD at 35°C. We expected to see more dramatic effects at 32–34°C because lowering temperature from 37.5 to 35°C significantly delayed SD onset following OGD in hippocampal slices (Obenaiat and Andrew 1998). However, at 30°C, SD was induced by OGD in only half of the neocortical slices. In those slices that underwent AD, there was a 2-min delay of onset compared with control slices at 35°C. In slices at up to 30°C where the AD was not elicited, there was no subsequent damage apparently because the tissue did not have to expend limited energy reserves on AD recovery. There is dramatic long-term protection following MCAO occlusion in rat neocortex by prolonged cooling between 30 min and 48 h postischemia (Corbett et al. 2000). We suggest this is at least partially due to suppression of peri-infarct depolarizations in the first 3–4 h poststroke. This could considerably reduce the onslaught of chemical cascades underlying delayed neuronal death, a process activated by and downstream from the AD and PIDs.

Lowering temperature blocks the AD during metabolic stress by reducing energy demand. At body temperature to be as effective, a drug would have to uncouple the AD during the period of energy deprivation. We have recently found this to be possible by bath application of certain sigma receptor (σR) ligands to neocortical slices during OGD (Anderson et al. 2000; Andrew et al. 2000). These ligands also block classic SD in normoxic slices (Anderson et al. 1999), implying that SD and AD initiation share common features. Indeed the current study shows that the AD is SD-like in that it arises suddenly at a focus, propagates across gray matter and is recorded as a negative voltage shift. We propose that the PID is a hybrid of the AD and SD, essentially an AD that can repolarize using the intermediate level of energy stores available in the penumbra. Theoretically then, PIDs may also be blocked by σR ligands. These ligands could prove to be protective if they penetrate the blood-brain barrier and are administered during the first 3 h following stroke.
IMAGING THE ANOXIC DEPOLARIZATION

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