Regional Differences in Hypoxic Depolarization and Swelling in Hippocampal Slices

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Kreisman, Norman R., Soheil Soliman, and David Gozal. Regional differences in hypoxic depolarization and swelling in hippocampal slices. J. Neurophysiol. 83: 1031–1038, 2000. Pyramidal neurons in the CA1 region of the hippocampus are highly vulnerable to damage from hypoxia-ischemia, whereas neurons in the CA3 region and the dentate gyrus are more resistant. A similar pattern of vulnerability to loss of synaptic and membrane function occurs in the in vitro hippocampal slice preparation, suggesting that intrinsic factors are important in acute neuronal damage. Simultaneous recordings of DC potential and imaging of changes in light transmittance were made on slices from the middle one-third of the hippocampus to characterize the initiation and spread of depolarization and swelling during hypoxia-aglycemia. Hypoxic depolarization (HD) and associated optical changes were initiated simultaneously in the stratum oriens of the CA1 region and thereafter spread to the stratum radiatum of CA1 and later to the upper (inner) blade of the dentate gyrus. A decrease in light transmittance was associated consistently with depolarization in all regions (n = 22 slices). Investigation of the sequence of activation in intact slices showed that activation of the dentate gyrus arose independently of activation of the CA1 region. This was confirmed by recordings made from minislices in which CA1, CA3, and dentate regions were physically separated. HD and optical changes were never observed in the CA3 region, despite exposure to 40–60 min of combined hypoxia and aglycemia. In contrast, exposure to hypoxia after pretreatment of slices with altered tonicity or ion composition, which triggered episodes of spreading depolarization (SD), provoked depolarization and optical changes simultaneously in both CA1 and CA3 regions. Similarly, pretreatment with agents that cause severe metabolic impairment, such as dinitrophenol (DNP), also rendered the CA3 regions. Pretreatment with agents that cause severe depolarization and optical changes simultaneously in both CA1 and dentate gyrus during hypoxia reveals that CA1 always depolarizes before the dentate gyrus (Aitken and Schiff 1986; Roberts and Sick 1988) and there is evidence that HD contributes to acute cellular damage (Obeidat and Andrew 1998). Simultaneous recordings of the extracellular potential in CA1 and dentate gyrus during hypoxia reveal that CA1 always depolarizes before the dentate gyrus (Aitken and Schiff 1986; Balestrino et al. 1989). However, the susceptibility of the CA3 region to HD varies among several reports, probably because of differences in experimental conditions (e.g., see Aitken et al. 1998; Davis et al. 1986; Obeidat and Andrew 1998).

Many of these experiments used recordings of extracellular potential from one or two microelectrodes to assess the localization of HD. Unfortunately, this punctate view cannot provide adequate information about the origin, spread, and spatial extent of HD. Recently, imaging techniques have been applied to the mapping of hypoxia- or glutamate-induced depolarization by measuring associated changes in light transmittance that are ostensibly due to cell swelling (Aitken et al. 1998; Andrew et al. 1996; Kreisman et al. 1996; Obeidat and Andrew 1998; Turner et al. 1995). HD is accompanied by cellular swelling because the dissipation of ion gradients across the cell membrane osmotically obligates the influx of water into the cell. These investigations have corroborated the particular susceptibility of CA1 to both HD and swelling. Moreover, recent imaging studies have localized where HD is initiated and have described the spread of HD in submerged hippocampal slices (Obeidat and Andrew 1998) and interfaced hippocampal slices

INTRODUCTION

Brain injury after ischemia shows a well-known pattern of regionally selective vulnerability (Kirino and Sano 1984; Pulsinelli et al. 1982). In the hippocampal formation, the CA1 region is highly vulnerable to damage from even brief episodes of ischemia, whereas the CA3 region and the dentate gyrus are more resistant (Schmidt-Kastner and Freund 1991). In the in vitro hippocampal slice preparation, a similar pattern of electrophysiological vulnerability emerges during acute hypoxia and/or aglycemia, suggesting the importance of intrinsic factors in acute neuronal damage. Irreversible blockade of synaptic transmission after acute hypoxia is an important physiological indicator of injury (Schiff and Somjen 1987; Schurr et al. 1989). Several laboratories have confirmed regional differences in the susceptibility to irreversible synaptic blockade, showing that CA1 is highly susceptible compared with the dentate gyrus or the CA3 region (Aitken and Schiff 1986; Crépel et al. 1992; Kass and Lipton 1986).

A putative factor contributing to hypoxic injury is the profound depolarization of neurons, and presumably glia, due to a substantial increase in membrane permeability triggered by hypoxia and/or hypoglycemia (Balestrino 1995; Hansen and Zeuthen 1981; Vyskočil et al. 1972). The depolarization, called hypoxic depolarization (HD), occurs within minutes after hypoxia-induced synaptic blockade. HD is heralded by a profound negative shift of the extracellular potential, virtually identical with that seen in spreading depression of Leão (Aitken et al. 1998; Somjen et al. 1990; Somjen et al. 1992). In hippocampal slices, synaptic transmission fails to recover after reoxygenation if HD lasts more than a few minutes (Balestrino et al. 1989; Roberts and Sick 1988) and there is evidence that HD contributes to acute cellular damage (Obeidat and Andrew 1998). Simultaneous recordings of the extracellular potential in CA1 and dentate gyrus during hypoxia reveal that CA1 always depolarizes before the dentate gyrus (Aitken and Schiff 1986; Balestrino et al. 1989). However, the susceptibility of the CA3 region to HD varies among several reports, probably because of differences in experimental conditions (e.g., see Aitken et al. 1998; Davis et al. 1986; Obeidat and Andrew 1998).
(Kreisman et al. 1996). In the present investigation we imaged changes in light transmittance to verify the regional susceptibility to HD in interfaced slices, as an extension of our earlier preliminary report. Additionally, we examined whether HD spreads from more susceptible to less susceptible regions or is initiated independently in each region, using both intact slices and minislices of individual regions. Also, we determined whether regions normally resistant to HD could be made vulnerable to HD by various treatments. Finally, we attempted to determine whether susceptibility to HD, or lack thereof, might be related to selective neuronal injury resulting from the preparation of hippocampal slices.

**Methods**

**Preparation of hippocampal slices**

Male Sprague-Dawley rats (200–500 g; Charles River) were anesthetized with ether and perfused through the heart with 60 ml of cold (4°C) bathing medium containing (in mM): 129 NaCl, 3.5 KCl, 2 MgSO₄, 1 NaHPO₄, 2.7 CaCl₂, 26 NaHCO₃, and 10 glucose (according to Kreisman and LaManna 1999). The rats were decapitated, the brain removed, and the tissue placed immediately in ice-cold bathing medium. Transverse slices (400–1000 μm-thick) were cut with a mechanical tissue chopper (Stoelting, Wood Dale, IL) from the middle one-third of the hippocampus to avoid septotemporal gradients of excitability (Bragdon et al. 1986). Slices were then placed in the wells of a holding chamber on filter paper (Whatman 50) thoroughly wetted with bathing medium and gassed with humidified 95% O₂:5% CO₂. The incubation medium was maintained at room temperature (23–24°C) and was replaced with fresh medium at 45-min intervals. In some experiments, slices were preincubated at room temperature in a modified bathing medium containing 400 μM ascorbate, with zero Ca²⁺ and 10 mM Mg²⁺ substituted for the usual 2.7 mM Ca²⁺ and 2 mM Mg²⁺. After 90–120 min of preincubation, one of the slices was transferred to the nylon mesh of an interface-style recording chamber containing normal medium, whereas others remained in the holding chamber. The temperature in the recording chamber was maintained at 33–34°C and the slice was subfused with standard bathing medium flowing at 0.6 ml/min. Warmed, humidified 95% O₂:5% CO₂ superfused the slice at a rate of 480 ml/min. Osmolarity of the standard bathing medium was 295–300 mOsm/l. Hypotonic media of various strengths were made by removing the appropriate amount of NaCl.

**Electrical stimulation and recording**

Viability of the CA1 region was tested by stimulating the Schaffer collaterals with constant-current pulses (400 μA; 0.2 ms) using glass micropipettes filled with 150 mM NaCl (tip resistance 5–20 MΩ). Only stable recordings of population spikes with a minimum amplitude of 3 mV were accepted. We attempted to test viability of the CA3 region by stimulating the fimbria and recording antidromic or orthodromic population spikes from the nearby CA3 stratum pyramidale. Although preovolleys were present, orthodromic population spikes were commonly absent. Thereafter, CA1 and CA3 recording electrodes were usually relocated to the stratum radiatum for recording extracellular DC potentials. Excitatory postsynaptic potentials (EPSPs) also were recorded extracellularly in CA1 s. radiatum in response to stimulating the Schaffer collaterals with constant-current pulses (50–100 μA; 1 ms) to produce a response that was 50–75% of maximum amplitude. Extracellular DC levels and optical signals (see following section) were recorded continuously on a strip-chart recorder, and evoked responses and optical signals were digitally acquired with MacLab software.

**Measurement and imaging of light transmittance**

Transmitted light from a stable quartz-halogen source was detected by a silicon photodiode (model 78-7821; Ealing), coupled to one ocular of a Nikon, SMZ-2 binocular dissecting microscope. The optical field was approximately 0.4 × 1.2 mm, which included the s. radiatum and s. pyramidale of the CA1 region. To calibrate the optical signal, basal light transmittance (T) was nulled at the beginning of the experiment, using the offset of a DC amplifier. The dark value was then determined by shutting off the light source. Variations in light intensity from zero (ΔT) were calculated as ΔT/T in percent.

Imaging of light transmittance was accomplished by coupling an 8-bit digital video camera (model CCD72, Dage MTI, Michigan City, IN) to the remaining ocular of the binocular dissecting microscope. Black levels and dynamic range of the video signals were adjusted manually, based on a histogram of light transmittance values obtained from each slice during normoxia. As with the photodiode measurements, relative changes in light transmittance (ΔT) were expressed as a percent of T. Images were captured at 0.05–3.0 Hz, depending on the individual experiment, using custom-designed imaging software (Synetic, Montreal, Quebec, Canada). Difference images were derived by subtracting the mean of five images at peak hypoxia from the mean of five images taken during normoxia. Pseudocoloring of the difference images was accomplished with Adobe Photoshop software. Areas of interest were manually drawn on an image of the slice, and ΔT/T was plotted as a function of time for each area.

**Induction of hypoxia**

Severe hypoxia was induced by switching the gas mixture superfusing the slices from 95% O₂:5% CO₂ to 95% N₂:5% CO₂. In several experiments, PO₂ in the bathing medium was measured polarographically, by using a platinum electrode polarized to −0.7 V relative to an Ag-AgCl wire connected to ground. The mean PO₂ values in the upper 1 mm of the bath during normoxia and hypoxia were 443 ± 19 mmHg and 20 ± 3 mmHg, respectively (n = 9).

**Histology**

Slices were incubated in the holding chamber at room temperature for 2 h and then at 33–34°C for 1 h before fixation overnight in 10% buffered formaldehyde. This was followed by incubation in 30% sucrose at 4°C for 24 h. Frozen sections were cut at 10 μm and mounted on gelatin-coated slides. Sections were stained with cresyl violet and the morphology of neurons in the CA1 and CA3 pyramidal layers was evaluated and scored according to the scheme described by Raley-Sussman et al. (1997). Reported values are the means of ratings made by two observers who were blinded to the treatment the slices received.

**Results**

**Optical and electrical responses to severe hypoxia in various hippocampal regions**

Within minutes after onset of severe hypoxia, simultaneous rapid decreases in light transmittance (plotted here as upward deflections of ΔT/T) and negative shifts of the extracellular potential (DC), indicative of hypoxic depolarization, occurred in the CA1 region in all 22 slices tested (see Fig. 1 for example). The optical response originated in the stratum oriens of the CA1 region and spread laterally in both directions, also spreading to the s. radiatum. The extent of lateral spread in the CA1 region was always greater than the extent of vertical spread, with a clear demarcation of swollen and normal areas at the borders of the CA2 region, the subiculum, and the upper blade of the
dentate gyrus. The most intense optical changes always occurred in the s. radiatum, followed by the s. oriens. In comparison, the optical responses in the s. pyramidalae were minimal (Fig. 1A). After a short delay, the optical response was also observed in the dendritic layer of the upper blade of the dentate gyrus in 20 of 22 slices, but in no case did the optical response involve the lower blade of the dentate gyrus (e.g., Fig. 1B). The mean latency (±SE) from onset of severe hypoxia to rapid HD and swelling was 3.0 ± 0.6 min in the CA1 region (n = 22 slices) and 3.7 ± 0.7 min in the upper blade of the dentate gyrus (n = 20 slices). Similar optical changes were observed in the subiculum after more prolonged hypoxia, but no attempt was made to quantify these latencies. Neither HD nor appreciable optical changes were observed in the CA3 region in these 22 slices, despite extending the period of severe hypoxia in five experiments (in three cases with superimposed aglycemia) for more than 40 min (e.g., Fig. 1, A–C). A summary of optical changes during various intervals of hypoxia in four regions of hippocampal slices is shown in Table 1.

The sequence of optical images generated in these experiments suggested that hypoxic swelling did not spread from the CA1 region to the dentate gyrus but rather was initiated independently in the two regions (Fig. 1A). To verify this, hippocampal slices were divided into three minislices by using microscissors to isolate the CA1, CA3, and dentate regions from one another (Fig. 2A; n = 3 slices). Both pseudocolored difference images (hypoxia minus normoxia; Fig. 2B) and plots of ΔT/T in the various minislices as a function of time (Fig. 2C) showed clearly that swelling occurred independently in the CA1 region and the upper blade of the dentate gyrus. Identically with intact slices, virtually no optical changes were observed in either CA3 minislices or minislices from the lower blade of the dentate gyrus during more than 20 min of severe hypoxia.

**TABLE 1. Light transmittance changes during hypoxia in various regions of the hippocampal slice**

<table>
<thead>
<tr>
<th>Slice Region</th>
<th>Hypoxia Duration</th>
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<tr>
<td></td>
<td>5 min</td>
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<tr>
<td>CA1</td>
<td>12.9 ± 1.6</td>
</tr>
<tr>
<td>CA3</td>
<td>2.6 ± 0.9</td>
</tr>
<tr>
<td>UBDent</td>
<td>6.8 ± 1.3</td>
</tr>
<tr>
<td>LBDent</td>
<td>0.6 ± 0.3</td>
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Changes in light transmittance (ΔT/T) are expressed as the percent change from the normoxic baseline transmittance level. Values are means ± SE in percent (n = 22 slices). UBDent, upper blade; dentate gyrus; LBDent, lower blade, dentate gyrus.

The results suggested that the CA3 region is more resistant to hypoxia than CA1, which is in agreement with data obtained in models in vivo of ischemia (Kirino and Sano 1984; Pulsinelli et al. 1982). However, previous reports have suggested that the preparation of hippocampal slices injures CA3 neurons compared with CA1 neurons (Johnston et al. 1992; Newman et al. 1995; Rice et al. 1994). Therefore, we undertook a series of experiments to test whether CA3 was in fact capable of depolarizing. This was attempted in 26 slices from an additional 17 rats by using a variety of treatments before and during induction of hypoxia, which were designed either to increase the intensity of the metabolic insult (e.g., by adding aglycemia or treatment with DNP to hypoxia) or to increase the excitability of the hippocampal slice (Table 2). Hypotonia was used to increase excitability, often in conjunction with increases in extracellular [K+] or removal of extracellular [Ca2+] or [Mg2+]. Hypotonia swells cells and thereby decreases in-
terstitial volume, which increases excitability via field effects (Andrew 1991; Taylor and Dudek 1984). For example, strong hypotonia is known to trigger episodes of SD in the CA1 region (Chebabo et al. 1995), but it is unknown whether the CA3 region also is susceptible to SD during hypotonia. Another goal was to make the interstitial volume in CA3 more like that in CA1 to test if that would render CA3 more vulnerable to hypoxic depolarization.

During normoxia, the treatments described occasionally led to episodes of SD in CA1 alone (3 of 26 slices) or in both CA1 and CA3 (4 of 26 slices; Table 2). The occurrence of SDs during normoxia predisposed both the CA1 and CA3 regions to depolarize subsequently during hypoxia in six of these seven slices (Table 2). The results of one of these experiments is shown in Fig. 3. Decreasing the osmolarity of the bathing medium from 300 mOsm/l to 200 mOsm/l caused a positive shift of 7–8 mV in the DC potential in both regions, probably because of the change in ionic strength of the bathing medium. This was followed in ~5 min by a prolonged negative shift in the baseline DC potential with superimposed episodes of SD in both the CA1 and CA3 regions. In this example, the initial SD occurred in CA1 seconds before it occurred in CA3, but the latency between the two responses decreased with successive episodes. The fourth episode of SD in this case occurred simultaneously in both regions. Measurements of \( \Delta T/T \) as a function of time also revealed simultaneous, rapid changes indicative of SD in both CA1 and CA3 (Fig. 4B). Images taken at the steady-state period between episodes of SD (i.e., at the 15-min mark of Fig. 4B) showed osmotic swelling primarily in CA1 and patchy swelling in CA3 (Fig. 4A). Hypoxia was induced 5 min after the four episodes of SD (Fig. 3). Hypoxic depolarization (HD) was triggered both in CA1 and CA3 within ~1 min after onset of hypoxia in the presence of the hypo-osmotic medium. CA3 depolarized before CA1 in this instance. Induction of hypoxia also produced rapid shifts in the optical traces, indicative of swelling, in CA1, CA3, and the upper blade of the dentate gyrus (Fig. 4D). Imaging at 8 min of hypoxia showed clear swelling of both the entire CA1 and CA3 regions (Fig. 4C). Similar responses were observed in three slices. In two other slices, treatment with DNP to deplete energy supplies resulted in rapid depolarization in both CA1 and CA3 during hypoxia, in one case despite the absence of preceding SDs (Table 2).

**Morphology of CA1 and CA3 pyramidal neurons and neurons of the dentate gyrus**

The morphology of neurons in the pyramidal cell layer of the CA1 and CA3 regions was evaluated to determine primarily whether CA3 neurons were preferentially damaged by the slicing procedure, as reported by others (Newman et al. 1995; **TABLE 2.** Effects of putatively noxious pretreatments and episodes of SD on the occurrence of HD during hypoxia

<table>
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<tr>
<th>Occurrence of SDs During Pretreatment</th>
<th>HD During Hypoxia</th>
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<tr>
<td>None ((n = 19))</td>
<td>None ((n = 2))</td>
</tr>
<tr>
<td>CA1 only ((n = 3))</td>
<td>CA1 only ((n = 1))</td>
</tr>
<tr>
<td>CA1 and CA3 ((n = 4))</td>
<td>CA1 and CA3 ((n = 2))</td>
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\(n = \) number of slices. Pretreatments included such insults as aglycemia, elevated extracellular \([K^+]\), zero extracellular \([Ca^{2+}]\), zero extracellular \([Mg^{2+}]\), 50–200 \(\mu\)m dinitrophenol, or combinations thereof. SD, spreading depolarization; HD, hypoxic depolarization.
Pyramidal neurons were classified in three categories, based on their appearance under the light microscope (according to Raley-Sussman et al. 1997). Class A neurons had intact, well-defined membranes, a clear uniform nucleus, distinct nucleoli, and a clear cytoplasm. Class B neurons had an indistinct nucleus, were darkly stained, and were shrunken and distorted in shape. Class C neurons had no distinct nuclear boundary and had either a vesiculated cytoplasm or were obviously swollen. Examples of the histological appearance of CA1 and CA3 pyramidal neurons are shown in Fig. 5, A and B. The percentage of class A neurons in CA3 pyramidale was lower than in CA1 pyramidale, whereas the percentage of class C neurons was higher in CA3 than in CA1, but all differences were relatively small in magnitude (Table 3). Some CA3 neurons contained vacuoles and had blebbing of their membranes (Fig. 5B). The tightly packed neurons of the upper blade of the dentate gyrus were round and had intact membranes and a distinct nucleolus (Fig. 5C). In contrast, neurons of the lower blade of the dentate gyrus were smaller than those of the upper blade, and many were less distinctly stained (Fig. 5D). However, the neurons of the lower blade were neither pyknotic nor swollen.

In four experiments, slices were preincubated in a modified bathing medium in which 400 μM ascorbate was added to the standard medium and zero Ca$^{2+}$ and 10 mM Mg$^{2+}$ substituted for the usual 2.7 mM Ca$^{2+}$ and 2 mM Mg$^{2+}$. This has been
slices classified in three histological categories

<table>
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<tr>
<th>Hippocampal Region</th>
<th>Histological Appearance</th>
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<tr>
<td></td>
<td>Class A</td>
</tr>
<tr>
<td>CA1</td>
<td>87.1 ± 2.6</td>
</tr>
<tr>
<td>CA3</td>
<td>78.1 ± 1.3*</td>
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</table>

Values are means ± SE in percent (n = 4–6 slices). * Significantly different from the CA1 value by Student’s t-test (P < 0.05). Histological classification was that of Raley-Sussman et al. (1997). All slices were taken from the middle one-third of the hippocampus.

shown to result in slices with improved morphology in CA3 pyramidal neurons (Newman et al. 1995; Rice et al. 1994). The rationale was that improved viability of the CA3 neurons might allow them to respond to hypoxia with HD. HD still failed to occur in the CA3 region of slices preincubated in this modified medium.

DISCUSSION

Four major findings have emerged from this investigation: 1) CA1 and, to a lesser extent, the upper blade of the dentate gyrus in hippocampal slices are susceptible to hypoxic depolarization, whereas the CA3 region and the lower blade of the dentate gyrus are resistant. 2) The magnitude and time course of hypoxic depolarization and changes in light transmittance indicative of swelling coincide, regardless of the hippocampal region being investigated. 3) Hypoxic depolarization and swelling of the upper blade of the dentate gyrus occur independently from the depolarization in the CA1 region; i.e., spread of the depolarization from CA1 is not required. 4) The hippocampal CA3 region becomes vulnerable to hypoxic depolarization and swelling when excitability is increased sufficiently to trigger episodes of spreading depression before induction of hypoxia or when metabolic activity is severely depressed.

Relative vulnerability of hippocampal formation regions in vitro to hypoxic depolarization

The order of vulnerability to hypoxic depolarization observed in vitro in the present investigation (CA1 > dentate > CA3) is similar to that reported in animal models of stroke in vivo (Kirino and Sano 1984; Pulsinelli et al. 1982; Schmidt-Kastner and Freund 1991). Furthermore, our results corroborate the longer latencies to hypoxic depolarization found by others in the dentate gyrus compared with the CA1 region of the in vitro hippocampal slice preparation (Aitkin and Schiff 1986; Balestrino et al. 1989). An important finding in our experiments was the virtually absolute resistance of both the CA3 region and the lower blade of the dentate gyrus to depolarization in response to hypoxia, aglycemia, or the combination of both insults. Indeed, even 60 min of combined, severe hypoxia and aglycemia failed to provoke depolarization or swelling in either region. The differential vulnerability of the upper and lower blades of the dentate gyrus in vitro has been reported previously by other investigators (Hara et al. 1990; Mitani et al. 1994). Failure of CA3 to swell during hypoxia was also recently reported by two laboratories (Aitken et al. 1998; Obeidat and Andrew 1998). Additionally, the spatial pattern of both intracellular calcium accumulation and depletion of ATP during hypoxia in hippocampal slices was identical with the depolarization and swelling observed in the present experiments—i.e., both CA3 and the lower blade of the dentate gyrus were resistant (Mitani et al. 1994). It should be noted that earlier investigations into the susceptibility of individual CA3 neurons to HD showed conflicting results, which appear to be related to both the stage of the animal’s development and the temperature at which the slices were preincubated (Davis et al. 1986; Janigro and Schwartzkroin 1987).

Several factors may contribute to the susceptibility of CA1 and the relative resistance of CA3 to hypoxic-ischemic injury (e.g., see Crépel et al. 1992). First, neurons in CA3 are larger and less tightly packed than neurons in CA1, resulting in a 30–50% smaller surface area-volume ratio, a 50% lower neuronal density, and a 50% greater interstitial volume (Boss et al. 1987; McBain et al. 1990; Pérez-Pinzón et al. 1995). Thus, even identical increases in hypoxia-induced ion conductances would produce smaller changes in Nernst and transmembrane potentials in CA3 neurons when compared with CA1 neurons, thereby leading to reduced depolarization and associated cell swelling in CA3 neurons. This concept is further supported by the finding that hypoxic increases in extracellular [K+] and hypoxic decreases in extracellular volume are consistently smaller in the CA3 region than in CA1 (Kawasaki et al. 1990; Pérez-Pinzón et al. 1995). Two additional factors that may minimize depolarization in CA3 are the relatively low density of N-methyl-D-aspartate receptors (Cotman et al. 1987) and the relatively high density of ATP-sensitive potassium channels in CA3 compared with CA1 (Mourre et al. 1989). Additionally, the higher level in CA1 of succinate dehydrogenase, a mitochondrial enzyme involved in aerobic production of ATP (Kuroiwa et al. 1996), could play a role because the postischemic decrease in succinate dehydrogenase activity is accentuated in CA1 compared with CA3. Finally, there is more Na+K+ATPase activity in CA3 compared with CA1 (Haglund et al. 1985), which likely improves the ability of CA3 to maintain transmembrane ion concentration gradients and consequently to resist depolarization.

Association of hypoxic depolarization and optical changes indicative of swelling

HD and rapid decreases in ΔT/T always corresponded spatially as well as temporally, which had been observed previously with SD (Snow et al. 1983). This was confirmed in two ways: 1) by examining tracings of the extracellular DC potential and ΔT/T and 2) by noting the exact position of microelectrodes in images of the slice relative to the regions undergoing marked changes in ΔT/T. The consistent coincidence of these regions suggests that it is reasonable to use the spatial extent of ΔT/T as an index of the spatial extent of depolarization. Until recently, the consistent correlation between changes in light transmittance and swelling strongly implied that cell swelling was the cause of the changes in ΔT/T (Andrew and MacVicar 1994; Andrew et al. 1996; Kreisman et al. 1995; Turner et al. 1995). However, recent evidence indicates that changes in cell volume can be dissociated from changes in light transmittance under certain circumstances in interfaced slices (Aitken et al. 1998). There is no such dissociation in submerged slices, wherein the optical responses are reversed in polarity (Obeidat and Andrew 1998), probably because the optical properties of...
submerged slices are simpler than in interfaced slices. Most important, the data herein obtained from interfaced slices and those data obtained from submerged slices (Obeidat and Andrew 1998) show that there is a consistent relationship between HD and changes in light transmittance, regardless of opposite polarities of the optical response in experiments using different configurations of the slice-bath interfaces.

**Optical changes associated with HD arise from independent foci rather than spread from CA1**

Examination of serial images during hypoxia showed that changes in light transmittance arose independently in the CA1 and upper blade of the dentate gyrus. The independence of origin was confirmed by imaging \( \frac{\Delta T}{T} \) in minislices where CA1, CA3, and the dentate gyrus were physically separated from one another but exposed simultaneously to the same hypoxic stimulus. These experiments show that spread of depolarization from the CA1 region during hypoxia is not necessary for induction of depolarization in adjacent structures, supporting the concept that HD arises independently from multiple foci (Aitken et al. 1998). However, the factors that determine where depolarization is initiated within a given region remain to be determined.

**Prior episodes of spreading depression make the CA3 region vulnerable to HD**

In our experiments, the resistance of the CA3 region to HD was overcome consistently by exposing hippocampal slices to conditions that triggered episodes of SD, such as severe hypotonia, elevation of extracellular \([K^+]_o\), or removal of extracellular \(Ca^{2+}\). Additionally, more severe insults to energy supply than hypoxia alone, such as pretreating slices with high doses of DNP, an uncoupler of the mitochondrial electron transport chain, occasionally fostered the production of HD in CA3. This indicates that severe depletion of energy supply is a critical factor rendering CA3 vulnerable to HD rather than the occurrence of SDs per se. Confirmation of this hypothesis necessitates additional experiments that are beyond the scope of this investigation. From the results discussed, three major conclusions can be made about the CA3 region in vitro: 1) the CA3 region in vitro is resistant to depolarization during severe hypoxia and/or aglycemia. This is supported by a recent histopathological investigation showing that CA3 neurons in vitro are resistant to hypoxic injury (Newman et al. 1995). 2) Both the capability of the CA3 region in hippocampal slices to depolarize and the histological appearance of CA3 suggest that this region is viable. 3) The association between electrophysiological and histological data suggests that the in vitro hippocampal slice may be a useful model for investigating early mechanisms contributing to selective vulnerability and resistance to hypoxic-ischemic injury.

Our conclusions regarding the CA3 region are of practical significance because some investigators have reported that neurons in the CA3 region of hippocampal slices often do not appear histologically as viable as neighboring CA1 neurons, or CA3 neurons from intact hippocampi fixed in situ (Newman et al. 1995; Rice et al. 1994). Significant improvement in the histological appearance of hippocampal neurons was reported previously after preincubation of slices in media containing: 1) zero \(Ca^{2+}\) plus 10 mM \(Mg^{2+}\) (Feig and Lipton 1990), 2) 3% dextran (Newman et al. 1995), or 3) 400 \(\mu M\) ascorbate (Rice et al. 1994). In the present experiments, the CA3 region did not become vulnerable to HD or swelling after slices were pretreated with media shown by others to improve the histological appearance of CA3 neurons. Additionally, the histological appearance of CA3 neurons in the present experiments was comparable to that in the treated slices described above. This may be because our slices were preincubated in the interface position at room temperature (Newman et al. 1992; Pohle et al. 1986).

The inability to record orthodromic population spikes in CA3 in our experiments also could be interpreted as indicating damage to the region. However, others have noted that population responses from the CA3 region are often small in amplitude or absent (Johnston et al. 1992), whereas intracellular recordings confirm that the pyramidal neurons and their synaptic connections are viable and functioning (Crépel et al. 1992; Davis et al. 1986; Takata and Okada 1995). The explanation for this discrepancy is probably related to both the lower density and nongeometric alignment of CA3 neurons, compared with CA1 neurons—i.e., the varied orientations of individual dipoles contributing to extracellular field potentials tend to cancel rather than reinforce each other. Also, the larger extracellular volume surrounding CA3 neurons (McBain et al. 1990; Pérez-Pinzón et al. 1995) tends to reduce the amplitude of CA3 field potentials. Additionally, more synaptic contacts to CA3 neurons are probably cut in the preparation of hippocampal slices because of the wider ramification of their dendritic trees, compared with CA1 neurons (Johnston et al. 1992). Therefore, the absence of orthodromic population spikes cannot be taken as definitive evidence for the reduced viability of CA3 neurons.

In conclusion, the order of vulnerability of identified regions of the in vitro hippocampal slice to HD and swelling is similar to the pattern of histopathology observed after hypoxia-ischemia in vivo. As such, the results of the present investigation support the use of the hippocampal slice preparation for the investigation of acute changes that may have significance for irreversible hypoxic-ischemic neuronal injury.

We thank Drs. Catherine Cusick, Richard Harlan, and Joseph Weber for help with histology and K. Le for technical assistance.

This investigation was supported by a grant from the American Heart Association, LA Affiliate to N. R. Kreisman and grants from the National Institutes of Health (HD-01072 and HL-62372) and the Maternal and Child Health Bureau (MCJ-229163), and a Career Development Award from the American Lung Association (CL-002-N) to D. Gozal.

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Received 14 July 1999; accepted in final form 21 October 1999.

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