Rapid and Slow Swelling During Hypoxia in the CA1 Region of Rat Hippocampal Slices

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Kreisman, Norman R. and Joseph C. Lamanna. Rapid and slow swelling during hypoxia in the CA1 region of rat hippocampal slices. J. Neurophysiol. 82: 320–329, 1999. The role of swelling in hypoxic/ischemic neuronal injury is incompletely understood. We investigated the extent and time course of cell swelling during hypoxia, and recovery of cell volume during reoxygenation, in the CA1 region of rat hippocampal slices in vitro. Cell swelling was measured optically and compared with simultaneous measurements of the extracellular DC potential, extracellular [K+], and synaptic transmission in the presence and absence of hypoxic depolarization. Hypoxia-induced swelling consisted of rapid and/or slow components. Rapid swelling was observed frequently and always occurred simultaneously with hypoxic depolarization. Additionally, rapid swelling was followed by a prolonged phase of swelling that was ~15 times slower. Less frequently, slow swelling occurred independently, without either hypoxic depolarization or a preceding rapid swelling. For slices initially swelling rapidly, recovery of both cell volume and the slope of field potentials was best correlated with the duration of hypoxia (r = 0.77 and 0.87, respectively). This was also the case for slices initially swelling slowly (r = 0.70 and 0.58, respectively). In contrast, the degree of recovery of cell volume was the same at 30 or 60 min of reoxygenation, indicating that prolonging the duration of reoxygenation within these limits was ineffective in improving recovery. Spectral measurements indicated that the hypoxia-induced changes in light transmittance were related to changes in cell volume and not changes in the oxidation state of mitochondrial cytochromes. The persistent impairment of synaptic transmission in slices swelling slowly (i.e., without hypoxic depolarization) indicates that swelling may play a role in this injury and that hypoxic depolarization is not required. Additionally, the correlation between the degree of recovery of cell volume and the degree of recovery of synaptic transmission during reoxygenation supports a role for swelling in hypoxic neuronal injury.

INTRODUCTION

Selected regions of the brain, including cerebral cortex, cerebellum, striatum, and hippocampus, can be damaged by episodes of ischemia lasting only several minutes (Pulsinelli et al. 1982; Schmidt-Kastner and Freund 1991). A critical factor in the triggering of ischemic brain injury is hypoxic depolarization (Vyskočil et al. 1972), which consists of a profound increase in membrane permeability, dissipation of transmembrane ion gradients, and depolarization of cell membranes (Hansen 1985; Siesjö 1992; Somjen et al. 1990). The combination of hypoxia, the increased membrane permeability, and activation of the Na-K pump all contribute to depletion of ATP (Kass and Lipton 1982; Lipton and Whittingham 1982; Siesjö 1992). Additionally, brain cells swell because of an associated osmotic influx of water (Hansen and Olsen 1980; Hossmann 1971; Korf et al. 1988; Nemoto 1982). Prolonged hypoxic depolarization leads to irreversible neuronal damage because of the associated rise in intracellular [Ca2+] (Choi 1988; Kass and Lipton 1982; Roberts and Sick 1988; Siesjö 1992; Somjen 1990).

Whereas hypoxic depolarization and elevations in intracellular [Ca2+] are accepted widely as playing a role in neuronal injury, the role of cell swelling is more controversial. Some investigators believe that swelling contributes to brain damage by compressing brain tissue and blood vessels in the bony vault of the skull (see Lutz and Nilsson 1994). However, swelling may play a role in neuronal injury in the absence of elevated intracranial pressure. Diffusion-weighted magnetic resonance imaging, which measures changes in diffusion of water molecules as an index of cytotoxic edema, has shown that focal swelling is an important marker of both the extent of injury and recovery of function after restoration of blood flow and oxygenation in animal models of global ischemia and reperfusion (Busza et al. 1992; Hossmann et al. 1994; Minematsu et al. 1992). Additionally, focal swelling can serve as a marker of impaired function because both electrophysiological and metabolic recovery are related spatially and temporally to recovery of normal volume (Hossmann et al. 1994). Moreover, results of investigations in brain slices, where intracranial compression is not a factor, suggest that swelling can contribute directly to irreversible damage (Balestrino 1995).

Hypoxic cell swelling has been confirmed in brain slices by measurements of extracellular volume (Hansen and Olsen 1980; Pérez-Pinzón et al. 1995; Rice and Nicholson 1991; Syková et al. 1994), extracellular resistance (Chebabo et al. 1995; Jing et al. 1994), optical properties of brain tissue (Kreisman et al. 1995a; Turner et al. 1995), and intracellular volume (Melzian et al. 1996). Increasing the osmolality of solutions bathing hippocampal slices with agents such as mannitol delays the onset of hypoxic depolarization (Balestrino 1995) and enhances posthypoxic recovery of orthodromic responses in the CA1 region of the hippocampus (Huang et al. 1996). Conversely, prior hypotonic cell swelling exacerbates the hypoxic injury to synaptic transmission (Payne et al. 1996). In the present experiments, we characterized the extent and time course of cell swelling during hypoxia and re-oxygenation.

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in the hippocampal slice preparation. Our rationale for using hypoxia in this preparation is that neurons most vulnerable to ischemia in vivo are also the first to be affected by hypoxia in vitro (Dong et al. 1988; Somjen et al. 1990). Swelling and extracellular potential were compared in the presence and absence of hypoxic depolarization. Additionally, we investigated the relationship between recovery of synaptic transmission and recovery from swelling during reoxygenation. Some of the results have been presented in abstracts (Kreisman et al. 1994, 1995b).

METHODS

Preparation of hippocampal slices

Male Sprague-Dawley rats from Charles River (200–500 g) were anesthetized deeply with ether and perfused through the heart with 60 ml of bathing medium (4°C). The rats were decapitated, and the brain was removed and placed immediately in iced bathing medium with the following composition (in mM): 129 NaCl, 3.5 KCl, 2 MgSO4, 1 NaH2PO4, 2.7 CaCl2, 26 NaHCO3, and 10 glucose (osmolarity was 295–300 mosMl). The hippocampi were removed from the brain, and 400–500 mg of tissue were cut transversely with a tissue chopper (Stoelting, Wood Dale, IL). Slices were incubated in an interface-style holding chamber for a minimum of 90 min at room temperature (23–24°C). The slices then were placed on the nylon mesh of an interface-style recording chamber or, on a few occasions, submersed below another mesh. The bathing medium was maintained at 32–34°C and was pumped through the chamber at 0.6 ml/min. Warmed, humidified 95% O2–5% CO2 flowed over the surface of the bathing fluid at a rate of 480 ml/min.

Electrical stimulation and recording

CA1 pyramidal cells were activated orthodromically by applying constant current pulses (400 μA; 0.2 ms; 20- to 60-s intervals) to bipolar stimulating electrodes placed on the Schaffer collaterals. Extracellular field potentials were recorded from either the stratum pyramidale or stratum radiatum with 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 acceptable. Thereafter, recording electrodes were usually relocated to the CA1 stratum radiatum for recording extracellular DC potentials and excitatory postsynaptic potentials (EPSPs). EPSPs were triggered by stimulating the Schaffer collaterals with constant current pulses (10–100 μA; 1 ms) to produce a response that was 50–75% of maximum amplitude. In some experiments, extracellular resistance was estimated by measuring the extracellular voltage response (Vx) to a 1-ms constant current stimulus. Extracellular DC levels and optical signals were recorded continuously on a stripchart recorder, and evoked field potentials were recorded on either magnetic tape or computer disk via a MacLab digital data recorder.

Measurement of extracellular \([K^+]\)

Extracellular \([K^+]\) was measured from double-barreled glass pipettes with tip diameters of 2–4 μm (modified from Kreisman and Smith 1993). The \(K^+-\)sensing barrel was filled with 5–10% trifluoroisobutyrylchlorosilane (Pfalz and Bauer, Waterbury, CT) in CCl4 and the electrode baked at 180°C for 2 h. Thereafter, the tip was filled with potassium liquid ion exchanger, IE190 (World Precision Instruments, Sarasota, FL) to a height of 1–2 mm. The sensing barrel was back-filled with 150 mM NaCl. Extracellular \([K^+]\) and DC potential were measured with an Axon Instruments Axoprobe 1-A differential amplifier. Calibrations were conducted in the recording chamber before and after experiments. Acceptable calibration slopes were 50–58 mV for the 10-fold change in \([K^+]\) between 3 and 30 mM.

Measurement and imaging of light transmittance

White light from a quartz-halogen source was delivered to the bottom of the recording chamber via a 3.2-mm-diam fiberscope bundle. Transmitted light was detected by a silicon photodiode (Ealing, model 78–7821), coupled to one ocular of a Nikon binocular dissecting microscope via a 6.4-mm-diam fiberscope bundle. The optical field was ~0.4 × 1.2 mm at ×80 magnification, which included the CA1 stratum radiatum and stratum pyramidale. To calibrate the optical signal, basal light transmittance (T) was set to 0 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 (ΔT) were calculated as ΔT/T in percent.

To image light transmittance, the photodiode was replaced with an 8-bit digital video camera (Dage, model CCD72, Dage MTI, Michigan City, IN). Black level and dynamic range were set 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 the basal transmittance (T). Images were captured by a framegrabber every 1–3 s, depending on the experiment, using National Institutes of Health Image or custom imaging software (Synetic, Montreal, Canada). Stored images were analyzed off-line. Areas of interest were demarcated on an image of the slice and sequential values of ΔT/T were plotted as a function of time for each area.

Spectroscopy

Spectral measurements were performed by collecting light from the ocular of the Nikon dissecting microscope, via a 3.2-mm fiber optic bundle, to a rapid-scanning spectrophotometer (World Precision Instruments, Sarasota, FL). Wavelength precision was checked by placing filters with narrow optical band-pass in the light path. The optical field for these measurements was a spot ~1.5 mm in diameter. Each reported spectrum is the average of seven raw spectra, each integrated over a 10-s period.

Control and measurement of bath oxygenation

Hypoxia was induced by switching the gas mixture superfusing the slice from 95% O2–5% CO2 to 95% N2–5% CO2. Graded levels of hypoxia were produced by proportionally mixing the gases with flowmeters. In several experiments, PO2 in the bathing medium was measured polarographically using a 250-μm-diam platinum electrode exposed only at the tip. The electrode was polarized to −0.7 V (relative to an Ag/AgCl wire connected to ground), where its current output was a linear function of PO2. Calibrations were conducted at room air and 100% N2. Mean PO2 at 1 mm below the surface of the bath was 443 ± 19 (mean ± SE) mmHg during “normoxia,” 98 ± 6 mmHg during “moderate hypoxia,” and 20 ± 3 mmHg during “severe hypoxia” (n = 9). All hypoxic episodes reported in RESULTS should be considered as severe hypoxia unless noted otherwise. All data were obtained from interface slices unless noted otherwise.

RESULTS

Hippocampal swelling occurs at two different rates during hypoxia

Responses to hypoxia and reoxygenation were assessed in the CA1 region of rat hippocampal slices by simultaneously recording the extracellular potential (DC), light transmittance (ΔT/T), and occasionally the extracellular potassium ion concentration ([K+]e). Hypoxia-induced swelling proceeded at
Hypoxia. Slow swelling occurred in 15 of 44 slices (34%) and was observed commonly in response to either severe or moderate depolarization never occurred. Hypoxia produced only slow swelling and classic hypoxic potential shifted.

Cellular potential (DC), and percent changes in light transmittance (ΔT/T) during hypoxia and reoxygenation in a rat hippocampal slice in an interface chamber. A decrease in ΔT/T indicates cell swelling (see long, downward arrows). A: example of rapid swelling during a 6-min episode of hypoxia (horizontal black bar). Arrow a, time of blockade of orthodromic population spikes in CA1 pyramidal; arrow b, transient escape of evoked population spikes from synaptic blockade. Population spikes disappeared again when hypoxic depolarization was triggered. B: example of slow swelling during a 12-min period of hypoxia (horizontal black bar). Arrow a, time of hypoxia-induced blockade of orthodromic activation of CA1 pyramidal neurons.

Two different rates in hippocampal slices, hereafter designated as rapid and slow swelling (Fig. 1). Rapid swelling was associated consistently with hypoxic depolarization, as indicated by the synchronous occurrence of sharp decrease in ΔT/T, a rapid negative shift in the extracellular DC potential, and an abrupt increase in extracellular [K⁺], which peaked at levels exceeding 20 mM (Fig. 1A). In contrast, slow swelling occurred only in the absence of classic hypoxic depolarization; the DC shift was either small or absent and increases in extracellular [K⁺] were limited in rate and magnitude (Fig. 1B). In five hypoxic slices showing slow swelling, [K⁺]o rose from a normoxic baseline value of 4.3 ± 0.2 mM to a plateau of only 6.9 ± 0.7 mM (P < 0.05 by a one-tailed Student’s t-test) while the DC potential shifted −1.2 ± 1.6 mV. In 10 submerged slices, hypoxia produced only slow swelling and classic hypoxic depolarization never occurred.

Rapid swelling occurred in 29 of 44 slices (66%) and was observed commonly in response to either severe or moderate hypoxia. Slow swelling occurred in 15 of 44 slices (34%) and was observed most often in response to moderate hypoxia. These percentages likely underestimate the true probability of rapid swelling and overestimate the true probability of slow swelling because we purposely attempted to enhance the occurrence of slow swelling in many experiments by exposing slices to moderate hypoxia and/or slowing the rate of onset of hypoxia. However, we did not investigate the role of these factors systematically because these manipulations did not guarantee that slow swelling would occur. It should be noted, for comparison, that durations of hypoxia tended to be longer for slices swelling slowly (mean 16.5 ± 2.3 min; n = 14) compared with those swelling rapidly (mean 9.2 ± 1.1 min; n = 22). Hypoxia was imposed usually for longer periods in slices responding with slow swelling because it was difficult to discern whether slow swelling had in fact occurred with shorter durations of hypoxia.

Slices in the rapidly swelling group actually displayed two rates of swelling, as indicated by curve fitting with a double exponential function (Fig. 2). The initial rate, which only lasted 20–60 s, had a time constant (τ) that was 6–17 times faster than the second time constant (τ) (Table 1). The peak ΔT/T reached during the initial rate was nearly 12%, whereas the maximum ΔT/T attained by the end of hypoxia was about double this value. In contrast, slices in the slow-swelling group displayed only a single time constant of swelling that was virtually identical to the second, slower time constant of swelling in the initially fast swelling slices (Table 1).

An example of a slice responding with rapid swelling is shown in Fig. 1A. A 6-min episode of severe hypoxia initially induced a small, slow increase in ΔT/T of 1.6% (indicating modest cell shrinkage), which was associated with both a slow rise in [K⁺]o and a slow negative shift in the DC potential from baseline. Synaptic transmission diminished and failed during this early period (arrow a) then recovered partially (arrow b) but failed again at the onset of hypoxic depolarization. Rapid swelling accompanied hypoxic depolarization, indicated by a sharp decrease in ΔT/T to 27.3% below baseline within 2 min. Simultaneously, [K⁺]o rose to a peak value of 28 mM, and the DC potential shifted −10 mV. Reoxygenation promptly reversed the direction of ΔT/T, indicating amelioration of swell-

**FIG. 1.** Tracings of extracellular potassium concentration ([K⁺]o), extracellular potential (DC), and percent changes in light transmittance (ΔT/T), during hypoxia and reoxygenation in a rat hippocampal slice in an interface chamber. A decrease in ΔT/T indicates cell swelling (see long, downward arrows). A: example of rapid swelling during a 6-min episode of hypoxia (horizontal black bar). Arrow a, time of blockade of orthodromic population spikes in CA1 pyramidal; arrow b, transient escape of evoked population spikes from synaptic blockade. Population spikes disappeared again when hypoxic depolarization was triggered. B: example of slow swelling during a 12-min period of hypoxia (horizontal black bar). Arrow a, time of hypoxia-induced blockade of orthodromic activation of CA1 pyramidal neurons.

**FIG. 2.** Demonstration of 2 rates of swelling in hypoxic slices responding with rapid depolarization. Plot shows light transmittance (ΔT/T) as a function of time from a typical hippocampal slice showing rapid hypoxic depolarization. Curve fitting with a single exponential function (light dashed line) vs. double exponential functions (heavy dashed line) showed consistently that the best fit had 2 time constants. This verified the separation of rapid and slow phases of swelling (see Table 1 for statistical comparisons).
ing. After 23 min of reoxygenation, \([K^+]_o\) already had recovered to baseline, whereas \(\Delta T/T\) returned only to 12.5% below baseline (i.e., a 54.2% recovery), indicating that hippocampal cells remained somewhat swollen. Population spikes did not yet show any recovery from hypoxic blockade.

A typical example of slow hippocampal swelling, induced in this case by severe hypoxia, is shown in Fig. 1B. Hypoxia initially caused a brief increase in \(\Delta T/T\) by 2.2% (indicating modest cell shrinkage) but \(\Delta T/T\) then decreased slowly for the duration of hypoxia, to a nadir of 34% below baseline at 20 min of hypoxia (indicating cell swelling). As in the previous example, reoxygenation abruptly suspended swelling. Arrow A marks the time of synaptic blockade. Synaptic blockade persisted during hypoxia in this slice, as in all slices responding to hypoxia with slow swelling. There was no transient recovery as in many slices showing rapid swelling. Additionally, extracelular DC potential decreased by 5 mV while \([K^+]_o\) increased slowly and moderately. Despite maintained hypoxia, the DC potential recovered to baseline while \([K^+]_o\) remained elevated. Twenty minutes of reoxygenation produced only a small (32.4%) recovery of \(\Delta T/T\), to a plateau at 23% below the original baseline. This indicates persistent swelling after reoxygenation.

In addition to using \(\Delta T/T\) as an index of cell swelling, the extracellular voltage response \((V_e)\) to a 1-ms constant current stimulus was measured in 13 slices. An increase of \(V_e\) (i.e., a decrease in the volume of the extracellular space) should accompany cell swelling and be correlated with \(\Delta T/T\). \(V_e\) increased linearly as a function of \(\Delta T/T\) (Fig. 3) with correlation coefficients ranging from 0.69 to 0.95.

### Recovery of hippocampal cell volume during reoxygenation

During reoxygenation, both \(\Delta T/T\) and population spike amplitudes often failed to recover fully to normoxic values. As expected, the degree of recovery of cell volume was inversely related to the duration of hypoxia. Measurements of changes in \([K^+]_o\), extracellular DC potential, and light transmittance (\(\Delta T/T\)) during two sequential hypoxia-reoxygenation episodes from the same slice help illustrate this point (Fig. 4). The first episode of hypoxia lasted 3 min, and the second episode lasted 4 min. Depolarization occurred within 2 min after onset of hypoxia as indicated by the rapid increase in \([K^+]_o\) and the simultaneous negative DC shift. There was a concomitant decrease in \(\Delta T/T\), indicative of cell swelling, which followed a time course similar to that of the DC trace. Reoxygenation after 80 s of hypoxic depolarization resulted in recovery and overshoot of \(\Delta T/T\), indicating a rebound decrease in cell volume. The second episode of hypoxic depolarization, which lasted

### Table 1. Time constants (Tau) and magnitudes of \(\Delta T/T\) for hippocampal slices responding to hypoxia with rapid followed by slow swelling versus slow swelling alone

<table>
<thead>
<tr>
<th></th>
<th>(n)</th>
<th>(\text{Tau 1, min})</th>
<th>(\text{peak } \Delta T/T) for (\text{Tau 1, %})</th>
<th>(\text{Tau 2, min})</th>
<th>(\text{Maximum } \Delta T/T, %)</th>
<th>(\text{Hypoxia Duration, min})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid swelling</td>
<td>29</td>
<td>0.3 ± 0.05</td>
<td>12.8 ± 1.3</td>
<td>5.8* ± 0.5</td>
<td>31.4 ± 2.4</td>
<td>12.6 ± 2.2</td>
</tr>
<tr>
<td>Slow swelling</td>
<td>13</td>
<td>6.2* ± 1.1</td>
<td></td>
<td></td>
<td>26.2 ± 2.6</td>
<td>20.1 ± 3.2</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from \(\text{Tau 1}\) in slices swelling rapidly (\(P < 0.001\) by 1-tailed Student’s \(t\)-test). \(\text{Tau} = \text{time constant.}\)

**FIG. 3.** Typical examples from 2 different experiments (\(A\) and \(B\)) showing strong correlations between the extracellular voltage response \((V_e)\) to constant current pulses, an index of extracellular volume, and the change in light transmittance (\(\Delta T/T\)), an index of cell volume. \(R\), 1st-order regression coefficient; \(p\), probability that the points fit the regression line by chance.

**FIG. 4.** Examples from a typical experiment showing effects of duration of hypoxic depolarization on changes in \([K^+]_o\), extracellular DC potential, and light transmittance (\(\Delta T/T\)) and their recovery after reoxygenation. Two episodes of hypoxia are shown from the same slice. First episode lasted 3 min (horizontal bar at bottom left) and the 2nd episode lasted 4 min (horizontal bar at bottom right).
140 s, produced a larger decrease in $\Delta T/T$ (i.e., greater swelling) than with the previous episode. Little recovery of $\Delta T/T$ accompanied reoxygenation, indicating persistent swelling of cells in the slice. Extracellular $[K^+]_o$ also failed to recover fully in this case.

Factors contributing to the reoxygenation-induced recovery from rapid and slow swelling were evaluated by making scatter plots and calculating first-order regression coefficients (Fig. 5).

The degree of recovery of cell volume from rapid swelling was related inversely to the duration of hypoxia ($r = 0.732$; $P < 0.001$; $n = 22$), the duration of hypoxic depolarization ($r = 0.640$; $P < 0.005$), and maximal $\Delta T/T$ ($r = 0.678$; $P < 0.001$). Recovery from rapid swelling tended to be good if the duration of severe hypoxia was <5 min but recovery was poor if the duration of severe hypoxia exceeded 10 min (Fig. 5, A and C). Increasing the duration of reoxygenation failed to enhance recovery (Fig. 5B and Table 2). Likewise, the degree of recovery from slow swelling was related inversely to both the duration of hypoxia ($r = 0.698$; $P = 0.017$; $n = 11$) and the maximal $\Delta T/T$ attained during hypoxia ($r = 0.670$; $P = 0.020$). As with rapid swelling, the degree of recovery from slow swelling was not enhanced by increasing the duration of reoxygenation (Fig. 5D and Table 3).

Comparisons were made of the magnitude of hypoxia-induced swelling and reoxygenation-induced recovery of cell volume in rapidly and slowly swelling slices that were exposed to equal durations of hypoxia (Table 4). Peak $\Delta T/T$ during hypoxia was significantly greater, and the percent recovery of $\Delta T/T$ during reoxygenation was significantly lower, in rapidly swelling slices. This comparison should be made with caution, however, because we often purposely attenuated both the degree of hypoxia and its rate of onset to enhance the probability of slices responding with slow swelling.

**Recovery of fEPSPs and population spikes during reoxygenation**

Initial slopes of fEPSPs from the CA1 stratum radiatum were measured in 13 of 22 slices responding to hypoxia with

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**TABLE 2. Recovery of fEPSP slope and light transmittance ($\Delta T/T$) in rapidly swelling slices as a function of duration of reoxygenation**

<table>
<thead>
<tr>
<th>Reoxygenation</th>
<th>30 Min</th>
<th>60 Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent recovery of fEPSP slope</td>
<td>71.7 ± 9.2</td>
<td>73.9 ± 8.7</td>
</tr>
<tr>
<td>Percent recovery of $\Delta T/T$</td>
<td>80.1 ± 7.1</td>
<td>76.9 ± 9.3</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.583 ($P = 0.018$)</td>
<td>0.855 ($P = 2.4 \times 10^{-5}$)</td>
</tr>
</tbody>
</table>

Values for percent recovery are means ± SE ($n = 16$). fEPSP, fast excitatory post-synaptic potential.

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**TABLE 3. Recovery of population spike amplitude and light transmittance ($\Delta T/T$) in slowly swelling slices as a function of duration of reoxygenation**

<table>
<thead>
<tr>
<th>Reoxygenation</th>
<th>30 Min</th>
<th>60 Min</th>
</tr>
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<tbody>
<tr>
<td>Percent recovery of population spike</td>
<td>58.7 ± 25.1</td>
<td>59.6 ± 24.6</td>
</tr>
<tr>
<td>Percent recovery of $\Delta T/T$</td>
<td>55.3 ± 18.1</td>
<td>54.0 ± 17.7</td>
</tr>
<tr>
<td>Correlation coefficient ($r$)</td>
<td>0.742 ($P = 0.151$)</td>
<td>0.730 ($P = 0.161$)</td>
</tr>
</tbody>
</table>

Values for percent recovery are means ± SE ($n = 5$).
rapid swelling. In all slices, fEPSPs were blocked within minutes of onset of hypoxia. Recovery of fEPSP slope during reoxygenation was related to recovery of $\Delta T/T$ (Figs. 6 and 7B, and Table 2). The degree of recovery of the initial fEPSP slope was correlated roughly with recovery of $\Delta T/T$ at 30 min of reoxygenation but the correlation improved considerably at 60 min of reoxygenation (Table 2). Despite the differences in correlation coefficients, mean recoveries of fEPSP slope and $\Delta T/T$ were similar at both 30 and 60 min of reoxygenation (Table 2). Likewise, recovery of population spike amplitudes and $\Delta T/T$ were similar to each other both at 30 and 60 min of reoxygenation in five slices that swelled slowly during hypoxia (Fig. 8 and Table 3). The degree of recovery of population spike amplitude was related generally to the degree of recovery of $\Delta T/T$ (compare Fig. 8, A and B). However, the degree of recovery was less in slowly swelling slices than in rapidly swelling slices (compare data in Tables 2 and 3), likely because slowly swelling slices were exposed to longer durations of hypoxia.

**Table 4. Effects of comparable durations of hypoxia and reoxygenation on slow versus fast swelling and recovery of cell volume**

<table>
<thead>
<tr>
<th>Duration, min</th>
<th>Peak Hypoxic $\Delta T/T$, %</th>
<th>Recovery of $\Delta T/T$, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slow swelling</td>
<td>16.5 ± 2.3</td>
<td>31.1 ± 2.5</td>
</tr>
<tr>
<td>Fast swelling</td>
<td>15.3 ± 1.3</td>
<td>34.0 ± 2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Hypoxic swelling in the various strata of CA1

The largest hypoxia-induced changes in $\Delta T/T$ occurred consistently in the stratum radiatum, with lesser changes in the strata oriens and pyramidale, respectively (Fig. 9, C and D). This order of swelling was similar to that observed in response to treatment of the slices with hypoosmotic media (Fig. 9A). Two phases of $\Delta T/T$ often were evoked by hypoxia in both rapidly and slowly swelling slices: a small increase in $\Delta T/T$ (shrinkage) followed by a larger decrease in $\Delta T/T$ (swelling). The extent of reoxygenation-induced recovery of $\Delta T/T$ in each layer of CA1 varied in different experiments (e.g., compare responses in Fig. 9, B–D).

**Spectral transmittance during hypoxia**

Transmission spectra were measured from two hippocampal slices during normoxia, hypoxia, isosmotic, and hypoosmotic conditions (Fig. 10). Transmission spectra during two separate episodes of normoxia taken 10 min apart were indistinguishable, indicating stable baseline transmittance over such time periods (Fig. 10A). In the same slice, spectral transmittance at 10 min of hypoxia decreased by as much as 10% compared with the immediately preceding period of normoxia, particularly at wavelengths > 525 nm (Fig. 10B). This was nearly identical to the shift in the transmission spectra during hypoosmotic swelling during normoxia (Fig. 10C), produced by decreasing osmolarity of the medium from 295 to 250 mosM/l. There was no evidence of absorbance changes at specific wavelengths characteristic of mitochondrial cytochromes.

**Discussion**

**Rapid versus slow swelling**

One of the most important findings of this investigation is that there are two rates of swelling in the CA1 region of
hippocampal slices during hypoxia. This was confirmed by the best fit of two exponential functions to the $\Delta T/T$ traces in response to hypoxia (Fig. 2 and Table 1). Fast swelling, as indicated by a profound and rapid change in $\Delta T/T$, was associated consistently with hypoxic depolarization, as indicated by a simultaneous negative shift of the extracellular potential and a sharp increase in extracellular $[K^+]$. In contrast, slow swelling occurred either in the absence of hypoxic depolarization (1/3 of the slices) or subsequent to hypoxic depolarization (2/3 of the slices). Hypoxic depolarization is triggered by a non-specific increase in membrane permeability and the associated efflux of $K^+$ and influx of $Na^+$, $Ca^{2+}$, and $Cl^-$ (Hansen and Zeuthen 1981; Vyskočil et al. 1972), a permeability change identical to that seen in spreading depression (Kraig and Nicholson 1978). Rapid swelling would be expected to accompany hypoxic depolarization and spreading depression because the influx of $Na^+$, $Ca^{2+}$, and $Cl^-$ should osmotically obligate the influx of water (Hansen 1985). In conjunction with these findings, the optical responses indicative of rapid hypoxic swelling observed here are remarkably similar to those reported during spreading depression in hippocampal slices (Snow et al. 1983).

Cell swelling during hypoxia has been inferred from repeated observations of increases in tissue impedance (Hossmann 1971; Jing et al. 1994; Korf et al. 1988), which are consistent with decreases in interstitial volume. Similar increases in impedance have been measured during severe hypoosmotic swelling in hippocampal slices (Chebabo et al. 1995) and during spreading depressions and hypoxic depolarizations (Jing et al. 1994). Other investigators measured hypoxia-induced decreases in the extracellular volume fraction (Hansen and Olsen 1980; Lundbæk and Hansen 1992; Pérez-

![FIG. 8. Effects of 30 and 60 min of reoxygenation on recovery of population spike amplitude (A) and $\Delta T/T$ (B) in the CA1 region of hippocampal slices that swelled slowly during hypoxia. Data are plotted as percent of control values. In B, positive changes in $\Delta T/T$ indicate cellular shrinking, 0 indicates normal levels of $\Delta T/T$, and negative values indicate cellular swelling.](http://jn.physiology.org/)

![FIG. 9. Changes in light transmittance ($\Delta T/T$) in various layers of the CA1 region in rat hippocampal slices during exposure to various insults, including: 260 mosM hypoosmotic medium (A), mild hypoxia (B), and severe hypoxia short and long durations (C and D, respectively). Swelling is indicated by a downward deflection of the $\Delta T/T$ trace. RAD, stratum radiatum; PYR, stratum pyramidale; OR, stratum oriens.](http://jn.physiology.org/)
Pinzón et al. 1995; Sykova et al. 1994), which were derived from changes in diffusion of an extracellular space marker (Rice and Nicholson 1991). Additionally, tissue swelling during hypoxia was confirmed recently by measuring changes in the intracellular concentration of a fluorescent dye (Melzian et al. 1996). Finally, brain tissue swelling during ischemia has been measured in situ by magnetic resonance imaging of the apparent diffusion coefficient of water (Busza et al. 1992; Hossmann et al. 1994; Minematsu et al. 1992). None of these investigations, however, differentiated fast versus slow swelling, probably because the time resolution of their measurements was set to measure only peak changes.

Inferences about a slow process, likely involving slow depolarization and swelling, can be made from existing reports of responses of neural tissues to hypoxia. Some tissues, such as spinal cord and peripheral nerves, respond to hypoxia with slow depolarization and seldom, if ever, respond with rapid depolarization (Collewijn and Van Harreveld 1966; Wright 1947). Fujiwara et al. (1987) reported slow transmembrane depolarization of 1–2 mV/min in 50% of the hippocampal neurons they tested during hypoxia. Membrane potentials at 20 min of hypoxia were depolarized ~25 mV from the resting level, which would be expected to inactivate voltage-gated conductances. Additionally, slow depolarization was observed in both hypoglossal and neocortical neurons during hypoxia (O’Reilly et al. 1995). Slow depolarization would be expected to be accompanied by slow swelling in response to qualitatively the same ionic fluxes described in the preceding text. This is supported in our experiments by the temporal association of moderate increases in extracellular [K+] and a small negative DC shift with slow changes in both ΔT/T and extracellular resistance. A virtually identical correlation between shrinkage of the extracellular space and a gradual decay of K+ homeostasis was reported during hypoxia in submerged striatal slices (Rice and Nicholson 1991). Finally, Croning and Haddad (1998) observed hypoxic changes in extracellular [K+] in submerged hippocampal slices that look identical to those recorded here during slow swelling. Proof of a causal relationship between slow depolarization and slow swelling would require recording of transmembrane potentials in conjunction with optical measurements. The factors that determine whether fast or slow depolarization and swelling will occur during hypoxia are unknown but likely involve a variety of ion channels, and possibly transport mechanisms, in the cell membrane.

Recovery of cell volume and synaptic transmission after rapid versus slow hypoxic swelling

Our results emphasize that incomplete recovery of synaptic transmission can occur both in slices responding to hypoxia with rapid depolarization and swelling or slow swelling (and presumably slow depolarization). Heretofore it generally had been accepted that the likelihood of permanent block of synaptic transmission after reoxygenation is related directly to the duration of rapid hypoxic depolarization and not the duration of hypoxia per se (Balestrino and Somjen 1986). However, these and other investigators also emphasize that the duration of hypoxic depolarization is not the only factor involved (Balestrino et al. 1988; Rader and Lanthorn 1989). Our findings indicate that both duration of hypoxia and duration of rapid hypoxic depolarization are important factors in failure of synaptic transmission to recover during reoxygenation. Our results show also that recovery of synaptic transmission is impaired after slow hypoxic swelling (and presumably slow depolarization). The correlation between duration of hypoxia and the degree of impairment on reoxygenation is similar to that observed for slices swelling rapidly. In support of our observations, others also have reported persistently impaired
synaptic transmission in the absence of conventional, fast hypoxic depolarization (Chen et al. 1996; Croning and Haddad 1998; Schiff and Somjen 1987). Additionally, neuroprotective agents can work independently of their effect to delay hypoxic depolarization (Chen et al. 1996), supporting a role for other factors in impaired posthypoxic synaptic transmission. Chen et al. (1996) also suggest that the persistent synaptic blockade during reoxygenation after slow hypoxic depolarization is more characteristic of the ischemic penumbra than the ischemic core because slow depolarization appears most often to be associated with moderate hypoxia.

Relationship of rapid and slow swelling to severity of hypoxia

The tendency of slow swelling to occur most often during more moderate degrees and slopes of hypoxia suggests that the rate of swelling is related to the severity of hypoxia. However, the relationship is not a simple one because sometimes severe hypoxia also led to slow swelling. Additionally, rapid swelling occurred during either severe or moderate hypoxia. Unfortunately, the unpredictability and relatively infrequent appearance of slow swelling made it difficult to investigate this relationship systematically in our experiments. In contrast with our results in interface slices, the few submerged slices we investigated always responded to hypoxia with slow swelling. Additionally, classic hypoxic depolarization was never observed in our submerged slices, in agreement with results of others (Croning and Haddad 1998). Spot checks of bath PO$_2$ in our submerged slices revealed lower PO$_2$ levels in normoxia and higher PO$_2$ levels in hypoxia than in our interface slices. These preliminary observations support the suggestion that slow swelling is related to milder hypoxia but do not rule out the possibility that other factors play a role.

Are recoveries of cell volume and synaptic transmission causally related?

Although the correlation between recovery of synaptic transmission and recovery from swelling observed here suggests a causal relationship, our data neither provide proof nor suggest an underlying mechanism. We speculate that swelling might contribute to posthypoxic depolarization, e.g., through dilution of intracellular [K$^+$], which would inactivate voltage-gated ion conductances and depress excitability. In fact, intraneuronal [K$^+$] is lower than normal after reoxygenation (Jiang and Haddad 1991; Kass and Lipton 1982), but some investigators argue that the low intracellular [K$^+$] during reoxygenation is insufficient to account for the persistent blockade of excitability (Kass and Lipton 1982). Another qualification is that hypoxic swelling alone does not depolarize either hippocampal or neocortical neurons (Ballyk et al. 1991; Rosen and Andrew 1990; Saly and Andrew 1993), but hypoxic swelling may have different consequences. Most importantly, measurements of membrane potentials from neurons exposed to hypoxia of several min duration show persistent posthypoxic depolarization despite prolonged reoxygenation (Fujiwara et al. 1987; O’Reilly et al. 1995; Rader and Lanthorn 1989). At minimum, resolution of this issue will require additional experiments in which simultaneous measurements are made of all the relevant variables.

Methodological considerations

One potential criticism of the use of light transmittance changes as an index of hypoxia-induced changes in cell volume is that other factors might affect light transmittance during hypoxia. The most likely interference is from changes in the oxidation-reduction state of mitochondrial cytochromes (Sick and LaManna 1995). There was no evidence of absorbance changes at specific wavelengths characteristic of mitochondrial cytochromes in spectra from our slices. If these changes occur, they are orders of magnitude smaller than the volume-related optical signal.

In conclusion, the persistent impairment of synaptic transmission in slices swelling slowly (i.e., without hypoxic depolarization) indicates that swelling may play a role in this injury and that hypoxic depolarization is not required. Additionally, the correlation between the degree of recovery of cell volume and the degree of recovery of synaptic transmission during reoxygenation supports a role for swelling in hypoxic neuronal injury.

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REFERENCES


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