Rapid Monitoring of Diffusion, DC Potential, and Blood Oxygenation Changes During Global Ischemia
Effects of Hypoglycemia, Hyperglycemia, and TTX

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Background and Purpose—The increasing interest in diffusion-weighted MRI (MRI) for diagnosis and monitoring of acute stroke in humans calls for a sound understanding of the underlying mechanisms of this image contrast in acute cerebral ischemia. The present study aimed to show that a rapid decrease in brain-water apparent diffusion coefficient (ADC) occurs coincident with anoxic depolarization and that this change is delayed by hyperglycemia and sodium channel blockade but accelerated by hypoglycemia.

Methods—Rats were divided into groups: normoglycemic, hypoglycemic, and hyperglycemic, and those given local tetrodotoxin (TTX) application. Cardiac arrest was effected by intravenous KCl injection during serial high-speed diffusion and blood oxygenation–sensitive gradient-recalled echo MRI. Brain DC potential was recorded simultaneously. Serial ADC maps were calculated from the diffusion-weighted data and fitted to a model function to measure the delay between cardiac arrest and rapid ADC decrease.

Results—The time of anoxic depolarization indicated by DC change agreed well with the rapid drop in ADC in all groups; both were accelerated with hypoglycemia and delayed by hyperglycemia. A more gradual ADC decline occurred before anoxic depolarization, which was more pronounced in hyperglycemic animals and less pronounced in hypoglycemic animals. Rapid drop in ADC was also delayed by local TTX application. Changes in gradient-recalled echo image intensity were not significantly different among groups.

Conclusions—While much of the ADC decrease in ischemia occurs during anoxic depolarization, significant but gradual ADC changes occur earlier that may not be due to a massive loss in ion homeostasis. (Stroke. 1999;30:2212-2222.)

Key Words: magnetic resonance imaging • cerebral ischemia, global • membrane potentials • rats

The reduction in apparent diffusion coefficient (ADC) of brain tissue during stroke has made diffusion-weighted MRI increasingly popular for the early detection and characterization of acute ischemic brain disease in stroke patients. The exact mechanisms behind these changes in brain water diffusion during stroke are still the subject of some discussion. However, a consensus has emerged that attributes ADC changes in ischemia to a net shift of water from the extracellular to the intracellular space as a result of anoxic cell membrane depolarization, although other mechanisms, such as changes in membrane permeability, may also play a role. Many authors have studied the changes in ADC immediately after ischemia in animal models and have shown that ADC reduces rapidly within the first few minutes of stroke and decays more slowly thereafter. In particular, high-speed diffusion measurements have shown an initial gradual decline in ADC followed by a rapid drop within the first few minutes after the onset of ischemia. Research has also shown that when the Na⁺/K⁺ pump is disabled by intraparenchymal ouabain, the ADC decreases, which supports a link between altered ion homeostasis and alteration in ADC. Failure of the transmembrane ion pumps and subsequent loss of cell membrane potential is immediately followed by a disruption of the ion homeostasis. The resulting ionic imbalance causes an osmotically driven flow of water into the cells, and the subsequent cell swelling reduces the ADC as detected by MRI.

Recently studies of global and focal cerebral ischemia using rapid diffusion-weighted line scanning in normoglycemic and hyperglycemic rats have found that the initial rapid decrease in ADC is not affected by increased preischemic plasma glucose levels, but that the subsequent ADC decline is prolonged in the hyperglycemic group. These findings are difficult to explain and seem to be in contrast to our own studies of remotely induced focal cerebral ischemia, which showed a delayed ADC decline in hyperglycemic rats compared with normoglycemic controls. An earlier study by Hansen that measured extracellular potassium concentra-
tions ([K+]i) showed that the time from cardiac arrest to a dramatic rise in [K+], (indicating anoxic depolarization) is significantly lengthened by hyperglycemia and reduced by hypoglycemia. With this in mind, one might expect similar changes in the ADC curves after cardiac arrest, if cell swelling due to anoxic depolarization is indeed a major mechanism behind the ischemic changes in water diffusion. In the present study, we have performed simultaneous rapid measurements of ADC (using echo-planar imaging), relative blood oxygenation level (from gradient recalled echo images), and brain DC potential (to indicate anoxic depolarization) during cardiac arrest–induced global ischemia at different preischemic plasma glucose levels. Cardiac arrest provides a convenient and simplified cerebral ischemia model, because CBF is known to go to zero at a precisely defined time. Varying plasma glucose levels from hypoglycemia to hyperglycemia have a well-known and measurable effect on the delay to anoxic depolarization. In addition, we performed experiments on normoglycemic animals by application of tetrodotoxin (TTX) topically to the cortex. TTX specifically blocks voltage-dependent sodium channels and delays the onset of anoxic depolarization and large-scale loss in ion homeostasis in regions into which the TTX has diffused.

Subjects and Methods

Animal Preparation

All animal procedures were performed within the guidelines for animal research of Stanford University School of Medicine. Male Sprague-Dawley rats (280 to 320 g, n = 20; Harlan) were anesthetized with halothane (0.75% to 1.5%) through a passive-flow face mask. Arterial and venous femoral catheters were inserted for delivery of KCl, blood pressure measurement, and sampling for arterial blood gas analysis. Physiological parameters were kept within the normal range. Temperature was maintained at 37 ± 1°C using a warm-air circulation system and measured using a rectal probe. A pulse oximeter probe was connected to 1 hind paw. DC potentials were recorded from a 150-μm-diameter silver/silver chloride electrode that was placed through a small burr hole 4 to 5 mm in front of the bregma. DC potential, blood pressure, temperature, heart rate, and capillary oxygen saturation were recorded and displayed continuously on a Macintosh computer connected to a computerized chart recorder system (MacLab, CB Sciences Inc). In addition, a spare digital line on the scanner, programmed to pulse at the start of each image set, was connected to the data-recording system to allow accurate temporal correlation of the MRI data, the DC potential, and the mean arterial blood pressure (MAP) recordings.

Four groups of rats were defined: group I, normoglycemic (n = 7); group II, hypoglycemic (n = 5); group III, hyperglycemic (n = 5); and group IV, normoglycemic given topical TTX (n = 3). Animals in group II were made hypoglycemic by intraperitoneal injection of 3 international units (IU)/kg of insulin 2 hours before the experiment. Animals in group III were made hyperglycemic by intraperitoneal injection of streptozotocin 36 hours before the experiment followed by intravenous injection of 1 mL of 50% glucose solution 1 hour before imaging. For group IV animals, symmetrical apertures were opened in the skull to either side of the midline, and the dura was removed in these regions to expose 2 approximately 5-mm-diameter regions of cortical tissue. About 1 hour before scanning, a solution of 10−4 mol/L TTX was applied to the left cortex and NaCl solution was applied to the right cortex. The DC recording electrode was placed ipsilateral to the site of TTX application through a separate burr hole; care was taken to avoid leakage of any fluid onto the electrode. Samples of arterial blood were drawn for blood gas analysis and glucose measurement shortly before initiation of global ischemia inside the magnet.

MR Measurements

MR experiments were performed on a 2.0-T GE/Bruker CSI Omega system (Bruker Instruments Inc) using a spin-echo echo-planar imaging (EPI) technique. All studies used a home-built 2.7-cm-diameter transmit/receive surface coil placed on top of the head. Multislice T1-weighted gradient-echo scout images were first acquired to localize the position of the DC electrode by means of its small susceptibility artifact. Next, the 2 coronal EPI imaging slices were positioned so that the anterior slice included the DC recording electrode. ADC was measured with a sequence of 3 diffusion-weighted EPI images (TE, 50 milliseconds; TR, 2 seconds; field of view, 40 mm; 1.5-mm slice thickness; 64 × 64 matrix; 2 coronal slices 3.5 mm apart; 1 average; diffusion weighting along the Z direction; and a low b value of 30 s/mm2 and a high b value of 1300 s/mm2 acquired twice). This was followed by gradient-recalled echo (GRE)-EPI imaging with a 30-millisecond echo time. This set of 4 images per slice was repeated continuously for up to 25 minutes. After each baseline image set (approximately 6.7 minutes), cardiac arrest was induced by an intravenous bolus injection of KCl solution.

Data Processing

Image analysis and display were performed using custom-written software (MRVision Co). The diffusion-weighted data were processed to generate serial ADC maps. The T2*-weighted GRE-EPI images were normalized to create images of the percentage change in image intensity from the baseline. The temporal resolution of the ADC and GRE measurements was 8 seconds, whereas the nominal spatial resolution was 1.5 × 0.63 × 0.63 mm2 over 2 coronal slices, with a nominal voxel volume of 0.6 μL. As has been pointed out previously, an important factor to consider when making such serial diffusion measurements is the stability of the ADC value over time, which determines the smallest ADC change that can be detected. This can be quantified by the coefficient of variation (CV), defined as the standard deviation (SD) of a series of images divided by the mean, times 100%. Therefore, we calculated CV on a pixel-by-pixel basis using the 50 baseline ADC images acquired in each experiment; this indicates the sensitivity for detecting small ADC changes and the stability of the ADC measurements from day to day under the influence of small variations in surface coil positioning, scanner stability, noise, etc.

The ADC versus time curves at each pixel position were analyzed by fitting a model function to the data to extract relevant parameters that characterize acute ADC changes after global ischemia. The model function consisted of 3 segments: an exponential decay, a more rapid linear decline, and then another slower exponential decay to the final ADC value. This function, described in detail in Figure 1, was not intended to model any particular underlying physiological process, but simply provides a convenient way of describing the data in terms of a reduced number of parameters. The 3 segments of the model function were chosen to be the simplest mathematical description of what we have observed to be the time course of acute ADC change in ischemia. The model function was fitted to the measured ADC time course for each pixel position in each slice with the use of an iterative nonlinear least-squares minimization procedure (simplex algorithm). The following subset of the model parameters was chosen and mapped over both imaging slices for all animals: Baseline ADC: initial ADC value during baseline scans.

\[ \text{Baseline ADC} = M_0 \]

\[ \Delta M_0 = \frac{M_v - M_0}{M_0} \]

\[ \tau_1; \text{fractional ADC decrease during exponential segment I (relative to baseline)} \]

\[ \tau_2; \text{duration of exponential segment I (up to the more rapid drop)} \]

\[ dM_1; \text{time constant of initial exponential segment I} \]

\[ M_C; \text{final plateau ADC values (as a fraction of baseline ADC)} \]

The \( \tau_1 \) parameter is particularly important, because it may be compared with the time of the DC potential change due to anoxic cell membrane depolarization. The T2*-weighted GRE-EPI image intensity versus time data were analyzed by measuring regions of interest (ROIs) in different anatomical areas and fitting the curves to a fifth-order polynomial function. From the fitted function, values for the time from...
Figure 1. Composite function used to model the ADC decay curves for each pixel of image data during global ischemia. Function consists of a fixed (user-defined) number of baseline time points followed by an exponential decay segment (I) of time constant \( \tau_1 \), a linear portion (II) to describe the more-rapid ADC drop, and another exponential segment (III) to model the slower ADC decline to its final plateau value. The 3 segments of the function are as follows:

**Exponential:**

\[
ADC = M_0 - dM_1 \times \left\{ \exp\left[\frac{-t}{\tau_1}\right] \right\} = M_0 - \frac{dM_1}{\tau_1} \times \exp\left[\frac{-t}{\tau_1}\right] - 1
\]

**Linear:**

\[
ADC = M_0 - dM_1 \times (t - T_0 - \tau_1) \times dM_1 / \tau_2
\]

**Exponential:**

\[
ADC_{III} = M_0 + (M_0 - dM_1 - dM_2) \times \exp\left[-\frac{(t - T_0 - \tau_1 - \tau_2)}{\tau_3}\right]
\]

where \( t \) is the time after the end of the baseline images, \( \tau_1 \) is the duration of the first exponential decay segment, \( dM \) is the fractional change during first segment (relative to \( M_0 \)), \( \tau_2 \) is the exponential decay constant of the first segment, \( \tau_3 \) is the duration of the linear segment, \( dM_2 \) is the fractional change during linear segment (relative to \( M_0 \)), \( \tau_3 \) is the exponential decay constant of the last segment, and \( M_0 \) is the final (plateau) ADC value.

Cardiac arrest until the maximum signal decrease (\( \tau_{abi} \)) and also the relative image intensity at this point (\( M_{abi} \)) were calculated.

Timing parameters from the ADC and GRE data (measured relative to the start of the MRI scan) were corrected to be relative to the actual time of cardiac arrest by comparing the MABP traces and the scan synchronization pulses recorded on the MacLab machine. The MacLab device records each data point (ie, digitized physiological measurement or scan synchronization pulse) along with the exact time of day (to the nearest millisecond) of that measurement at a rate of 40 values per second for each channel. The time axis for all the plots of the physiological variables was calculated by subtracting the time of day of the first scan synchronization pulse (indicating the start of MRI data acquisition) from the time of day of each of the recorded data points. Subsequent visual observations of these plots of the time point of the sudden MABP drop at cardiac arrest and the time point of DC potential drop gave timing values as offsets from the start of MRI data acquisition. All timing parameters in the present article are shown after subtraction of the time point of sudden MABP drop for each animal.

The parametric maps resulting from the ADC curve-fitting procedure were further analyzed in 2 ways. First, all pixels within the brain on the \( \tau_1 \) parametric maps for both imaging slices for which the baseline CV was less than approximately 5% were collected for groups I through III and displayed as histograms of the \( \tau_1 \) parameter. Second, ROIs were defined in the cortex, subcortical white matter, deep gray (basal ganglia) matter, and cortex beneath the DC recording electrode. These ROIs were transferred to the calculated parameter images, and the regional average values of baseline CV, ADC, and the fitting coefficients \( \tau_1, dM_1, \tau_3 \), and \( M_1 \) were measured. The ROI measurements were made on parameter maps formed by pixel-by-pixel fitting of the model function rather than by being averaged before the fitting procedure, to reduce the effects of partial volume averaging on the accuracy of the fitted curves. For each anatomic region, the measured values for groups II and III were compared individually with group I by use of Student’s t test. Also, the values for \( \tau_3 \), measured in the cortex under the DC electrode were compared with the time of rapid DC potential drop within each group.

**Results**

**Physiological Data**

Physiological parameters remained within the normal range throughout the experiments and did not differ significantly between groups. The values for plasma glucose concentration were as follows: for the normoglycemic group, 5.5±1 mmol/L; for the hypoglycemic group, 2.1±0.5 mmol/L; and for the hyperglycemic group, 30.5±9.0 mmol/L. The hypoglycemic and hyperglycemic values were significantly different from the normoglycemic value (\( P < 0.001 \)) but were similar to the values previously reported by Hansen.¹⁹

**MABP and DC Potential**

Blood pressure began to decrease immediately after the end of the KCl injection and took 6.4±2.9 seconds to fall to <10% of the starting value. Overall, usable DC recordings were obtained from 13 of the 20 animals studied. In 7 animals, disruption of the DC trace occurred as a result of electrical interference from the gradient and radiofrequency pulses of the MRI scanner, despite heavy filtering, and also as a result of degradation of the electrode-brain contact during the experiment. Nevertheless, on 65% of the traces, a rapid drop in DC was observed after cardiac arrest, attributable to anoxic cell membrane depolarization. The size of this DC change was 4.7±2.4 mV, and it lasted 12±7 seconds. EEG traces were obtained from 13 of the 20 animals studied. Despite much interference, we were able to estimate the times at which the EEG fell to zero after cardiac arrest to be 9.0±4.2, 7.7±3.1, 12.5±4.5 seconds for groups I, II, and III respectively. The difference among the groups was not statistically significant.

**Magnetic Resonance Imaging**

The surface coil probe gave images with good signal-to-noise ratio over the whole brain. The CV of the ADC data measured over the 50 baseline images was consistently <5% except at the very edge of the brain and at the base of the brain because of increased distance from the NMR surface coil (which reduces the signal-to-noise ratio in that region).

Images and curves for a group I animal (normoglycemic) are shown in Figure 2. The blood pressure rapidly fell to zero immediately on KCl injection, whereas the drop in DC potential associated with anoxic depolarization occurred about 1.5 minutes later. Single-voxel curves are shown from 4 different locations, as indicated on the ADC map. Figure 2, plot 1, was measured from the edge of the brain beneath the DC recording electrode. As expected, the ADC curve is similar in shape to the model function shown in Figure 1, with the steepest part of the decay coincident with the sudden drop in DC potential. The gradient-echo signal was noisy here because of the susceptibility artifact from the electrode. A similarly shaped ADC curve is seen in Figure 2, plot 2, from another cortical area with the same gradual ADC decline followed by a rapid drop and a slow final decline. In contrast, the GRE signal intensity dropped immediately on KCl injection, quickly reached a minimum value, and then slowly climbed back toward baseline. A voxel located in...
the deeper structures (Figure 2, plot 3) showed a much more gradual ADC decline without a clear drop; however, the GRE signal curve is similar to that seen in the cortical region. Another type of curve seen in a few voxels is shown in Figure 2, plot 4: in this voxel, the ADC appeared to drop rapidly on KCl injection (as indicated by an arrow on the plot) to an intermediate plateau value followed by another steep decline coincident with the DC change. However, the GRE signal was the same as in other cortical areas. This type of ADC curve, with an early, rapid drop, has been reported previously\(^{15}\) but was observed in only a minority of voxels in the present study.

### Diffusion Changes

Although some variation in the ADC traces was observed in different brain regions, a more striking difference was seen among the groups. Examples of the ADC versus time curves for each of the 3 groups are graphed in Figure 3. The plots are from a single voxel in a similar location in the cortex of the anterior slice (contralateral to the recording electrode) in each animal. The general characteristics of the ADC traces are the same, as described by the model in Figure 1; however, the rapid drop in ADC occurs much earlier in the hypoglycemic animal and much later in the hyperglycemic animal.

Observations regarding the shape of the ADC curves are more accurately parameterized by the curve-fitting procedure. Figure 3 also shows parametric maps resulting from fitting the ADC curves for both slices from a representative animal in each of groups I to III, whereas Table 1 shows ROI values measured from these maps averaged over all animals in each group. The baseline CV was $<5\%$ in all ROIs measured and...
did not show any significant variation among groups. The baseline (preischemic) ADC values were similar across groups, although they varied anatomically, as illustrated by the relatively constant pixel values in the ADC maps (Fig. 3, left) and similar ROI values in Table 1. Thus, any potential osmotic effects of varying plasma glucose concentration on brain-water diffusion were not significant.

The maps of $t_1$ (Figure 3, right side, middle images) show markedly lower pixel intensities in group II and markedly higher intensities in group III compared with normoglycemic group I, which indicates that the rapid drop in ADC occurs sooner with hypoglycemia and later with hyperglycemia. In addition, significant structure is found within each $t_1$ map, with a tendency towards higher values in pixels containing more white matter and in areas close to the base of the brain. This finding is supported by the ROI measurements, which show significantly reduced $t_1$ for hypoglycemic animals in all brain regions in increased $t_1$ for hyperglycemic animals. Subcortical white matter (Table 1, region C) also shows longer $t_1$ values than gray-matter regions for all groups. A similar but more striking regional variation of the ADC profile in global ischemia has also been reported in neonatal rat brain. Most importantly, however, there is good agreement between the onset of rapid ADC decline indicated by $t_1$ and the DC potential decrease; this holds true for all 3 groups, as seen in Table 1, region B.

The other 2 parameters describing the initial gradual decline in ADC show a trend similar to that of $t_1$ with hypoglycemia and hyperglycemia, as shown in Table 1. The values of $dM_1$ were lower in group II and higher in group III, which indicates that less initial ADC decline precedes the rapid drop in hypoglycemic animals but that the ADC declines further in hyperglycemic animals compared with the normoglycemic controls. The $t_c$ of the initial ADC decline segment is also lower in group II and higher in group III than in group I, which indicates a more-rapid initial decay with hypoglycemia and a more-gradual decay with hyperglycemia. The maps of $M_3$ show little variation across the groups, except for a slight reduction in the cortex of group II. However, the ROI measurements indicate a small but significantly lower final relative ADC in the cortex of group II and in all brain regions of group III compared with group I.

A histogram analysis of the $t_1$ values of all brain voxels in groups I through III is shown in Figure 4, which shows our own DC measurements and the previously reported [K$^+$], measurements relative to the distribution of $t_1$ and the
dependence of these quantities on preischemic blood glucose levels. In groups I and II, the width of the distribution of $\tau_1$ values is mostly due to anatomic variation of the time of onset of the rapid ADC decrease; whereas in group III, the tails of the distribution are increased because of fitting errors. (In pixels in which the ADC decline does not show a well-defined “sudden drop,” the fitting algorithm generally picks a value of $\tau_1$ around the midpoint of the time course of the ADC decline.) The vertical lines passing through each plot indicate the mean and SD of the time of the DC potential drop measured in the present study, which agrees well with the onset of anoxic depolarization as measured previously by Hansen and shown at the top of each graph. The onset of anoxic depolarization, as indicated by both DC and [K+]e, falls within 1 SD of the means of the distributions of $\tau_1$ for each group.

<table>
<thead>
<tr>
<th>Region</th>
<th>Baseline ADC, $\times 10^{-3}$ mm²/s</th>
<th>$\tau_1$, s</th>
<th>$dM_1$, Fractional Change</th>
<th>$\tau_0$, s</th>
<th>$M_3$ (Relative to Baseline)</th>
<th>DC Decrease, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Cortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>0.70±0.04</td>
<td>79±10</td>
<td>0.14±0.04</td>
<td>52±19</td>
<td>0.66±0.03</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>0.75±0.05</td>
<td>54±17*</td>
<td>0.10±0.03*</td>
<td>39±14*</td>
<td>0.64±0.03†</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>0.75±0.03*</td>
<td>146±32*</td>
<td>0.19±0.01*</td>
<td>259±130*</td>
<td>0.62±0.02*</td>
<td>\ldots</td>
</tr>
<tr>
<td>B: Under DC electrode</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>0.72±0.05</td>
<td>83±12</td>
<td>0.14±0.04</td>
<td>47±12</td>
<td>0.66±0.03</td>
<td>84±15‡</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>0.76±0.1</td>
<td>47±26†</td>
<td>0.11±0.06†</td>
<td>30±1†</td>
<td>0.60±0.04</td>
<td>42±13§</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>0.76±0.05</td>
<td>195±19*</td>
<td>0.23±0.04†</td>
<td>290±150*</td>
<td>0.61±0.03</td>
<td>205±36§</td>
</tr>
<tr>
<td>C: Subcortical WM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>0.85±0.07</td>
<td>100±8</td>
<td>0.13±0.02</td>
<td>64±15</td>
<td>0.66±0.02</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>0.84±0.07</td>
<td>61±15*</td>
<td>0.08±0.03*</td>
<td>34±8*</td>
<td>0.66±0.03</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>0.86±0.05</td>
<td>197±22*</td>
<td>0.19±0.03*</td>
<td>282±130*</td>
<td>0.65±0.03†</td>
<td>\ldots</td>
</tr>
<tr>
<td>D: BG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>0.82±0.06</td>
<td>72±5</td>
<td>0.12±0.01</td>
<td>41±6</td>
<td>0.68±0.01</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>0.76±0.11</td>
<td>41±18*</td>
<td>0.08±0.03*</td>
<td>29±8*</td>
<td>0.66±0.02</td>
<td>\ldots</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>0.75±0.24</td>
<td>146±47*</td>
<td>0.16±0.03*</td>
<td>258±154*</td>
<td>0.64±0.03†</td>
<td>\ldots</td>
</tr>
</tbody>
</table>

Values are mean±SD. 
†n=5; §n=3.

Region A encompassed most of the cortical region on both hemispheres and both slices. Region B was a small, 2×2-pixel area directly beneath the DC recording electrode on the anterior slice. Region C included subcortical white matter. Region D was defined in deep gray matter regions, including basal ganglia in both hemispheres on the posterior slice. $\tau_1$ is the time from cardiac arrest to the rapid drop in ADC. $dM_1$ is the fractional change in ADC (compared with baseline) up to the rapid drop. $\tau_0$ is the exponential time constant of the initial ADC decay up to the rapid drop. $M_3$ is the relative ADC value (compared with baseline) that is reached at the end of the scan. A significant difference between hyperglycemic and hypoglycemic vs normoglycemic is indicated by $^*P<0.05$ and $†P<0.01$. Values for $\tau_1$ and DC decrease (arrows) under the recording electrode (B) are not significantly different within each group. n=7, n=5, and n=5 for normoglycemic, hypoglycemic, and hyperglycemic animal groups, respectively.

![Figure 4](http://stroke.ahajournals.org/)

Figure 4. Histograms of the $\tau_1$ parameter for all voxels in all slices for each of the 3 experimental groups. Long vertical lines indicate the mean (solid) and SD (dashed) times of the DC drop for each group measured. Short vertical lines at the top of each plot indicate the time points of the rapid change in [K+]e, concentration, measured by Hansen, which indicate anoxic depolarization. Mean $\tau_1$ values for all brain voxels in each group were as follows: normoglycemic, 99±25 seconds; hypoglycemic, 60±29 seconds; hyperglycemic, 177±94 seconds.
TABLE 2. Results of the ROI Analysis of the GRE-EPI Data for the Regions Defined on the ADC Parametric Maps*

<table>
<thead>
<tr>
<th>Region</th>
<th>( \tau_{GRE} ), s</th>
<th>( M_{GRE} ) (Relative to Baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>26±14</td>
<td>0.92±0.02</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>18±9</td>
<td>0.93±0.02</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>16±11†</td>
<td>0.92±0.03</td>
</tr>
<tr>
<td>B: Subcortical WM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>28±14</td>
<td>0.93±0.02</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>30±27</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>37±41</td>
<td>0.91±0.03</td>
</tr>
<tr>
<td>C: BG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normoglycemic</td>
<td>28±12</td>
<td>0.92±0.01</td>
</tr>
<tr>
<td>Hypoglycemic</td>
<td>48±43</td>
<td>0.93±0.02</td>
</tr>
<tr>
<td>Hyperglycemic</td>
<td>19±12</td>
<td>0.90±0.03</td>
</tr>
</tbody>
</table>

Values are mean±SD. \( \tau_{GRE} \) is the time between KCl injection and the point of maximum decrease of gradient echo EPI signal intensity. \( M_{GRE} \) is the signal intensity relative to baseline at the point of maximum decrease (ie, at time \( \tau_{GRE} \)).

*Values could not be measured close to the DC electrode because of susceptibility artifacts.
†Signal difference from the normoglycemic group (P<0.05).

GRE Signal Changes
In contrast, the GRE signal intensity curves show the same rapid drop and gradual return toward baseline in all 3 groups, as can be seen in the graphs in Figure 3. Table 2 shows data from the GRE images at the same ROI locations. Analysis of the GRE-EPI signal intensity curves showed little variation from group to group. The time between cardiac arrest and the maximum decrease in signal intensity, \( \tau_{GRE} \), was around 30 seconds overall and did not vary significantly between groups (except for slightly decreased \( \tau_{GRE} \) in the cortex in group III). In addition, the relative signal intensity at the minimum, about 0.92, was not significantly different between groups or between brain regions.

TTX Application
Results from group IV animals, which received TTX, are shown in Figure 5. The TTX was applied directly to the brain surface through an opening in the skull posterior and ipsilateral to the DC electrode, at approximately the position of the posterior slice. Some distortions are apparent in the ADC maps; these are susceptibility artifacts from the aperture in the skull. The baseline ADC value was not affected by the surgery or the TTX application (ipsilateral, 0.78×10⁻³±0.08×10⁻³ mm²/s versus contralateral, 0.76×10⁻³±0.06×10⁻³ mm²/s). However, a local increase in \( \tau_1 \) was observed in all animals; it extended about 2.5 mm into the brain below the site of TTX application (indicated by arrows in Figure 5). The mean value of \( \tau_1 \) here was 118±4 seconds, which is significantly longer (P<0.01) than the value measured at the corresponding contralateral location: 75±7 seconds. The drop in DC potential occurred at 71±2 seconds. The local delay in rapid ADC decrease is clearly seen in the plots (Figure 5, right side), which were measured from single voxels in animal 1. The ADC trace measured just below the electrode (Figure 5, trace 2) shows a rapid decrease coincident with the DC drop (Figure 5, trace 1). In contrast, the ADC drop is delayed by about 50 seconds close to the site of TTX application (Figure 5, trace 3) in the cortex of the posterior slice, whereas at a similar contralateral location (Figure 5, trace 4), the ADC drop is again coincident with the DC change. Unlike \( \tau_1 \), the size of the predepolarization ADC decline was the same when measured in a small ROI in the TTX region (\( dM_1 \), 0.14±0.04%) and contralateral cortex (\( dM_1 \), 0.14±0.03%).

Discussion
The main findings of this study are as follows.

Rapid diffusion measurements of rat brain during permanent global ischemia were fitted to a model function on a pixel-by-pixel basis to extract the major characteristics of the ADC change throughout the brain. In particular, the time point of rapid ADC drop after cardiac arrest was significantly accelerated in hypoglycemic animals but delayed in hyperglycemic animals compared with the normoglycemic control group.

The onset of the rapid change in ADC after cardiac arrest exactly coincides with a sudden drop in DC potential in all groups and is probably due to anoxic cell membrane depolarization.

A significant but more gradual decrease in ADC precedes the rapid drop associated with anoxic depolarization. Both the duration and magnitude of this predepolarization ADC decrease increase with hyperglycemia but decrease with hypoglycemia. These ADC changes are probably due to oncotic water shifts after progressive ion accumulation combined with intracellular lactacidosis as a consequence of anaerobic glycolysis.

Application of 10⁻⁷ mol/L TTX to the cortex of normoglycemic animals locally delays rapid ADC decrease and anoxic depolarization by approximately 50 seconds.

The time course of blood-oxygenation changes, as measured with gradient-echo MRI by the blood oxygen level–dependent (BOLD) effect, is not affected by variation in preischemic blood glucose levels and simply reflects the rapid exhaustion of the oxygen store of the blood after cardiac arrest.

The use of the noninvasive MRI methodology provides both the time resolution required to follow the dynamic metabolic changes in ischemia and a spatial resolution good enough to allow measurements from different anatomic regions and to detect spatially variable effects, as in the focal TTX application.

Parameterization of Water Diffusion Changes
The ADC traces for all brain voxels were fitted to a model function. This function generally fitted well to the data and gave \( \tau_1 \) values (time to rapid ADC drop) close to those determined by visual inspection of the curves. Regional measurements on the parametric maps of \( \tau_1 \) show a significant delay in rapid drop in ADC in hyperglycemic animals in all brain areas, consistent with a picture of delayed anoxic depolarization due to increased substrate availability; conversely, the reduced energy availability after cardiac arrest in hypoglycemic animals leads to earlier mass cell depolarization and an earlier rapid drop in ADC (ie, reduced \( \tau_1 \)). The overall distribution of \( \tau_1 \) values for all brain voxels shows the
same effect, with a shift of the distribution towards lower $\tau_1$ with hypoglycemia and higher $\tau_1$ with hyperglycemia, as seen in the histograms in Figure 4, right side. The width of the distributions of $\tau_1$ (see Figure 4) reflects the spread in $\tau_1$ values over the brain and was mainly due to anatomic variation in the ADC time course during global ischemia in different brain regions (group III animals had a wider distribution because of the smoother ADC decay curves, which caused increased fitting error). Nevertheless, pixels with $\tau_1$ values close to the median value displayed a distinct but delayed rapid drop in ADC, which was correctly measured by the curve-fitting procedure.

Other parameters characterizing the ADC changes for each pixel were also measured and highlight the aspects of postischemia water diffusion that change in response to altered plasma glucose concentration (specifically, $\tau_1$, $dM_1$, and $\tau_c$).

**Comparison of Potential Drop in ADC and DC**
The onset of potential change in DC was accelerated with hypoglycemia and delayed with hyperglycemia; the exact timing of the changes agreed well with the timing of anoxic cell membrane depolarization determined by previous measurements of $[K^+]_e$ (see also Figure 4). More importantly, the time of decrease in DC was the same as the time of rapid ADC drop, $\tau_1$, when measured from beneath the electrode for groups I through III. This strongly supports the notion that the rapid decrease in ADC during ischemia is caused by rapid cell swelling that occurs as a result of anoxic depolarization.

**ADC Changes Preceding Anoxic Depolarization**
In addition to the rapid drop in ADC, significant declines in ADC occurred at a more gradual rate (but clearly preceding anoxic depolarization). This predepolarization ADC decrease, $dM_1$, scaled with plasma glucose concentration from 10% to 14% to 19% (expressed as a percentage of the baseline ADC value) and occurred more slowly; $\tau_c$ varied from 40 to 50 to 260 seconds in cortex for groups II, I, and III, respectively. Increasing plasma glucose levels delay anoxic depolarization; however, the degree of ATP depletion at depolarization has been shown to remain constant at about 30% of baseline. The slowed ATP reduction in hyperglycemia may prolong ion pump activity and result in a slowed

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**Figure 5.** Left, ADC and $\tau_1$ maps for both slices for each of the 3 animals in group IV (topical TTX application). A local increase in $\tau_1$ can be seen below the site of TTX application in all 3 animals (arrows). Right, Plots of data from animal 1: cardiac arrest is indicated by the vertical dashed line. 1, DC potential and EEG are shown. Single-voxel ADC traces were measured from the areas indicated on the $\tau_1$ maps for animal 1 below the DC electrode (2), below the site of TTX application (3), and contralateral to the site of TTX application (4).
accumulation of $[K^+]$, and a slower rate of water shift into the cells. This may explain the slower predepolarization ADC reductions (longer $\tau_1$) as the plasma glucose level increases. It may also explain the larger $dM_1$ value: increased plasma glucose concentration may simply allow more time (longer $\tau_1$) for osmotically driven water to enter the cells, so that the total accumulation of intracellular water at the point of anoxic depolarization is greater, although this accumulation seems to occur more slowly.

Although predepolarization $K^+$ leakage may be the cause of some gradual water shifts into the cells, the slow change in $[K^+]$, is still much smaller than the rapid increase that occurs with membrane depolarization.\textsuperscript{19,25} Hyperglycemia is known to dramatically increase the accumulation of lactate to the point of anoxic depolarization,\textsuperscript{25} and this suggests that an additional cause of water shift into the cells may be a rise in the intracellular osmolarity in the vicinity of anaerobic glycolysis and, consequently, lactacidosis.\textsuperscript{26} Lactacidosis is known to increase cell volume,\textsuperscript{27} and rapid lactate increase has been measured soon after ischemia in vivo.\textsuperscript{28} In dynamic spectroscopy and diffusion measurements in cat brain with 36-second time resolution, Decanniere et al\textsuperscript{10} found that decreased diffusion coincided with increased lactate concentration for about the first 2 to 2.5 minutes after cardiac arrest, after which the diffusion decreased faster, presumably due to anoxic depolarization. Interestingly, in a previous study that compared ADC and metabolic changes in a rat model of focal ischemia,\textsuperscript{29} tissue acidosis was shown to correspond to an ADC reduction threshold of 10%, whereas ATP depletion occurred at a larger ADC reduction of at least 23% from baseline. The fact that 2 different thresholds seem to exist for ADC decrease due to acidosis and energy failure is in line with the biphasic nature of the time-resolved ADC changes in our global ischemia model, although the size of the ADC changes is somewhat larger in our model. Although lactacidosis is unlikely to be the only cause of slow decrease in ADC before anoxic depolarization (hyperglycemia accelerates the rate of acid accumulation,\textsuperscript{24} but we observed a slower rate of ADC change), it may well be a significant contributor to the total intracellular water shifts that precede membrane depolarization.

Comparison With Earlier Studies

The findings of this study appear to be inconsistent with earlier work by Huang et al,\textsuperscript{15,16} which showed large ADC changes that preceded anoxic depolarization. This inconsistency is not easy to explain, but it may be related to 2 differences in the methods used. First, Huang and colleagues used a 1-dimensional line-scan method at 4.7 T with pixels positioned reproducibly at particular anatomical sites but did not measure DC potential in the same animals. They relied instead on literature values for the timing of anoxic depolarization. The present study at 2 T used multislice echo-planar imaging to obtain cross-sectional views of the brain with a 5-fold smaller voxel volume than in the earlier works and measured DC potential simultaneously. This mapping method demonstrated variation in the exact timing of the rapid drop in ADC, both in different brain regions and among individual animals, and it was important to compare the DC potential traces with the ADC profiles measured close to the recording electrode. Thus, regional variation among the ADC changes combined with the 5-fold larger voxels used in the work of Huang et al\textsuperscript{17} (giving a greater chance of partial volume averaging) may account for some of the apparent discrepancy between the studies.

In the present study, we occasionally observed a 5% to 10% drop in ADC within 30 seconds of cardiac arrest (Figure 2, right, trace 4) in only a few pixels and in only some animals. This observation is similar to the group mean ADC change previously reported.\textsuperscript{15} One possible explanation is that the very early ADC changes may be partly caused by magnetic susceptibility effects\textsuperscript{10} from increased deoxyhemoglobin in pixels containing veins or larger venules. (Our susceptibility-weighted GRE data also showed a rapid drop within 30 seconds of cardiac arrest.) Such susceptibility effects would be more pronounced with larger voxels and a higher magnetic field.

The second important difference between this work and earlier studies by Huang et al is the method of data analysis. Previously, the time of initial ADC decline was defined as the time point at which ADC fell to <2 SD of the baseline value,\textsuperscript{15} and this was not found to vary between normal and hyperglycemic animals. In the present study, we used a model function constructed by following observations of the shape of typical ADC curves during cardiac arrest. In this model, ADC begins to decline immediately after KCl injection, as a slowly decaying exponential function. The time point at which ADC decline is first detected depends on the noise in the baseline ADC value, which is a strong function of the experimental parameters. Therefore, we chose to characterize the ADC curves in terms of more obvious features such as the rapid drop, although we acknowledge that ADC probably does begin to decline, slowly, immediately after cardiac arrest.

TTX Application

TTX application caused delayed drop in ADC only in a localized region of cortex beneath the application site. Again, this finding supports the hypothesis that the rapid drop in ADC is caused by anoxic depolarization that is locally delayed in the area into which TTX has diffused in the interval between application and imaging. The time point of rapid ADC drop, $\tau_1$, was similar to the time of DC change in the contralateral hemisphere (NaCl application) as before. One exception to this was the third animal in group IV, which showed decreased $\tau_1$ anterior to the site of the trepanation for TTX application. The cause of this is uncertain but may be due to a degree of local ischemia resulting from the surgery.

Because the main effect of TTX is the blockade of sodium channels, it is likely that reduced sodium influx in the region of TTX infusion reduces the amount of ATP used by Na\textsuperscript+-K\textsuperscript+-ATPase, which thus reduces the overall rate of ATP depletion so that it takes longer to reach the critical threshold of 30% ATP level for anoxic depolarization. Another effect of TTX is the suppression of neuronal activity, which results in reduced glycolysis and a reduction in lactic acid production.\textsuperscript{22} Both reduced Na\textsuperscript{+} influx and reduced lactate production may slow the rate of water shift into the cells. The observation that the size of gradual ADC decrease ($dM_1$) is
the same with or without TTX infusion, despite the fact that anoxic depolarization is significantly delayed by TTX, indicates that the rate of ADC decline is reduced, consistent with a slower shift of water into the cells.

**Blood Oxygenation Changes**

Gradient-echo MRI has been shown to be uniquely sensitive to changes in microvascular blood oxygenation in the brain.\(^{31,32}\) This BOLD image contrast arises as a result of the paramagnetic nature of deoxyhemoglobin.\(^{33}\) Increased deoxyhemoglobin concentration in tissue leads to reduced T2* relaxation times\(^ {34}\) and, hence, signal decrease in a GRE MRI. During ischemia, a rapid increase in deoxyhemoglobin concentration occurs, which causes immediate signal drop in T2*-weighted image intensity in global\(^ {15}\) and focal ischemia,\(^ {36}\) as a result of the BOLD effect. In general, the BOLD effect is a complex function of arterial blood oxygenation, cerebral blood flow, cerebral blood volume, and cerebral oxygen utilization. In our study, the GRE signal intensity dropped to a minimum of about 92% within 30 seconds of cardiac arrest and then slowly returned to baseline. Because CBF is zero, cells will continue to extract oxygen from the blood until virtually all the hemoglobin is deoxygenated (after approximately 30 seconds). Thus, the time from arrest to this maximum GRE signal change simply reflects the delay until the start of anaerobic glycolysis.\(^ {37}\)

Once all of the hemoglobin in the blood has been deoxygenated, the slow return of GRE signal intensity may be explained by a gradual decrease in CBV as blood is forced out of the cranial cavity by hydrostatic pressure (due to the lack of incoming perfusion pressure). The available oxygen in the blood remaining in the brain after cardiac arrest is used up so rapidly that there is no time for the effects of differing plasma glucose concentration to become apparent. Thus, no variation in the BOLD signal would be expected or was observed among groups I, II, and III.

**Magnetic Resonance Imaging**

High-speed MRI provides sufficient temporal resolution to follow the changes in water diffusion and hemodynamics during global ischemia. The data clearly show the importance of imaging studies, because significant variation was observed between the ADC time courses on individual slices, most notably between voxels containing mostly gray matter and voxels containing mostly white matter. In particular, imaging allows for measurement of spatially varying effects such as that observed in animals receiving topical TTX and will be important in future studies that involve focal cerebral ischemia.

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**References**

The present investigation studied the time course of the anoxic depolarization after global cerebral ischemia as a function of plasma glucose level in rats. The authors took advantage of the high sensitivity of water diffusion changes, measured in MRI, to both ischemia-induced changes of ion and water homeostasis and decrease of extracellular space. The MRI experiment was combined with simultaneous recording of the DC potential. The main findings are that the delay between cardiac arrest and onset of anoxic depolarization is increased by hyperglycemia but shortened by hypoglycemia. During this delay phase, slight changes of ADC were observed, which were more pronounced for hyperglycemic animals.

This study is a demonstration of state-of-the-art experimental design. While many experimental studies rely exclusively on MRI signal changes for indirect interpretation, here the combination of MR data with independent techniques provides further information for the analysis of the patho logical situation. But more importantly, it is this combination of MRI with independent, established techniques that allows evaluation of the observed MR changes and interpretation of them in terms of physiological alterations. It was only the simultaneous DC recording that enabled the authors to solve and explain the seeming discrepancy of their MRI results with those of Huang and colleagues.2

Upon cardiac arrest the authors described a biphasic behavior of the ADC change with a slight ADC decrease followed by a rapid and pronounced ADC drop. Via DC potential, recording the rapid ADC drop was convincingly related to the anoxic depolarization, in full temporal agreement with an earlier study by Hansen,3 who showed that the delay between cardiac arrest and increase of extracellular potassium concentration, as an indicator for anoxic depolarization, was increased by hyperglycemia but reduced by hypoglycemia.

The first phase of slight ADC alteration described by the authors was less pronounced in hypoglycemia animals. This gradual ADC decrease, preceding the rapid drop associated with anoxic depolarization, is interpreted as resulting from the intracellular lactacidosis as a consequence of anaerobic glycolysis. Its presence before the rapid ADC drop is rightly assumed to be due to increased substrate availability, thus supporting the energy metabolism and thereby delaying the breakdown of ion homeostasis. Interestingly, a similar amount of ADC decrease has been described in an earlier investigation on focal cerebral ischemia4 for the acidic but viable periphery of the ischemic territory. It would be of great interest to pursue this aspect further, as it appears to indicate a general correlation of slight ADC change with viable tissue of a disturbed metabolism.

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Rapid Monitoring of Diffusion, DC Potential, and Blood Oxygenation Changes During Global Ischemia: Effects of Hypoglycemia, Hyperglycemia, and TTX
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