Stroke Onset Time Using Sodium MRI in Rat Focal Cerebral Ischemia

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Background and Purpose—Thrombolytic therapy with intravenous tPA must be administered within 3 hours after stroke onset. However, stroke onset time cannot be established in 20% to 45% of potential patients. We propose that the rate of increase of the brain concentration of sodium ([Na]$^+$) after stroke, monitored using sodium MRI in a rat model of cortical ischemia, is linear in each individual animal, can locate the ischemic region, and can be used to estimate onset time.

Methods—After induction of focal cortical ischemia in rats under isoflurane anesthesia, [Na]$^+$ time course maps were acquired continuously on a 3 T whole body scanner from 2 to 7 hours after occlusion followed by T2-weighted proton images. Microtubule-associated protein-2 immunostained brain sections were used to verify the location of the infarct.

Results—The ischemic region identified with microtubule-associated protein-2 corresponded to the region of maximum [Na]$^+$ increase ($P<0.001$; $n=5$), and all of the animals demonstrated high linearity. [Na]$^+$ increased at a mean rate of 25±4.7%/h in ischemic tissue ($P=0.013$) but not in normal cortex (1.0±1.1%/h; $P=0.42$). The mean onset time error was 1±4 minutes ($n=4$).

Conclusions—These results of sodium MRI show that the region of maximum [Na]$^+$ increase corresponds to the ischemic region. Although [Na]$^+$ increases at a different rate in each animal, the increase is linear, and, therefore, onset time can be estimated. These findings suggest that this method can be used as a ticking clock to estimate time elapsed after vascular occlusion. (Stroke. 2006;37:883-888.)

Key Words: cerebrovascular disorders ■ infarction, middle cerebral artery ■ MRI ■ sodium ■ stroke

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troke is a serious debilitating disease; however, the only US Food and Drug Administration–approved therapy for acute ischemic stroke involves thrombolysis with tissue plasminogen activator (tPA), and only a small percentage (2% to 6%) of stroke patients actually receive this therapy. tPA or prourokinase must be administered within 3 or 6 hours, respectively, after stroke onset. Reanalysis of the combined data from 5 major tPA thrombolysis studies suggests that the time limitation for tPA could be extended to 4 to 5 hours. Because of this time limitation for thrombolysis, the most important clinical question is, “When did the symptoms of the stroke occur?” Twenty to 45% of potential patients cannot estimate onset time and, therefore, cannot be considered for thrombolysis. Unfortunately, clinically accepted neuroimaging tools cannot determine onset time. However, an animal study using tissue sampling suggests that the brain concentration of sodium ([Na]$^+$) could be used as a marker for determining the duration of ischemia in evolving stroke.

Recently, twisted projection imaging (TPI) for sodium MRI has emerged as a viable method that can be used in both experimental settings and humans, producing sodium images of diagnostic quality in acceptable imaging times (10 minutes) at clinical magnetic field strengths (1.5 and 3.0 T) using commercially available scanner hardware. Based on the ability of MRI to determine a sodium time course in individual subjects, we propose that, after the onset of ischemia, [Na]$^+$ increases linearly in ischemic cortex in each individual animal, and the onset time can be estimated from the rate of [Na]$^+$ increase. In order to optimize the region selection for [Na]$^+$ determination, we also investigated whether the ischemic region corresponded to the area of maximum sodium increase.

Methods

Animal Preparation

Animal procedures were approved by the animal committee of the institution. Sprague-Dawley rats (~400 g, $n=7$) were anesthetized using 2% isoflurane and maintained with 0.8% isoflurane in 70% N$_2$O and 30% O$_2$, administered via endotracheal tube and ventilated by a volume-controlled respirator (Model 681, Harvard Apparatus).
Mean arterial blood pressure was recorded continuously using a femoral artery catheter. Rectal temperature was maintained at 37°C, and arterial blood gases (PaO₂ and PaCO₂) and pH were determined. Pancuronium in normal saline was infused IV at 0.3 mg/kg/h (delivered at 1 mL/h) to maintain immobilization and hydration.

Experimental stroke was induced by direct surgical transection of the middle cerebral artery (MCAT), combined with permanent bilateral occlusion of the common carotid arteries as performed in our laboratory. Two animals were used as sham surgical controls with bipolar coagulation performed at a site immediately adjacent to the middle cerebral artery. In these 2 sham animals, the rate of [Na⁺]ᵢ increase in the ipsilateral and contralateral cortex were both zero and not different than each other.

MRI
Sodium 3D and proton multislice 2D data were acquired on a 3 T whole body scanner (General Electric Medical Systems). The animal’s head was positioned inside a 5-cm-diameter, 5-cm-long dual-tuned (²³Na and ¹H), dual-quadrature, birdcage radiofrequency coil. A TPI pulse sequence (projections=398, averages=8, repetition time/echo time=100/0.4 ms, data acquisition time=5.3 minutes, field-of-view=5×5×5 cm, voxel size=0.48 mm³, and resolution element=1.5 mm³) was used to acquire 18 to 54 serial [Na⁺]ᵢ images every 5.3 minutes during 2 to 5 hours within the 2.3- to 9.6-hour time window after ischemia. T2-weighted images (data acquisition time=4 minutes, average=1, repetition time/echo time=2000/120 ms, voxel size=0.061 mm³, and slice thickness=1.6 mm) were acquired at the end of the study for anatomical reference.

Image Processing
Edema-corrected infarct volume and location were determined with microtubule-associated protein-2 (MAP2) immunohistochemistry with anti-MAP2 antibody (HM2, Sigma). MAP2-immunoreactive sections were digitized (Imaging Research Inc), and the area of the study for anatomical reference.

As the initial step to investigate linearity in the rate of sodium increase in ischemic cortex, the serial sodium images were used to generate a parametric image of the slope by performing a linear regression of [Na⁺]ᵢ versus time after stroke onset. Volumetric image stacks from the 4 data sets, [Na⁺]ᵢ, [Na⁺]ᵢ-slope, T2-weighted, and aligned MAP2 images, were transferred to the image analysis program AMIDE. Seven ischemic core regions-of-interest (ROIs) were identified in the [Na⁺]ᵢ-slope images at isocountour levels of 95% to 65% in steps of 5% of the maximum slope. The [Na⁺]ᵢ (as a percentage of the mean [Na⁺]ᵢ) from the homotopic normal cortex) over time in each of these slope isocountour ROIs was then extracted from the original [Na⁺]ᵢ data sets and plotted versus time after stroke onset. The onset time error (OTE), which is the difference from the actual onset time, was estimated as the intersection of the line fits of the ischemic and normal cortex ROIs from each of these slope isocountour ROIs. For each animal, the ischemic cortex ROI with the minimum absolute value of OTE was chosen for additional analysis. The volumes of the ROIs representing 95% of the maximum [Na⁺]ᵢ-slope were superimposed on the MAP2 infarct areas to assess whether the maximum [Na⁺]ᵢ-slope ROI could precisely locate the ischemic core.

Statistics
Repeated-measures linear regression analyses using mixed linear models were performed to describe the relationship between [Na⁺]ᵢ and time after stroke. Rates of increase of [Na⁺]ᵢ for the ischemic cortex were obtained using a mixed-effects model specifying that [Na⁺]ᵢ follows a linear regression over time after stroke, with random slope and intercept for each animal. The same statistical model was fit separately for the normal cortex. SAS Proc Mixed software (version 8) was used to fit the regression models. The time course of [Na⁺]ᵢ in each of these ischemic and normal cortex ROIs was analyzed for linearity by comparing linear and quadratic regressions. Repeated-measures ANOVA was performed to test for differences between physiological variables. Paired or unpaired t tests were used for all of the other comparisons. Values are expressed as mean±SEM, except for slopes and intercepts for which the error term is SE of the estimated regression parameter. Statistical significance was assumed when P<0.05.

Results
Physiological Variables
The physiological variables were within normal limits (Table 1), with no significant differences over time or between animals.

Maximum [Na⁺]ᵢ-slope Predicts the Location of the Ischemic Core
A typical set of [Na⁺]ᵢ, [Na⁺]ᵢ-slope, T2-weighted, and MAP2 images from animal No. 3 is presented in Figure 1 showing the superposition of grayscale and pseudocolor coded images and ROIs of normal cortex, infarct, and maximum slope. Although the presence of high-extracranial [Na⁺] at the site of the skin incision and tissue dissection for the craniotomy (Figure 1A, lower left) and over what is presumably the temporal ridge (upper left, just outside of the brain) is obvious, the [Na⁺]ᵢ-slope image clearly localizes intracranially over the ischemic cortex (Figure 1B). The 90%-maximal [Na⁺]ᵢ-slope isocontour ROI (see Table 2) showing the maximum [Na⁺]ᵢ increase (Figure 1B) clearly overlies the ROI for the MAP2 infarct region and vice versa (Figure 1C).

To quantitatively show that the slope image can be used to locate the ischemic core, we analyzed the overlap of the infarct ROI, identified by MAP2 immunohistochemistry, and

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time MCAT, min</th>
<th>MAPB, mm Hg</th>
<th>pH</th>
<th>Paco₂, mm Hg</th>
<th>PaO₂, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before MCAT</td>
<td>−105±14</td>
<td>100±5</td>
<td>7.524±0.020</td>
<td>32±2.7</td>
<td>111±9.8</td>
</tr>
<tr>
<td>After MCAT</td>
<td>32±13</td>
<td>102±5</td>
<td>7.484±0.020</td>
<td>33±2.7</td>
<td>97±9.8</td>
</tr>
<tr>
<td>MRI start</td>
<td>99±5</td>
<td>98±5</td>
<td>99±5</td>
<td>93±5</td>
<td>92±5</td>
</tr>
<tr>
<td>1 h</td>
<td>98±5</td>
<td>99±5</td>
<td>93±5</td>
<td>92±5</td>
<td></td>
</tr>
<tr>
<td>2 h</td>
<td>98±5</td>
<td>99±5</td>
<td>93±5</td>
<td>92±5</td>
<td></td>
</tr>
<tr>
<td>3 h</td>
<td>98±5</td>
<td>99±5</td>
<td>93±5</td>
<td>92±5</td>
<td></td>
</tr>
<tr>
<td>4 h</td>
<td>98±5</td>
<td>99±5</td>
<td>93±5</td>
<td>92±5</td>
<td></td>
</tr>
<tr>
<td>MRI end</td>
<td>84±5</td>
<td>7.483±0.029*</td>
<td>32±3.2*</td>
<td>108±12*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=5, except for *where n=4). No significant differences between animals or over time.
the 95%-maximal \([Na^+]_{bc}\)-slope ROI by comparing the volume percentage of each slope ROI inside and outside the infarct ROI. The volume percentages of the 95%-maximal \([Na^+]_{bc}\)-slope ROIs that intersect with the infarct volume ROI for each animal are shown in Table 2, with the mean of 78%±6% (P<0.001; n=5, 1-tailed paired t test versus the volume percent outside the infarct ROI). In addition, in 4 of 5 animals, every voxel (100%) in the 95%-maximal \([Na^+]_{bc}\)-slope ROIs touched some portion of MAP2 infarct ROI (in animal No. 4, 87%). These results show that the slope and infarct ROIs overlap, and that the maximum \([Na^+]_{bc}\) slope occurs within the eventual infarct.

**Linear \([Na^+]_{bc}\) Increase**

The time courses of \([Na^+]_{bc}\) of ischemic and normal cortex ROIs for a typical animal are shown in Figure 2A. The rate of increase for this animal is 19±2.2%/h, and there is no indication of deviation from linearity. After 6.8 hours, \([Na^+]_{bc}\) reached 217% of the value in the normal homotopic cortex. The \([Na^+]_{bc}\) in the homotopic cortex remained stable with the slope not different than zero (P=0.15).

The rates of sodium increase in ischemic cortex for each animal are plotted in Figure 2B, and the population means in the ischemic and normal cortex are listed in Table 2. The ischemic cortex slopes for animal No. 1 and No. 2 differ from the mean population slope (P=0.03), indicating that a common slope model is not appropriate for the estimation of onset time. \([Na^+]_{bc}\) increased at a mean rate of 25±4.7%/h in ischemic tissue (P=0.013) but not in normal cortex (population slope=1.0±1.1%/h; P=0.42). No deviation in linearity of the rate of \([Na^+]_{bc}\) increase was noted in any of the animals, as shown in Figure 2. Quadratic fits were not significant (P=0.50), supporting linearity.

One animal (No. 5) was not used for slope or OTE analysis, because excessive bleeding after MCAT caused high-extracranial [Na+] compromising the intracranial [Na+] determination via the partial volume effect. However, even in this animal, the 95%-maximal \([Na^+]_{bc}\)-slope ROI localized the ischemic region as shown in Table 2.

**Onset Time Estimation**

Using the serial \([Na^+]_{bc}\) data, we estimated the OTE as the intersection of the linear regressions for ischemic and normal

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>[Na(^+)](_{bc})-Slope ROI Threshold (%)</th>
<th>[Na(^+)](_{bc}) Slope ROI</th>
<th>[Na(^+)](_{bc})</th>
<th>Rate of Increase</th>
<th>[Na(^+)](_{bc})-Slope ROI Overlap With Infarct</th>
<th>% Overlap</th>
<th>% Touch</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95%</td>
<td>-2.1±0.80 0.86 35±4.6 0.03</td>
<td>13</td>
<td>90</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>65%</td>
<td>5.4±0.44 0.58 15±4.6 0.03</td>
<td>-6.4</td>
<td>76</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>90%</td>
<td>-0.40±0.80 0.15 29±4.6 0.29</td>
<td>0.17</td>
<td>94</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>80%</td>
<td>2.2±0.08 0.55 30±4.6 0.32</td>
<td>-3.2</td>
<td>71</td>
<td>87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>...</td>
<td>... ... ... ...</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population mean</td>
<td>1.0±1.1 0.42</td>
<td>25±4.7 0.013 1±4</td>
<td>78±6&quot;*</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IC indicates ischemic cortex; NC, normal cortex. The error is SE for the rate of increase estimates and SEM for OTE and % overlap. For individual IC, P value is for the difference from the population mean. For the population mean and for NC, P value is for the difference from zero. Animal No. 5 was not used for slope or OTE analysis. *P<0.001 vs volume percent outside infarct ROI.
cortex, as shown in Figure 2A. The mean OTE was 1±4 minutes (n=4) as shown in Table 2.

Discussion
The main findings of this study were as follows: (1) the linear increase in [Na⁺] br can be demonstrated directly by sodium MRI during the first hours after experimental stroke in each individual animal (Figure 2); (2) the region with the most rapid [Na⁺] br increase corresponded to the ischemic zone, as confirmed by MAP2 immunohistochemistry (Figure 1 and Table 2); and (3) the onset time can be estimated (Table 2). Using the maximum rate of [Na⁺] br increase to localize the ischemic region is a more direct method than that used in our previous work9 in which low apparent diffusion coefficient (ADC) slope (red circles) and NC (green diamonds) ROIs in Figure 1, with corresponding linear regressions for the IC (red solid line) and NC (green dashed line) against the time after arterial occlusion (Ta). The orange vertical dashed line segment represents the OTE (0.17 minutes) determined by the intersection of the 2 linear regressions. (B) The individual time courses in IC for each animal, with the numbers indicating individual animals, along with the population mean. The population slope is significantly different than zero (P=0.013).

The Linear Accumulation of Sodium in Focal Cerebral Ischemia
The ability to assess the [Na⁺] br slope in individual animals is a major advantage of sodium MRI. The linear time course of [Na⁺] br, including both intracellular and extracellular components, extends over long periods (2 to 7 hours) after stroke onset (Figure 2). Although the individual [Na⁺] br slopes were statistically different, the mean rate of sodium increase determined by sodium MRI (25±4.7%/h) compared favorably with the results of direct tissue analysis with flame photometry from grouped animals (23±4.2%/h)9 and with the range of 16% to 35%/h from other works as compiled by Wang et al.9 These previous studies used a separate animal at each time point, making it impossible to determine whether each animal possessed a consistent linear time course.

Often the most severe ischemia does not occur at the onset of clinical symptoms in human stroke, whereas in experimental stroke caused by arterial occlusion, it definitely does. For a stroke with a delayed start or with a “waxing and waning” progression with intermittent severe ischemia, [Na⁺] br will only increase when a certain threshold of perfusion has been reached. In this regard, the [Na⁺] threshold proposed by Thulborn et al11 might be an alternate concept that avoids the issue of timing.

Mechanism of Sodium Increase
The individual linearity suggests that the mechanism of [Na⁺] br accumulation is not related to the transport of Na⁺ across the blood-brain barrier; the Na⁺/K⁺ transport ratio changes from 3 to 6 hours after onset,15 the time during which there is a transition from cytotoxic to vasogenic edema.16 However, our data show that the rate of [Na⁺] br increase remains stable during the same transition period. Small between-animal differences in the trickle flow to the ischemic core17 or in the efflux of sodium via the paravascular fluid pathway18 could be the source of the variability in the [Na⁺] br rate and control the Na⁺ accumulation rate,19 based on the vascular compression associated with cytotoxic edema-induced brain swelling and the corresponding extracellular space decrease by 50%.20 Most of the variability in our [Na⁺] br time course data are attributable to within-animal factors (62%) presumably because of random experimental errors. The source of variation between animals is primarily attributable to slope (38%).

Our hypothesis that the maximum [Na⁺] br slope precisely locates the infarct was satisfied because the 95%-maximal [Na⁺] br slope ROI does overlap significantly (P<0.001) with the infarct ROI. However, all of the maximum slope voxels were not inside the MAP2 infarct. In 4 of 5 animals, the 95%-maximal [Na⁺] br-slope ROIs were at the rostral portion of the infarct, suggesting that the maximum rate of [Na⁺] br is localized at the site of maximum Na⁺ delivery via trickle flow or, alternatively, the site of maximum swelling and most limited Na⁺ egress.
Onset Time Determination

Three findings of this study suggest that the estimation of onset time is possible. First, the linear $[\text{Na}^+]_b$ increase in each individual is a prerequisite for the estimation of onset time. Although our data did not cover the period between 0 and 2 hours after stroke, data from other studies$^{21-23}$ during both early (0 to 3 hours) and late (up to 7 hours) periods after stroke using either punch or magnetic resonance methods do show high and consistent linearity over the entire period. Secondly, the ischemic region can be located from the maximum rate of $[\text{Na}^+]_b$ increase, greatly simplifying the choice of the region on which to base this estimation. Third, because the rate of $\text{Na}^+$ increase differs between individuals, an individual estimate of the rate of $[\text{Na}^+]_b$ increase, without the assumption of a common slope, can be used to estimate onset time. Our data analysis indicates a mean OTE of $1\pm4$ minutes.

To find the minimum absolute value of OTE, we chose from 7 isocontour ischemic ROIs defined by decreasing percentages of the maximum slope. The determination of the slope threshold for the minimum OTE might be complicated by the extracranial contamination because of surgical exposure for the craniotomy required for MCAT. In human stroke, with the larger brain, increased signal-to-noise ratio, no extracranial $[\text{Na}^+]_b$, and minimal contribution via the partial volume effect to intracranial $[\text{Na}^+]_b$, the most reasonable estimate of onset time would be based on the ROI with the 95% threshold. Individual slope estimates based on measurements of $[\text{Na}^+]_b$ from both normal and ischemic cortex over a 20- to 30-minute period before and after other imaging studies could potentially be used to estimate time after stroke onset in the human brain using this method.

Sodium MRI for Stroke

There have been limited measurements of $[\text{Na}^+]_b$ using quantitative MRI in 3 ischemic nonhuman primates$^{11,21,23}$; I study showed linearity both before and during the same time period as this study.$^{23}$ Sodium MRI can be performed in 10 minutes with acceptable image quality$^{10,11}$ as demonstrated 4 to 23 hours after human stroke onset.$^{11}$

The onset time estimate from the constant rate of sodium increase proposed in this work has no analogues with other imaging methods, such as magnetic resonance diffusion-weighted imaging and perfusion-weighted imaging.$^{24}$ Moreover, the limitations of diffusion-weighted imaging/perfusion-weighted imaging for characterizing ischemic versus penumbral tissue in stroke are recognized.$^{25,26}$ Although other multimodal computed tomography or MRI methods have been proposed to select patients who might benefit from thrombolysis after the 3-hour time window,$^{27,28}$ consensus for the use of these methods has not been reached.$^{29}$ Knowing the MRI dynamics of $[\text{Na}^+]_b$ might remove some limitations of present magnetic resonance methods, provide a complementary and unique measure of tissue viability and potential salvageability, and eventually expand the rigid time window beyond 3 hours after onset.$^{30}$

In summary, this study of experimental focal cerebral ischemia using sodium MRI demonstrated that the linear increase in $[\text{Na}^+]_b$ can be observed in each individual rat with the maximum rate of increase in the ischemic region and that the individual onset times of ischemia can be estimated. This work suggests that sodium MRI could be applied to human stroke to estimate the time-after-stroke onset, because imaging and sodium quantification is easier in larger brains. The practical application of this “ticking clock” might increase the number of patients eligible for thrombolytic therapy.

Acknowledgments

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References

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