Regional Brain Sodium, Potassium, and Water Changes in the Rat Middle Cerebral Artery Occlusion Model of Ischemia

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Middle cerebral artery occlusions (MCAo) in rats produce infarcts in the pyriform and frontoparietal cortex, extending into the lateral basal ganglia and parasagittal cortex. We estimated tissue H2O concentrations from wet and dry weight measurements and determined Na and K concentrations by atomic absorption spectroscopy in these areas of rat brains. Tissue samples were analyzed at 2, 4, and 24 hours after MCAo and sham MCAo, compared with normal values measured in unoperated rats. In the pyriform and frontoparietal areas, H2O concentrations increased to 34 and 7% greater than normal by 2 hours, and 89 and 94% by 24 hours after MCAo. Na concentrations rose in these areas to 73 and 37% greater than normal by 2 hours, and 281 and 330% by 24 hours. K concentrations did not change until 4 hours, but fell to 62 and 34% of normal in these areas by 24 hours. Such large ion shifts indicate severe tissue destruction. In the parasagittal cortex and basal ganglia areas, the ion and water changes were smaller and did not become significant until 24 hours after MCAo. Rates of Na entry into the infarct site were greatest at 0-2 hours, while the rates of K loss peaked later, between 2 and 4 hours. The difference in Na influx and K efflux resulted in net ion shifts that correlated highly with water entry, yielding a correlation coefficient of 0.992 (p < 0.001) and a slope indicating that 1 ml of water entered the tissue with each 145 μmoles of ions. These findings strongly suggest that net ion shifts cause the early edema of regional brain ischemia. To explain the dominance of Na gain over K loss, we reject the hypothesis of a selective increase of blood-brain barrier Na permeability and propose instead that glial buffering of K ions released by ischemic neurons reduced the driving force for K clearance during the first 2-4 hours after MCAo. (Stroke 1987;18:751-759)

The middle cerebral artery (MCA) occlusion (MCAo) model in rats possesses several major advantages over other models of cerebral ischemia. First, unlike global ischemia models,1-3 occlusion of cerebral arteries produces regional losses of blood flow, allowing ischemic sites to be compared with perfused tissues in the same brain. Second, the effects of severe ischemia can be investigated in animals with relatively low mortality rates. Third, unlike MCAo models in larger mammals such as cats and primates, rats can be studied in large numbers.

In an earlier study,4 we showed that tissue ion shifts in injured spinal cords can serve as quantitative indices of the severity and time course of cell damage. Here, a similar approach was applied to the rat MCAo model of regional ischemia. The time course and regional distribution of Na, K, and water changes at 2, 4, and 24 hours after unilateral MCAo were compared with measurements in sham-occluded and unoperated rats. We report below earlier and larger changes in brain Na, K, and water shifts than described previously.5-15

Na influx exceeded K efflux, resulting in net ion shifts that correlated well with water movements into the tissue. To explain the net ion shift, we suggest a role of glial buffering in retarding K loss from ischemic tissues.

Materials and Methods

Rat MCA Occlusion Model

Sprague-Dawley rats weighing 250–350 g were anesthetized with 40 mg/kg of pentobarbital given i.p. To expose the MCA, we removed the temporalis muscle and the zygoma and retracted the jaw downward to allow access to the infratemporal skull. Under 25 X magnification, the mandibular nerve was followed to the foramen ovale. A 3 x 2 mm craniectomy was made with a microdrill anterior to the foramen. The dura was opened with a hooked 25-gauge needle. To locate the infarct, 0.8 ml of 4% Evans blue solution was injected i.v. through the femoral vein after opening the dura. The left MCA was coagulated with bipolar radiofrequency currents applied with fine forceps at the level of the olfactory tract. The artery was divided with microscissors to ensure complete occlusion. In some rats, this procedure was stopped just after the dural opening; the MCA was not occluded. These rats served as sham-occluded controls.

General Experimental Protocols

In preliminary studies, we examined 3 groups of rats for histologic changes at 2, 4, and 24 hours after MCAo. The brains were fixed by intraaortic perfusion.
of formalin, serially sectioned, and stained with hematoxylin and eosin. Somatosensory evoked potentials were recorded in these rats, using epidural screw electrodes that were implanted 1 mm posterior to the coro-
nal sutures and at the frontal midline, referenced against a ground electrode placed at the base of the skull. Cortical potentials activated by sciatic nerve stimulation were averaged for 128 sweeps of 100 msec duration with a signal averager (TN3500, Tracor Northern, Middleton, Wisc.).

We measured Na, K, Ca, and water shifts in 3
groups of 8 rats subjected to MCAo, 3 groups of 8
sham-occluded rats, and 1 group of 6 unoperated rats.
The Ca measurements will be reported separately. The sham-occluded controls allowed us to rule out ion changes due to anesthesia, surgery, or Evans blue injection. Both sham-occluded and MCAo rats were decapitated 2, 4, and 24 hours after surgery. The unoperated rats were simply decapitated and analyzed immediately.

The brains were removed within 90 seconds after
decapitation and cooled in a deep freezer at -85° C. A
4-mm thick coronal slice of brain was then cut with a
double razor blade assembly behind the frontal pole. The slices were examined for the presence of Evans blue, and the stain locations were noted. Brain tissues were sampled by coring with a hollow 3-mm i.d. metal tube with sharp edges; each tissue sample was placed in a ceramic crucible (Coors, Colo.). The areas sampled included the pyriform cortex (Area 1), frontoparietal cortex (Area 2), parasagittal cortex (Area 3), and basal ganglia (Area 4). Figure 1 illustrates the coronal locations of the samples.

Tissue samples were obtained from both hemi-
spheres in each rat. The hemisphere on the operated
(left) side will be referred to as ipsilateral and the other
controlateral. For each area sampled in the lesioned hemisphere, ion and water measurements were made at 3 times. These were then compared with 3 types of controls: sham-occluded, contralateral, and unoperated. All comparisons of lesioned hemispheres with the sham-occluded and controlateral controls were matched for site and time. The ion values measured in the unoperated rats were pooled. The mean ion and water concentrations in unoperated control rats will be referred to as normal.

**Ion and Water Analyses**

The tissue samples were weighed on a chemical
balance (Mettler AE163, Zurich) to obtain wet weight (W) with 0.1 mg precision; average W was 36.6 mg. The tissues were then dried in a desiccating oven at 105° C for 24 hours, placed in a desiccating jar under vacuum for 20 minutes, and reweighed to obtain dry weight (D). Water concentrations expressed in milliliters per gram wet tissue weight ([H₂O]w) were calculated as (W - D)/W. Water concentrations in milliliters per gram dry tissue weight ([H₂O]d) were calculated as (W - D)/D.

To prepare the tissue samples for atomic absorption spectroscopic measurements of Na and K, we first digested the dried samples in 1 ml of concentrated nitric acid at 45° C for 90 minutes. After drying the tissue at 95° C, 1 additional ml of concentrated nitric acid was added to dissolve the digested tissue. A 0.1-
ml aliquot was removed from each crucible and added to 10 ml of deionized water. The Na and K determinations were made on this solution by atomic absorption spectroscopy (Perkin-Elmer 2280, Norwalk, Conn.) at 589.6 and 766.5 nm, using a hollow Na-K cathode lamp, a premix burner, and an acetylene-air flame. All the ion measurements were bracketed with 2 standards, one containing only the diluent and the other the diluent plus a known concentration of Na or K. Standard and sample concentrations were adjusted to fall within the linear range of the instrument. Flame conditions and detection wavelengths were optimized for sensitivity and linearity.

**Data Analysis**

Ion concentrations will be expressed as a function of W and D. Wet weight concentrations of Na and K, i.e., [Na]w and [K]w, have units of μmol/g W; dry weight concentrations, i.e., [Na]d and [K]d, have units of μmol/g D. Mean concentrations were calculated for each sampled site and time. Changes in ion and water concentrations, i.e., Δ[Na]d, Δ[K]d, and Δ[H₂O]d, were obtained by subtracting mean concen-
to estimate the rates of change, we subtracted the concentrations measured in time-matched and site-specific controls from the mean concentrations measured in the lesioned hemisphere. Negative values indicate losses, while positive values indicate gains. To estimate the rates of change, we subtracted the $\Delta [Na]_d$, $\Delta [K]_d$, or $\Delta [H_2O]_d$ from 2 times and divided the difference by the number of hours between the times, yielding $\mu$mol/hr.

We used the two-tailed unpaired $t$ test to evaluate the differences between means in lesioned and sham-occluded control rats; lesioned and contralateral hemispheres were compared with the paired $t$ test. Note that because so many paired and unpaired $t$ tests were carried out, a number of the comparisons may fortuitously be significant. We therefore elected to use a more rigorous standard for judging significance than would normally be applied. In the discussion, we will focus on those statistical comparisons that exceed the statistical criterion of $p < 0.005$. Analysis by Bonferroni correction suggests that this is a sufficiently conservative criterion to rule out any spurious correlations.

Relations between ion and water changes were assessed by linear regression analysis, yielding the slope and correlation coefficient ($r$) of regression. The significance of the correlation was estimated by calculating the $t$ value from $r \times \sqrt{(n-2)/(1-r^2)}$, where $n$ is the number of points. The following conventions will be used to express data variability: When sample means are presented, standard deviations (SD) will be listed. When differences between 2 sample means are compared, we will illustrate variability by the pooled standard error of the means (SEM) from which the $t$ values were calculated.

**Results**

The general results of MCAo will be described first, followed by the water and ion concentrations in the sham-occluded and normal controls, and then the water, Na, and K concentrations in rats with MCAo. Table 1 lists the normal values of ion and water concentrations measured in normal rats. Table 2 lists the ion and water concentrations in rats with MCAo. In Figures 2–9, all ion and water concentrations are in terms of D, and all changes are expressed relative to sham-occluded controls. Data that are significantly different by unpaired $t$ test at $p < 0.005$ will be emphasized.

**Clinical Course, Somatosensory Evoked Potentials, and Pathology**

All the rats awoke within 2 hours after surgery. A mild right hemiparesis was usually present in rats with MCAo but was resolved in 75% by 4 hours and in 90% by 24 hours. Cortical somatosensory evoked potentials (SEPs) recorded before the MCAo showed a characteristic positive-negative-positive complex at 7.6 (± 0.4) msec onset latency, 25–50 $\mu$V amplitude, and duration of 14.5 (± 0.4) msec. MCAo produced a consistent loss of cortical SEP in the lesioned side within 30 seconds. SEPs, however, typically returned by 24 hours, albeit with reduced amplitudes. Latencies of the responses were usually not affected. SEP from unlesioned hemispheres either did not change or increased slightly in amplitude. Sham occlusion did not alter the latencies or amplitudes of SEP components within the first 25 msec after the stimuli.

Histologic examination revealed ischemic changes in the brains as early as 2 hours after MCAo. At 24 hours, a broad zone of infarction was evident in the lesioned hemisphere, most prominent in the pyriform cortex and frontoparietal cortex and occasionally involving the lateral third of the basal ganglia. Evans blue staining of the MCA was not evident until 4 hours after MCAo, but the site of the surgery often became stained shortly after surgery. By 24 hours, an intense blue stain usually surrounded a pale infarct site at the pyriform cortex. Gross histopathologic changes were visible in the pyriform cortex in 8 of 9 rats and in the frontoparietal cortex in all 9 rats examined at 24 hours. None of the sham-occluded rats showed the gross histopathologic changes seen in the MCAo rats. Although most sham-occluded rats showed Evans blue staining of blood vessels and the operation site, there was no visible blue stain in the MCA territory.

**Tissue Ion and Water Concentrations in Normal and Sham-Occluded Controls**

Normal, unoperated rats had reproducible water, Na, and K concentrations. Table 1 lists the mean ± SD concentrations, measured in 48 tissue samples from 6 rats, expressed in terms of both W and D. The standard coefficients of variation ranged from 2 to 9%, i.e., 1.6% for $[H_2O]_w$, 5.5% for $[Na]_w$, 2.3% for $[K]_w$, 8.7% for $[H_2O]_d$, 10.1% for $[Na]_d$, and 7.5% for $[K]_d$. In general, the dry weight concentrations were more variable than the wet weight concentrations.

Sham occlusion tended to increase tissue ion and water concentrations slightly. Figure 2 (top) shows the $[H_2O]_d$ measured in rats subjected to sham occlusions. Some values in Areas 1 and 3 in both hemispheres were greater than the normal value of 3.67 ± 0.32 ml/g. Although $t$ tests suggest that these values may be different ($p < 0.05$), the differences are small and probably inconsequential. Mean $[Na]_d$ also tended to increase with time in Area 1 in both hemispheres but was not significantly different ($p > 0.05$) from the normal value of 253.6 ± 25.6 $\mu$mol/g, as shown in Figure 4 (top). Some mean $[K]_d$ values in sham-occluded rats appeared to be higher than the normal value of

| Table 1. Brain Water and Ion Concentrations in Unoperated Rats |
|-----------------|-----------------|-----------------|
|                 | Wet weight      | Dry weight      |
|                 | concentrations  | concentrations  |
| $H_2O$          | 0.782 ± 0.013   | 3.67 ± 0.32     |
| Na              | 53.27 ± 2.93    | 253.6 ± 25.6    |
| K               | 112.08 ± 2.68   | 524.2 ± 39.5    |
| $K + Na$        | 165.34 ± 2.46   | 776.8 ± 26.8    |

Values are mean ± SD, ml/g or $\mu$mol/g of wet or dry tissue wt, from 8 areas in 6 rats.
**Tissue Water Changes**

Large increases in \([H_2O]_d\) occurred after MCAo. Figure 2 shows the distribution of \([H_2O]_d\) measured in rats with sham occlusion and MCAo. Table 2 lists the mean \([H_2O]_d\) measured in the ischemic hemispheres and the statistical comparison of these values against normal, sham-occluded, and contralateral controls. In Area 1, mean \([H_2O]_d\) rose to 34, 52, and 89% greater than normal at 2, 4, and 24 hours, respectively, after MCAo. In Area 2, mean \([H_2O]_d\) rose more slowly to 7, 14, and 94% greater than normal at 2, 4, and 24 hours, respectively.

Figure 3 shows the mean \(\Delta[H_2O]_d\) calculated by subtracting time- and site-matched sham-occluded values from the measurements made in the lesioned hemisphere. The mean \(\Delta[H_2O]_d\) indicated significant \((p<0.001)\) water entry into Areas 1, 2, and 4 from 2 to 24 hours and in Area 3 from 4 to 24 hours. Areas 1 and 2 in the lesioned hemisphere respectively gained 0.88 and 0.47 ml/g by 2 hours, 1.08 and 0.46 ml/g by 4 hours, and 2.81 and 3.67 ml/g by 24 hours. The rates of water entry into the brain were greatest at 0–2 hours after MCAo (Figure 3, bottom). At 0–2 hours in Area 1, the tissue gained water at the rate of 438 \(\mu l/g/hr\). At 2–4 hours, the rates of water gain were much smaller. Areas 3 and 4, in fact, appeared to have lost water at 2–4 hours. For example, Area 4 lost water at the rate of 164 \(\mu l/g/hr\) between 2 and 4 hours after MCAo. Between 4 and 24 hours, all sampled sites in the lesioned hemisphere gained water.

**Tissue Sodium Changes**

Figure 4 shows the distribution of \([Na]_d\) in rats with sham occlusion and MCAo; Table 2 lists the mean values of \([Na]_d\). Mean \([Na]_d\) in Areas 1 and 2 rose significantly \((p<0.001)\) above sham-occluded controls. Table 2 shows the mean \([Na]_d\) values of Area 1 and 2 rose significantly \((p<0.001)\) above sham-occluded controls.

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**Table 2. Mean Dry Weight Concentrations of Water, Sodium, and Potassium in Lesioned Hemispheres**

<table>
<thead>
<tr>
<th>Sampled site</th>
<th>2 hours</th>
<th>4 hours</th>
<th>24 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>4.952 ± 0.144*§ $</td>
<td>5.579 ± 0.093*§ $</td>
<td>6.937 ± 0.087*§ $</td>
</tr>
<tr>
<td>Area 2</td>
<td>3.950 ± 0.076*§ $</td>
<td>4.181 ± 0.067*§ $</td>
<td>7.130 ± 0.063*§ $</td>
</tr>
<tr>
<td>Area 3</td>
<td>3.673 ± 0.025*§</td>
<td>4.076 ± 0.099*†</td>
<td>4.291 ± 0.052*§ $</td>
</tr>
<tr>
<td>Area 4</td>
<td>3.762 ± 0.049*§</td>
<td>3.739 ± 0.053*§</td>
<td>4.128 ± 0.064*§</td>
</tr>
<tr>
<td><strong>Sodium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>439.29 ± 87.55*§ $</td>
<td>517.11 ± 122.81*§ $</td>
<td>962.70 ± 94.28*§ $</td>
</tr>
<tr>
<td>Area 2</td>
<td>347.03 ± 67.21*§ $</td>
<td>415.54 ± 96.72*§ $</td>
<td>1086.99 ± 142.57*† $</td>
</tr>
<tr>
<td>Area 3</td>
<td>268.69 ± 19.83*§</td>
<td>301.52 ± 33.02*§</td>
<td>431.22 ± 104.76*†</td>
</tr>
<tr>
<td>Area 4</td>
<td>271.90 ± 21.55*§</td>
<td>255.45 ± 12.06*§</td>
<td>368.72 ± 66.72*§</td>
</tr>
<tr>
<td><strong>Potassium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area 1</td>
<td>517.26 ± 86.14*§</td>
<td>563.16 ± 46.71*§</td>
<td>324.60 ± 56.35§</td>
</tr>
<tr>
<td>Area 2</td>
<td>463.86 ± 21.78*§</td>
<td>414.51 ± 41.97*§</td>
<td>176.42 ± 35.77*§</td>
</tr>
<tr>
<td>Area 3</td>
<td>510.28 ± 11.68*§</td>
<td>541.12 ± 11.17*§</td>
<td>424.34 ± 51.32*§</td>
</tr>
<tr>
<td>Area 4</td>
<td>500.95 ± 10.48*§</td>
<td>509.00 ± 4.27*§</td>
<td>447.69 ± 41.03*§</td>
</tr>
</tbody>
</table>

Values are mean ± SD, ml/g dry tissue wt for H2O and \(\mu mol/g\) dry tissue wt for Na and K, from 8 rats.

*Different from normal, unoperated controls, \(p<0.005\).

†Different from homologous areas in the contralateral hemispheres, \(p<0.005\).

§Different from homologous sham-occluded controls, \(p<0.005\).
controls at all times studied. In Areas 3 and 4, [Na]d values were significantly ($p < 0.005$) greater than sham-occluded controls only at 24 hours. Figure 5 (top) shows the mean $\Delta$[Na]d relative to sham-occluded controls; the right ordinate gives the changes in terms of percent of normal rats. In Area 1, mean $\Delta$[Na]d was greater than normal by 73, 105, and 281%, respectively, at 2, 4, and 24 hours after MCAo. By 24 hours, mean $\Delta$[Na]d in Areas 2, 3, and 4 exceeded normal by 330, 71, and 46%. The rates of Na accumulation were smaller, not exceeding normal by 330, 71, and 46%. By 24 hours, mean $\Delta$[Na]d in Areas 2, 3, and 4 exceeded normal by 330, 71, and 46%.

Figure 5 (bottom) shows the mean rates of $\Delta$[Na]d relative to sham-occluded controls. In Areas 1 and 2, respectively, $\Delta$[Na]d was 165 and 104 $\mu$mol/g at 2 hours, increasing to 644 and 833 $\mu$mol/g by 24 hours. The highest rate of Na accumulation occurred between 0 and 2 hours in Areas 1 and 2, averaging 83 and 52 $\mu$mol/g/hr, respectively (Figure 5, bottom). Later, the rates of Na accumulation were smaller, not exceeding 22 $\mu$mol/g/hr in any area. Between 4 and 24 hours, Area 2 gained 34 compared with 21 $\mu$mol/g/hr in Area 1, accounting for the greater [Na]d and $\Delta$[Na]d in Area 2 at 24 hours. These represent very large increases in tissue Na. For example, in Area 2, $\Delta$[Na]d values were $>3$ times higher than normal at 24 hours.

$\textbf{Tissue Potassium Changes}$

Figure 6 shows the mean [K]d values in rats after sham occlusion and MCAo. In general, the K data were much more variable than the Na measurements, although the intrinsic error from the measurement of tissue K in uninjured brain is quite comparable with that of Na measurements. For example, as shown in Table 1, the K measurements in normal tissue have a coefficient of variability of 2.3% for wet and 7.6% for dry weight concentrations, compared with 20–35% in the lesioned hemisphere. Part of the variability is due to the surgery itself. For example, sham-occluded rats had greater variability of K values measured in Area 1 (Figure 6, top) than normal rats. Most of the variability, however, stems from the tendency of K levels to behave reciprocally in adjacent areas of the brain. For example, almost without exception during the first 2–4 hours after MCAo, when [K]d was lower than normal in Area 1, it was higher in the adjacent Areas 2 or 4. Vice versa, when [K]d was low in Area 2, it tended to be higher than normal in Area 1.

Due to the increased variability as well as the elevated [K]d values in Area 1 of sham-occluded controls, the statistical analyses revealed significant $\Delta$[K]d ($p < 0.005$) only at 24 hours in Areas 1 and 2 (Figure 7, top). At 24 hours after MCAo, [K]d in Areas 1 and 2 was 38 and 66% less than normal. The rates of K loss peaked at 4 hours in Areas 1 and 2 (Figure 7, bottom). The amount of K lost from the lesioned hemisphere...
was small during the first 2 hours after MCAo; no more than 40 μmol/g D, or about 8% of normal tissue K, was lost in any area, an insignificant amount (p < 0.1).

This pattern is strikingly different from the tissue Na changes, where the maximum rates of Na entry occurred at 0–2 hours and Δ[Na]d was 104–165 μmol/g, or 41–65% greater than normal by 2 hours (p < 0.001).

Net Ion Shifts

Na and K ions together constitute >95% of the inorganic ions in brain tissues. Therefore, sums of Δ[K]d and Δ[Na]d represent reasonable approximations of net ion shifts in the brain. Figure 8 shows net ion shifts and the rates of net ion shifts. By 24 hours, mean net ion shift in Area 2 was 509 compared with 418 μmol/g in Area 1. This represents a net increase of 65% over normal ionic strength (776.8 ± 26.8 μmol/g, Table 1). In Areas 3 and 4, (Δ[Na]d + Δ[K]d) was <70 μmol/g D at all times sampled.

The rates of net ion shifts peaked between 0 and 2 hours after MCAo, reaching 73.5 μmol/g/hr in Area 1, 32.6 μmol/g/hr in Area 2, and 34.7 μmol/g/hr in Area 3. In the remainder of the sites, net ion shifts were considerably smaller. Between 2 and 4 hours, all 4 areas sampled on the lesioned side showed no net ion gain, and some may have even lost ions. For example, Areas 2 and 4 showed rates of net ion changes from −20.4 to −38.4 μmol/g/hr, consistent with a decrease in the rate of Na gain while the rate of K loss increased.

To assess the relation between ion and water shifts, we did linear regression analysis of (Δ[Na]d + Δ[K]d) vs. Δ[H2O]d. Figure 9 shows a scatterplot of mean net ion shift vs. water changes in all 4 areas in the ischemic hemisphere and for each time period. The linear regression line had a slope of 145.5 μmol/g and a highly significant correlation coefficient of 0.992 (p < 0.001). The units of the slope are μmol ions/ml water.

Discussion

Tissue Ion Shifts and Cellular Damage

Atomic absorption spectroscopy measures total amounts of ions in the tissue sampled. Change in the ion contents therefore unambiguously reflects movement of the ions into or out of the sample site. Our data indicate massive movements of Na and K in the ischemic MCA territory. The changes are earlier and larger than those reported previously for the rat MCAo model. For example, although Gotoh et al observed increases in brain water contents as early as 3 hours after MCAo in rats, they did not detect any Na or K changes before 12 hours because they sampled entire hemispheres. In contrast, using small tissue samples, we...
found significant Na and water accumulations by 2 hours and K losses by 4–24 hours.

The magnitudes of the K and Na shifts indicate severe tissue damage in Areas 1 and 2. Since extracellular K ionic activity levels are normally <4 mM, total tissue K concentration is 104 μmol/g W and extracellular volume fraction is typically about 20%, >99% of tissue K probably resides within cells. At 24 hours after MCAo, 45–60% of the tissue K was gone from Areas 1 and 2. Most of the cells in the tissue consequently must have completely equilibrated with surrounding tissues or, more likely, an even larger proportion of the cells lost a major fraction of their intracellular contents.

The amounts of Na accumulated at the infarct site likewise suggest massive cell damage. If all the cells in the tissue were disrupted and the tissue was fully equilibrated with the surrounding extracellular fluids and blood, the maximum Na concentration resulting from passive diffusion is limited to about 150 mM, the concentration of Na in blood and extracellular fluids. In Area 2, mean Na concentration was close to this value, i.e., 135 μmol/g W at 24 hours after MCAo. To achieve such high Na concentrations, at least 90% of the cell volume at the infarct site must have equilibrated with surrounding tissues, cerebrospinal fluid (CSF), or blood. Thus, even by the most conservative estimates, the vast majority of cells in Areas 1 and 2 must be dead by 24 hours after MCAo.

Relation of Tissue Sodium and Potassium Changes

In our experiments, nearly all tissue samples in the lesioned hemisphere showed increases of Na and...
er than the loss of \([K]d\). In most areas, more than half of the Na ions entering did not exchange with K ions leaving. In some cases, the losses of \([K]d\) were negligible while the increases of \([Na]d\) were highly significant. For example, at 2 hours, \(\Delta[Na]d\) was 9 and 4 times greater than the magnitude of \(\Delta[K]d\) in Areas 1 and 2. Similar dominances of tissue Na gains over K losses have been reported in other ischemia models.8-10,13-15

The \(\Delta[Na]d\) and \(\Delta[K]d\), however, mislead because they represent cumulative ionic changes from the time of MCAo. For example, an initial large net ion influx at 0–2 hours may mask a smaller net ion efflux at 2–4 hours. Analysis of the rates of \(\Delta[Na]d\) and \(\Delta[K]d\) indeed suggests that this occurs. The greatest rate of Na gain occurred in Areas 1 and 2 during the first 2 hours, i.e., 82.6 and 51.8 \(\mu\)mol/g/hr at 0–2 hours compared with 21 and 33 \(\mu\)mol/g/hr at 2–4 hours. In contrast, rates of \(\Delta[Na]d\) suggest negligible K loss or even K gain in Areas 1 and 2 at 0–2 hours but large K losses at 2–4 hours, i.e., −48.8 \(\mu\)mol/g/hr in Area 2. Thus, in Area 2, Na influx exceeded K efflux at 0–2 hours and vice versa at 2–4 hours.

**Net Ion and Water Shifts**

The observations of Na influx into and K efflux from the lesion site are consistent with known changes of extracellular Na and K activities in ischemic brain. Extracellular K activity rises rapidly in brain within minutes after the onset of ischemia, from <4 to >50 mM, while intracellular Na falls from 155 to <70 mM.17 These extracellular ion changes produce substantial ion gradients between the lesion site and surrounding brain tissues, CSF, and vascular compartments. Na and K ions diffuse down these gradients, resulting in Na influx into and K efflux from the lesion site. Since the formation of these extracellular ionic derangements precedes the development of edema, it is reasonable to consider them potential causes of the early edema of ischemia.

Electroneutrality considerations require that every Na ion that enters the tissue must exchange with a similarly charged ion or be accompanied by an oppositely charged ion. In the former case, exchange results in no net ion flux. In the latter case, a net influx of Na and anions results, increasing interstitial ionic strength. Enough water should enter to equalize interstitial ionic osmolality with fluids in surrounding brain, blood, and CSF, i.e., approximately 155 \(\mu\)mol/ml. Our findings of large net ion shifts in ischemic rat brains, a high correlation between net ion shifts and water movement, and a regression slope indicating that 1 ml of water accompanied each 145 \(\mu\)mol of Na or K entering the tissue strongly support this scenario of events in the rat MCAo model. We therefore conclude that the early edema of regional brain ischemia is caused by the net ion shifts.

**Mechanisms of Net Ion Shifts**

What causes the net ion shift? Brain Na concentration is 53 \(\mu\)mol/g W compared with 155 \(\mu\)mol/ml in blood and extracellular fluids of surrounding tissues. Brain K concentration is 112 \(\mu\)mol/g W compared with 4 \(\mu\)mol/ml in blood. Therefore, injuries causing intracellular and extracellular compartments to equilibrate should produce opposed Na and K gradients of about 100 mM between the lesion site and surrounding tissues. Na ions should exchange with K and produce no net ion shift. Yet, our data suggest that more Na enters than K leaves the lesion site. On average, 2–3 times as much Na accumulated at the lesion site as the amount of K lost. For Na gain to exceed K loss to this extent, a barrier must favor Na influx over K efflux, or the driving forces for Na entry must be greater than for K clearance.

Gotoh et al15 proposed that a selective increase in Na permeability of the blood–brain barrier (BBB) is responsible for the greater Na accumulation in the rat MCAo model. This hypothesis is untenable for the following reasons. Even if the BBB were to become selectively permeable to Na ions, the lesion site can exchange ions with surrounding tissues and CSF. There is no known extracellular barrier that favors Na diffusion over K to the extent observed. In fact, studies of intrabrain diffusion of K and Na suggest that K diffusion may be faster than Na diffusion.18,19 Also, in severely ischemic tissues, the amount of ions and water that can be supplied or cleared by blood flow should be limited. Intra-tissue diffusion of Na and K is likely to dominate over transvascular ion transfer in ischemic tissues.

We sought an alternative explanation of the net ion shift based on possible differences in the driving forces for Na influx and K clearance. Glial cells are well known to be more permeable to K than Na ions.16-20 Assuming that glial cells occupy about the same volume as neurons and that the extracellular volume fraction is about 20%, K ions can potentially distribute into an extraneuronal compartment 3 times larger than the compartment available for Na ions. Therefore, the K gradient that develops between the extracellular space of the infarct site and surrounding tissues may be one-third the gradient for Na entry into brain. We consequently hypothesize that glial cell buffering of K ions released into the extracellular space by ischemic neurons reduces the driving force for K loss. As glial cells die, however, the glial compartment becomes less selective for K, and therefore we expect greater losses of K with time. Our data support this expectation.

**Predictions of the Glial Buffering Hypothesis**

Several testable predictions follow from the glial buffering hypothesis. First, glial buffering should be associated with rapid clearance of the released extracellular K. The interstitial K gradient between the lesion site and surrounding tissues or blood should diminish more rapidly than the Na gradient. Such extracellular ion changes should be easily demonstrated by ion-selective microelectrode recordings of Na and K in the MCAo model.

Second, if glial buffering of K is the cause of a net ion shift, any injury that simultaneously destroys both
glial and neuronal compartments should reduce net ion shifts at the lesion site. In such an injury situation, the entire tissue compartment becomes available for both K and Na redistribution. The driving forces for Na entry and K loss should be similar, and therefore we expect Na gains and K losses to be similar.

Third, agents that decrease K entry into glial cells should decrease edema. For example, lowering the K or Cl permeability of glial cells should impair glial buffering of extracellular K, enhance Na-K exchange between the lesion site and surrounding tissues, and reduce the net ion shift. By contrast, such agents should have little effect on edema resulting from extravasation of protein through the BBB or a selective increase in Na permeability of the BBB.

Ionic Edema

Edema is a common reaction of nervous tissues to injury. Tissue swelling has been reported to occur as early as 5 minutes after an ischemic event and has been attributed to cytotoxic cellular swelling. Katzman et al identified an intermediate stage of edema which manifests as tissue water content increases before the appearance of BBB breakdown, calling it "ischemic" edema to distinguish from "vasogenic" edema, which is associated with increases in vascular permeability to serum protein. Our findings of a large net ion influx peaking at 0–2 hours after MCAo and a linear correlation between water entry and the net ion shift strongly argue for a causative role of the ion shifts in the early edema observed in ischemia. The net ion influx alone is sufficient to explain the edema. We suggest replacing the term "ischemic" edema with "ionic" edema, a name that not only better expresses the mechanisms underlying the phenomenon but does not restrict the phenomenon to ischemia.

Ionic edema should be readily distinguishable from vasogenic edema by the absence of large molecular penetration across the BBB. The presence of large tissue K losses and Na gains, a net ion shift, and increase in tissue water differentiates ionic edema from cytotoxic edema, a term that perhaps should be reserved for microscopic observations of cellular swelling resulting from ionic redistributions within the tissue. Finally, unlike vasogenic edema, which can occur with BBB breakdown in the absence of cellular damage, and unlike cytotoxic edema, which may be reversible, ionic edema should directly reflect the extent of cellular damage.

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