Cerebral Glucose Metabolism During the Recovery Period After Ischemia — Its Relationship to NADH-Fluorescence, Blood Flow, EcoG and Histology

Kortaro Tanaka, M.D., Eors Dora, M.D., Joel H. Greenberg, Ph.D., and Martin Revich, M.D.

SUMMARY Local cerebral glucose utilization (ICMRgl), NADH fluorescence, cerebral blood flow (CBF), electrocortical activity (ECOg) and histology were studied during a 4 hr recovery period following 2 hrs of left middle cerebral artery (MCA) occlusion in cats. Changes in relative reduced pyridine nucleotides and CBF were measured by fluororeflectometry, ECOG was obtained from the left middle ectosylvian gyrus (MEG), and ICMRgl was measured at the end of the recovery period autoradiographically with 14-C-2-deoxyglucose. A sham group was comprised of 4 cats. The ten animals subjected to the stroke were classified into 3 groups based on the mean amplitude of the ECOG at the end of the ischemic period. At the end of the recovery period, the relative reduced pyridine nucleotides showed a 22.5% oxidation (oxidation of NADH), a 66.2% reduction (reduction of NAD) and a 3.0% reduction compared to the sham group in the severe, moderate and mild groups, respectively. LCMRgl of the left MEG in the severe group was 64.2% of the corresponding sham value, whereas ICMRgl in the moderate and mild groups were 124.8% and 132.0% of the sham, respectively. CBF at the end of the recovery period ranged from 28.1% to 83.0% of the sham value, although there was no significant difference among these groups. Histologically, a large portion of the neurons in the left MEG in the severe group showed ischemic neuronal changes, while the damage was less severe in the moderate and mild groups. On the basis of these data, it is suggested that a relative substrate deficiency and/or a loss of mitochondrial enzymatic pool size may occur in the animals comprising the severe group. Conversely, anaerobic glycolysis may be activated in the moderate group, while the mild group exhibits an increase in glucose metabolism that is most likely aerobic. A gradient in the magnitude of changes in ICMRgl was noted from the central MCA territory to the surrounding brain regions in the ischemic hemisphere. In addition, there was a mild, but statistically significant (p < 0.05), depression in ICMRgl with no histological damage in the non-ischemic hemisphere of the severe group.

Stroke Vol 17, No 5, 1986

UNDER NORMAL CONDITIONS, the energy production of brain tissue is almost totally dependent upon oxidative metabolism in the mitochondria. The mitochondrial mechanism of synthesizing energy-rich compounds in brain, however, seems to be very vulnerable to ischemia and it has been demonstrated that the dysfunction of brain mitochondrial metabolism deteriorates further during recirculation after incomplete ischemia. In addition, histopathological studies have shown that structural mitochondrial alterations are the first sign of ischemic cellular damage in brain tissue. Since the pyridine nucleotide coenzyme, reduced nicotin-amine adenine dinucleotide (NADH), stands at the negative end of the chain of mitochondrial respiratory components, the pyridine nucleotide fluorescence correlates well with the ability of mitochondria to carry out energy-linked functions such as the production of adenosine triphosphate (ATP) and the removal of reducing equivalents. The method of surface fluororeflectometry, which enables us to continuously monitor the alterations of pyridine nucleotide fluorescence, vascular volume and cerebral blood flow (CBF) in the same volume of tissue, has been recently applied to various animal studies, and a significant reduction...
in the relative levels of reduced pyridine nucleotides (RLRPN) has been noted immediately after occlusion of the middle cerebral artery (MCA) in cats. This finding suggests that cerebral oxidative metabolism is very sensitive to ischemia. The recovery process of mitochondrial function after ischemia, however, still awaits further investigation.

Several studies have reported posts ischemic derangements of cerebral glucose metabolism ranging from foci of activation to marked suppression. The wide-range of abnormalities of glucose metabolism seem to correlate well with functional and structural tissue damage, although the exact underlying mechanism for the varied glucose metabolic response after ischemia is still unknown.

In the present study, 14-C-2-deoxyglucose (14-C-2DG) autoradiography, fluororeflectometry and histological evaluation were performed in the same animals subjected to 4 hours of recirculation following 2 hours of MCA occlusion. The results demonstrate alterations of the RLRPN ranging from a significant reduction to a hyperoxidation, which appear to have a close relationship with both local cerebral glucose utilization (ICMRgl) and histological damage. The time course of changes in the fluororeflectometric data and the electrocortical activity (ECoG) during the ischemic insult as well as during the subsequent recovery period has been presented in a separate publication. The present communication focuses on the events at the end of the recovery period.

Methods

Adult male cats weighing between 2.5 kg and 3.5 kg were anesthetized with intraperitoneally administered sodium pentobarbital (40 mg/kg). The animals were immobilized with 5 mg/kg gallamine triethiodide following the tracheostomy, and were artificially ventilated with a Harvard respirator. Femoral arteries and veins were cannulated, and an additional catheter was placed retrogradely into the left lingual artery for repeated bolus injections of isotonic, oxygenated dextran solution (0.1 ml-0.3 ml) used to obtain cerebrocortical blood content (i.e., cerebrocortical vascular volume, CVV) and reduced pyridine nucleotide fluorescence were measured photoelectrically through the cranial window. The brain cortex was excited at 366 nm light, and both the reflected light (366 nm) and the emitted fluorescence (450 nm) were measured by photomultiplier tubes (EMI, United Kingdom) using appropriate optical filters (for reflectance, Corning 5874 glass filter; for fluorescence, Kodak Wratten 2C and 47 gelatine filters). The measurement of the changes in the relative levels of pyridine nucleotides in vivo by surface fluorometry is based on the data of Chance et al, who showed that NADH and NADPH, but not NAD and NADP, have a strong fluorescence at 450 nm when excited at 366 nm. Artificial changes in fluorescence, caused by alterations in the tissue blood content, was avoided by subtracting a fraction of the reflected signal from the fluorescence signal. This fraction was determined by injecting dextran into the lingual arterial catheter (artificial hemodilution) and adjusting the subtraction circuit so that the corrected fluorescence signal did not change. Mean transit time of cortical blood flow (tm) was determined by area-over-height analysis of the hemodilution-induced cortical reflectance curves. Cerebrocortical blood flow (CBF) was calculated by dividing CVB by tm. The diameter of the excited area was 1 mm, and most of the optical signal is obtained from the cortical surface down to a depth of 0.3 mm.

Copper screws were fixed into the temporal bone of the contralateral hemisphere for the measurement of the electrocorticogram. The pO2, pCO2 and pH of the arterial blood were determined frequently with a Radiometer blood gas analyzer (BMS3, MK2). The rectal temperature of the animal was maintained at 37°C by a thermostatically controlled infrared lamp (73A, Yellow Springs Instrument). Arterial blood pressure was monitored with a transducer (Model P23De, Statham) connected to the femoral artery catheter and end tidal carbon dioxide concentration was monitored with an infrared medical gas analyzer (LB-2, Beckman Instruments). Cerebrocortical NADH fluorescence and reflectance, ECoG of each hemisphere, and arterial blood pressure were recorded on a multi-channel polygraph (Hewlett-Packard). Mean transit time was determined every 15-30 min.

The proximal portion of the left middle cerebral artery (MCA) was exposed via a transorbital approach using a modified O’Brien and Waltz technique. After a control period, ischemia was initiated in a series of ten cats by occluding the proximal MCA trunk with a miniature Mayfield clip. In four control animals, a sham-insult was initiated by lightly touching the MCA with a glass rod. Following 120 min of MCA occlusion, the clip was removed, and re-expansion of the MCA trunk was visually confirmed. At 3 hr and 15 min after release of the clip, 250 ìCi of 14-C-2DG (New England Nuclear) was injected intravenously as a bolus for the determination of ICMRgl during the recovery period. Arterial blood samples were drawn during the next 45 min, initially at 15 to 30 sec intervals and later at 1 to 10 min intervals. Plasma aliquots of these samples were assayed for 14-C concentration using a liquid scintillation counter (Packard Instruments) to define the time course of arterial plasma 14-C-2DG activity. Larger samples were taken at 10 min intervals for the determination of the arterial plasma glucose level. At 45 min following the injection of 14-C-2DG, the animal was killed by the intravenous administration of saturated potassium chloride solution,
and the brain was quickly removed and cut into 4 mm thick coronal blocks. Alternate blocks were immersion-fixed in 10% formalin for 10 days, and processed for histological evaluation by light microscopy. The sections cut from paraffin-embedded blocks were stained with Nissl or hematoxylin-eosin. The remainder of the blocks were frozen in Freon-22 (DuPont) at −55 degrees C. Twenty μm thick sections were cut from these blocks at −20 degrees C in a cryostat (American Optical Company), and were placed on glass coverslips and dried on a hot plate (60 degrees C) for 5 min. The dried sections were then placed in apposition to X-ray film (SB-5, Eastman Kodak) together with calibrated 14-C standards for 10 days. The resultant autoradiograms were subjected to quantitative densitometric measurements by means of a computerized image analysis system consisting of a rotating drum scanner (Optronics) and an image analyzer (Grinnell Systems). 30

For the calculation of ICMI Rgl, the operational equation of Sokoloff et al. 29 as modified for a changing plasma glucose level by Savaki et al. 31 was used. The value of the lumped constant of normal anesthetized cats (0.411) 32 was employed, since preliminary data obtained in cats 30 suggest that the lumped constant for postischemic brain might be similar to that for the normal brain (see below). The rate constants for glucose and deoxyglucose as determined in the rat 29, 31 were employed, since the similarity of the values between rat and monkey 34 suggests that the rate constants for the cat will also be similar. The rate constants for the postischemic brain might be different from those in the normal brain. The effect of this difference on the calculation of ICMI Rgl will be discussed below. When the relationship of ICMI Rgl with the fluororeflectometric data was studied as shown in figure 2 and figure 3, ICMI Rgl was calculated from the upper 0.3 mm of the cortical area under the light spot so as to represent the same region as measured by the fluororeflectometer.

Histological changes in each anatomical structure were graded into four categories. 35, 36 The first category was histologically normal (Grade 0). The second category showed slight ischemic changes such as shrunken cell bodies with triangular, darkly stained cytoplasm and loss of discrete Nissl substance with only widely scattered neurons affected (Grade 1). The third category exhibited moderate changes with a typical medium-power microscopic field containing several affected neurons (Grade 2). In the fourth category, a large portion of neurons were affected, which were often accompanied by edematous neuropil (Grade 3). By using the 4 mm thick alternate blocks for histology, it was possible to observe the histological changes in the region adjacent to the area from which the fluororeflectometric data and ICMI Rgl were obtained.

Results

Animals were classified into three groups depending on the mean amplitude of the EEG on the contralateral side at the end of the recovery period constituted the severe group, whereas those with the EEG amplitude more than 70% of that on the contralateral value constituted the mild group. The animals with an EEG amplitude between 20% and 70% of the EEG on the contralateral side were classified as the moderate group. Using this criteria, five cats were in the severe group with an average EEG amplitude that was 7.4 ± 1.7% (mean ± SEM) of the corresponding sham value at the end of study, while the moderate and mild groups consisted of three and two animals respectively, with average EEG amplitudes of 39.6 ± 7.5% and 86.1 ± 10.8%, respectively. There was no apparent seizure activity present in the EEG in either hemisphere in any of the animals. The EEG amplitude of the non-ischemic side was slightly depressed during the study as compared to the sham animals. At the end of the study, the mean EEG amplitudes of the severe, moderate and mild groups were 78.9 ± 11.2%, 72.6 ± 16.4% and 77.8 ± 23.4% of the sham values, respectively.

Physiological Data

As shown in table 1, blood gases and mean arterial blood pressure (MABP) were within the normal range throughout the study in all animals, and there was no statistical difference among the four groups of animals (the Bonferroni method). 39

Local Cerebral Glucose Metabolism of the Sham Group

The local CMRI Rgl of various anatomical structures of the sham animals are listed in table 2. Those structures belonging to the cerebral gray matter are grouped into 3 territories: central MCA territory, peripheral MCA territory and non-MCA territory, as shown in the table. This classification is based on the distribution of the MCA. 19 There was neither any focal abnormalities nor any differences in ICMI Rgl between the hemispheres in any specific structure.

RLRPN and EEG in the Left Middle Ectosylvian Gyrus

The EEG amplitude of the left middle ectosylvian gyrus at the end of the study was correlated with the RLRPN (fig. 1). RLRPN is expressed as the change from the control period to the end of the study from which is subtracted the corresponding change in the sham animals. It was necessary to subtract the changes seen in the sham animals since there was a gradual reduction (20.5%) in RLRPN of this group toward the end of the study due to lightening of anesthesia. Accordingly, the upward shifts from the zero line in the future figures represent changes toward reduction and oxidation, respectively, as compared to the sham group. As is evident in this figure, the animals in the severe group exhibited a mild oxidation in the RLRPN. By contrast, the animals in the moderate group always showed considerable reduction, and the animals in the mild group exhibited a near normal redox state. These findings suggest a close relationship between the degree of depression in EEG amplitude and mitochondrial functional state.
TABLE 1  Physiological Data

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>At 1 hr during ischemia or sham-insult</th>
<th>During recovery period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>at 1 hr</td>
<td>at 3 hr</td>
</tr>
<tr>
<td>Experimental group (n = 10)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild group (n = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>93.5±0.5</td>
<td>92.5±1.5</td>
<td>105.5±10.5</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>30.8±2.3</td>
<td>30.8±2.3</td>
<td>31.5±0.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.371±0.070</td>
<td>7.385±0.055</td>
<td>7.315±0.015</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>137.5±12.5</td>
<td>130.0±30.0</td>
<td>137.5±12.5</td>
</tr>
<tr>
<td>Moderate group (n = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>107.0±10.0</td>
<td>118.0±2.5</td>
<td>117.5±5.4</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>32.2±2.2</td>
<td>31.4±2.8</td>
<td>35.3±3.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.343±0.047</td>
<td>7.310±0.043</td>
<td>7.354±0.044</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>133.0±8.4</td>
<td>141.7±8.2</td>
<td>141.7±8.2</td>
</tr>
<tr>
<td>Severe group (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>97.2±4.0</td>
<td>113.2±6.1</td>
<td>105.2±2.6</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>30.9±1.1</td>
<td>9.8±1.1</td>
<td>30.0±0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.367±0.027</td>
<td>7.376±0.017</td>
<td>7.345±0.009</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>135.0±4.5</td>
<td>151.0±9.8</td>
<td>151.0±7.5</td>
</tr>
<tr>
<td>Sham group (n = 4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>106.7±3.0</td>
<td>105.2±2.4</td>
<td>103.7±1.0</td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td>32.2±2.2</td>
<td>33.0±0.7</td>
<td>31.7±0.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.378±0.009</td>
<td>7.360±0.011</td>
<td>7.386±0.018</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>136.2±5.5</td>
<td>150.0±4.1</td>
<td>162.5±4.8</td>
</tr>
</tbody>
</table>

Values are mean ± standard error.  

n = number of animals; MABP = mean arterial blood pressure.

GLUCOSE METABOLISM, RLRPN AND HISTOLOGY IN THE LEFT MIDDLE ECSTOSYLVIAN GYRUS

Figure 2 shows the relationship among lCMRgl, RLRPN and histology in the left middle ectosylvian gyrus. The ICMRgl is expressed as a percentage of the sham value, whereas the scale of the RLRPN is the same as in figure 1. The histological grade is indicated on the side of each data point. The animals in the

TABLE 2  Local Cerebral Metabolic Rate for Glucose in Sham Animals and Animals Recovering from Cerebral Ischemia

<table>
<thead>
<tr>
<th></th>
<th>Sham group</th>
<th>Mild group</th>
<th>Moderate group</th>
<th>Severe group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lt</td>
<td>Rt</td>
<td>Lt</td>
<td>Rt</td>
</tr>
<tr>
<td>Central MCA territory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>middle ectosylvian gyrus</td>
<td>30.2±2.9</td>
<td>28.0±3.6</td>
<td>39.9±1.1</td>
<td>32.0±0.8</td>
</tr>
<tr>
<td>anterior sylvian gyrus</td>
<td>26.4±1.7</td>
<td>26.3±0.4</td>
<td>34.7±3.9</td>
<td>29.6±0.4</td>
</tr>
<tr>
<td>caudate nucleus</td>
<td>32.7±2.2</td>
<td>34.0±1.2</td>
<td>37.7±5.0</td>
<td>36.8±0.8</td>
</tr>
<tr>
<td>Peripheral MCA territory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>middle suprasylvian gyrus</td>
<td>27.2±2.1</td>
<td>26.4±2.5</td>
<td>31.6±1.1</td>
<td>29.5±0.8</td>
</tr>
<tr>
<td>anterior suprasylvian gyrus</td>
<td>26.7±1.4</td>
<td>25.6±0.7</td>
<td>33.4±4.3</td>
<td>30.3±2.5</td>
</tr>
<tr>
<td>anterior ectosylvian gyrus</td>
<td>28.3±2.3</td>
<td>28.3±1.0</td>
<td>30.1±3.8</td>
<td>30.1±0.4</td>
</tr>
<tr>
<td>Non-MCA territory</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sigmoid gyrus</td>
<td>26.2±1.8</td>
<td>27.1±1.8</td>
<td>28.5±1.8</td>
<td>30.9±0.8</td>
</tr>
<tr>
<td>cingulate gyrus</td>
<td>30.1±1.6</td>
<td>28.4±1.2</td>
<td>34.2±0.6</td>
<td>34.0±1.4</td>
</tr>
<tr>
<td>parahippocampal gyrus</td>
<td>22.4±2.3</td>
<td>24.9±3.6</td>
<td>25.1±0.8</td>
<td>26.3±1.3</td>
</tr>
<tr>
<td>Cerebellar gray matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>upper hemisphere</td>
<td>22.7±1.0</td>
<td>22.8±1.1</td>
<td>24.8±3.6</td>
<td>25.9±4.4</td>
</tr>
<tr>
<td>lower hemisphere</td>
<td>22.6±1.2</td>
<td>22.5±0.8</td>
<td>25.5±2.8</td>
<td>24.9±2.9</td>
</tr>
</tbody>
</table>

Values are mean ± standard error in μmol/100 g/min.  

MCA = middle cerebral artery; Lt = the left hemisphere; Rt = the right hemisphere.
group. There was no correlation between 1CBF and histologic damage, nor between 1CBF and ICMRgl.

Local Cerebral Glucose Metabolism in Various Regions of the Experimental Group

The ICMRgl values in individual cerebral structures of the animals subjected to 2 hr MCA occlusion followed by a 4 hr recirculation period are listed in table 2. The structures within each territory group exhibited similar changes in ICMRgl in all experimental groups when compared to the sham group. The data from each structure was converted to a percentage of the sham value of the corresponding structure and all structures comprising the territory were averaged to obtain a mean ICMRgl value (as a percentage of the sham). Statistical analysis was performed for the multiple intergroup comparisons using an analysis of variance and the Bonferroni method.37

1. Central MCA territory. On the ischemic (left) side, the mild group showed a statistically significant increase in ICMRgl in the central MCA territory as compared to the same (p < 0.01) with an average value of 126.2 ± 6.5% of the sham (fig. 5). The moderate group exhibited a similar enhancement of ICMRgl (123.7 ± 7% of the sham group). In contrast, the severe group showed a significantly depressed ICMRgl (p < 0.001) with an average value of 63.5 ± 1.9% of the sham level. On the non-ischemic side, the mild group showed a slight but not significant increase in ICMRgl, while the severe group exhibited a mild, but statistically significant (p < 0.05) decrease with an average value of 88.9 ± 4.3% of the sham. LCMRgl severe group invariably showed a moderate decrease in ICMRgl of between 53.8% and 75.3% of the sham along with an oxidation of the pyridine nucleotides. These changes were accompanied by moderate (grade 2) or severe (grade 3) histologic damage. On the other hand, the animals in the other groups exhibited a normal or mildly activated ICMRgl with less severe or no histologic damage. It is noteworthy that there was a rather large difference in the RLRPN between the mild and moderate groups in spite of the similar level of ICMRgl. Even though immersion fixation was utilized in this study, artifactual darkly stained neurons were not prevalent as evidenced by close examination of stained sections from the contralateral hemisphere (fig. 3).

Blood Flow, Glucose Metabolism and Histology in the Left Middle Ectosylvian Gyrus

Figure 4 shows the relationship among 1CBF, ICMRgl and histology in the left middle ectosylvian gyrus. Both 1CBF and ICMRgl are expressed as a percentage of the corresponding sham values. CBF in all animals was below the sham level. The animals with ICMRgl increased to a level greater than the sham animals showed a 1CBF value ranging from 42.3% to 56.2% of the sham, whereas there was a wide scatter in 1CBF (28.1%–83.0%) in the animals of the severe group.
of the moderate group was intermediate between the other two groups.

2. Peripheral MCA territory. In the peripheral MCA territory of the ischemic side, both the mild and moderate groups showed increases in ICMRgl of 21.2 ± 6.0% (p < 0.02) and 17.6 ± 5.9% (p < 0.02) above the sham level, respectively (fig. 6). These increases were slightly smaller than those observed in the central MCA territory. In contrast to these changes, ICMRgl in the severe group did not show any consistent change from the sham level, although the average value was significantly higher than the values in the

**Figure 3.** Photomicrographs from animals classified as mild (a,b), moderate (c,d) and severe (e,f) based on the reduction of the relative amplitude of the EEG. The sections on the right (b,d,f) are from the occluded (left) cortex in the middle cerebral artery territory while the sections on the left are from homologous regions from the same section in the control (right) hemisphere. Note an increasing pyknosis of neurons and vacuolation of the neuropil from the mild to the severe animals. There are negligible fixation artifacts as seen by examination of the control hemisphere. (H + E and original magnification ×225).

**Figure 4.** The relationship between local cerebral blood flow (ICBF), local cerebral metabolic rate for glucose (ICMRgl) and histology in the left middle ectosylvian gyrus. The ICBF (ordinate) and ICMRgl (abscissa) are expressed as percentage of the sham value.

**Figure 5.** Local cerebral metabolic rate for glucose (ICMRgl) of the mild, moderate and severe groups in the central MCA territory of each hemisphere. The left side (Lt) was ischemic, whereas the right side (Rt) was not ischemic. Each data point is expressed as a percentage of the sham value, and represents mean ± standard error of ICMRgl of the middle ectosylvian gyrus, the anterior sylvian gyrus and the caudate nucleus.
Figure 6. Local cerebral metabolic rate for glucose (ICMRgl) of each group in the peripheral MCA territory of each hemisphere. Each data point represents the mean ± standard error of ICMRgl of the middle suprasylvian gyrus and the anterior ectosylvian gyrus.

central MCA territory ($p < 0.001$, Student's non-paired t-test) which exhibited a depression in glucose utilization. On the non-ischemic side, only the mild group showed a change, with an increase of $12.2 ± 2.4\%$ above the sham level; this was not a statistically significant change, however.

Figure 7. Local cerebral metabolic rate for glucose (ICMRgl) of each group in the non-MCA territory of each hemisphere. Each data point represents the mean ± standard error of the ICMRgl of the sigmoid gyrus, the cingulate gyrus and the parahippocampal gyrus.

3. Non-MCA territory. Figure 7 displays ICMRgl in the non-MCA territory. There were no significant changes except for a small depression in the non-ischemic side of the severe group where ICMRgl was $90.8 ± 4.0\%$ of the sham ($p < 0.05$).

4. Cerebellar hemispheres. There were no significant changes in ICMRgl in either hemisphere of any group in the cerebellum (fig. 8).

Histological Damage and Glucose Metabolism

Figure 9 shows the distribution of ICMRgl in the gray matter of the postischemic hemisphere plotted against the histological damage in the same regions. The regions with slight damage showed an average ICMRgl of $118.7 ± 4.1\%$ of the sham value, whereas the regions with severe damage had a significantly depressed ICMRgl with an average value of $69.8 ± 2.8\%$ of the sham. The ICMRgl of the regions with moderate histologic damage was intermediate between those of the mildly and severely damaged regions. The regions with no histologic changes had glucose utilization rates ranging from $83.0\%$ to $131.5\%$ of the sham value with an average value of $105.3 ± 2.2\%$.

Discussion

The calculation of ICMRgl in postischemic tissue using normal values for the rate constants and the lumped constant of deoxyglucose should be viewed with caution, since there might be some changes in these parameters under pathological conditions. As discussed elsewhere, however, no systematic evaluation of the rate constants in postischemic brain has been made. Preliminary data in a diffuse ischemia model in the cat suggest that the lumped constants for
2-DG is elevated significantly during ischemia, but returns toward a normal level during the subsequent recovery period. Further studies are needed to document the changes in these constants that may occur during ischemia as well as during the recirculation period both as a function of flow and histologic damage.

One of the striking findings of the present study is the oxidation of the pyridine nucleotides (so-called hyperoxidation) as compared to the sham in the animals with significantly depressed ECoG activity as well as severe histological damage. The ICMRgl in these animals was moderately depressed, while ICBF showed a rather wide scatter (28.1%-83.0% of the flow of the sham group). Since the fluorometric technique in the brain measures mostly the changes in mitochondrial NADH fluorescence, the above findings may suggest that there existed a serious derangement of energy production related to mitochondrial oxidative metabolism. The following possibilities can be raised to explain the observed hyperoxidation of the RLRPN:

1. Deficiency of substrate relative to oxygen supply;
2. Decreased mitochondrial enzymatic pool size;
3. Uncoupling of oxidative phosphorylation;
4. Seizure activity;
5. Increased oxygen availability to the tissue.

Kogure et al. have suggested that posts ischemic tissue may have difficulty further oxidizing pyruvate resulting in a lack of electron supply to the mitochondrial respiratory chain. On the other hand, Duckrow et al. speculated that a block in the glucose metabolic pathway would have to exist after the phosphorylation of glucose but before the tri-carboxylic acid cycle, and would cause a relative substrate deficiency. In the scheme outlined by Kogure et al., ICMRgl would be enhanced due to the activation of glycolysis; in our study, however, ICMRgl was depressed. This finding supports the hypothesis of Duckrow et al., since if there is a block in the metabolic pathway, intermediate metabolites would accumulate which in turn would induce an inhibition of hexokinase activity by a feedback mechanism.

The loss of NAD from the tissue, with subsequent inhibition of NADH generation, as suggested by Welsh et al. may have resulted from the disruption of the plasma and mitochondrial membranes accompanying the severe histologic damage. In addition, the acid-catalyzed destruction of NADH which is accelerated by tissue acidosis both during ischemia and recovery, may have contributed to the hyperoxidation of the pyridine nucleotides.

Based on fluorometric data along with a measured increase in oxygen consumption following transient ischemia, Rosenthal et al. have suggested that the primary pathogenic mechanism of ischemic damage may be the uncoupling of oxidative phosphorylation. With an uncoupled mitochondrial system, however, glucose utilization of the tissue should also be activated. This activation would be caused by an increased flux through the glycolytic pathway stimulated by a decrease in the cytosolic phosphorylation potential of free adenine nucleotides and the consequent stimulation of phosphofructokinase. Our finding of a depressed ICMRgl does not support the existence of uncoupled mitochondrial metabolism, an interpretation consistent with the view of Duckrow et al.

Although energy-consuming seizure activity can produce hyperoxidation of the redox state, the ECoG recordings did not show any apparent seizure activity. Likewise, increased tissue oxygenation is not likely to play a role in the hyperoxidation since the ICBF values were always lower than the sham level throughout the study.

In the moderate group, which exhibited an increased glucose metabolism (106.2%-145.6% of the sham), the RLRPN was significantly reduced in comparison to the sham group (p < 0.005, the Bonferroni method). These changes were accompanied by slight to moderate histologic damage. An increase of ICMRgl during posts ischemic recirculation has been noted in several animal models, although the pathophysiological mechanism responsible for this metabolic alteration is still controversial. The reduction in the RLRPN might be explained by a number of mechanisms: (1) tissue hypoxia, (2) enhanced glycolysis and (3) decreased neuronal activity.

While PaO2 remained normal throughout the study, ICBF at the end of the recovery period was between 42.3% and 56.2% of the sham level, which may produce mild tissue hypoxia accompanied by a reduction in the RLRPN. Under normal conditions, the threshold of CBF at which anaerobic glycolysis is activated is 40% - 45% of the control. In the animals in the moderate group, ICBF was decreased to below 35% of the sham during the ischemic period. This reduction in flow, along with data showing an aggravation of mitochondrial dysfunction and a posts ischemic persistence.
of anaerobic glycolysis,\textsuperscript{31} suggests that the enhanced IC\textsubscript{MRgl} in the moderate group was largely due to anaerobic glycolysis, and was responsible for the reduction of the RLRPN. Decreased neuronal activity indicated by the moderately depressed ECoG amplitude may also have contributed to the reduction of the RLRPN.

In the mild group, the RLRPN was within the normal range, and was accompanied by a normal or mildly depressed ECoG along with a mildly increased IC\textsubscript{MRgl}. Histologic damage either was not present or was slight. This implies that mitochondrial function as well as energy metabolism was not damaged in these animals, even though the recovery of IC\textsubscript{BF} was not complete. It should be noted that there was no difference in IC\textsubscript{BF} and IC\textsubscript{MRgl} at the end of the recovery period between the moderate and mild groups, while they did differ in RLRPN and ECoG activity. These differences presumably result from events occurring during the ischemic event. Indeed, the average IC\textsubscript{BF} of the mild group 60 min after MCA occlusion was 37.0 ± 3.7\% of the control, which was higher than the corresponding value of the moderate group (23.6 ± 5.4\%), while during the remainder of the ischemic period, IC\textsubscript{BF} of the mild group gradually increased and in the moderate group decreased further. The slightly increased IC\textsubscript{MRgl} along with a normal RLRPN may indicate that glucose utilization is stimulated aerobically in parallel with an increased tissue oxygen extraction such that the balance between the substrate flow and the oxygen supply in the mitochondrial respiratory chain remains normal. The increased energy production might have been needed for cellular repair following the ischemic insult.\textsuperscript{31}

Since the middle ectosylvian gyrus is invariably the core of the ischemic insult induced by MCA occlusion in cats,\textsuperscript{15, 39} the changes in ECoG activity recorded from this gyrus can be regarded as an indicator of the severity of the ischemic damage. Consequently, the animals were classified into 3 groups depending upon the ECoG amplitude. In order to establish the extent of derangement in glucose metabolism in the whole brain in terms of the severity of the insult, as judged by ECoG activity, IC\textsubscript{MRgl} in various regions was examined (figs. 5–8).

In the central MCA territory, the IC\textsubscript{MRgl} of the non-ischemic hemisphere in the severe group exhibited a slight, but statistically significant, depression. A similar depression of IC\textsubscript{MRgl} in the contralateral hemisphere in the non-MCA territory (fig. 7) was also observed although this metabolic depression was not accompanied by any histological cell damage. This finding seems to be comparable to the mild suppression of cortical glucose utilization of the non-ischemic hemisphere observed during MCA occlusion by Ginsberg et al.\textsuperscript{33} Examination of the EEG records from that study indicates that their animals correspond to the severe group of our study. In their pooled data, however, IC\textsubscript{MRgl} was depressed by 30\% of the sham value, whereas in our study depression was only 10\%. Additionally, in the present study, there was no sign of metabolic depression in the peripheral MCA territory, whereas Ginsberg et al\textsuperscript{33} noted a depression in this territory similar to that observed in other regions. These findings suggest that the metabolic depression of the non-ischemic (contralateral) hemisphere may gradually recover during the reperfusion period, with a regional inhomogeneity in this recovery process. Indeed, it has been reported from clinical studies that the depression of oxygen consumption and CBF, which seems to correspond to the metabolic depression, continues only temporarily and then tends to recover in the non-ischemic hemisphere after the onset of stroke.\textsuperscript{53–55}

Recently, crossed cerebellar diaschisis has been demonstrated in which CBF and oxygen consumption\textsuperscript{53–54} and IC\textsubscript{MRgl}\textsuperscript{57} are depressed in the cerebellar hemisphere contralateral to a supratentorial infarction in patients. There was no indication in the present study, however, of any metabolic depression in the contralateral cerebellar hemisphere (fig. 8). The absence of contralateral cerebellar effects may be attributed to differences in the ischemia model since in our studies reperfusion follows a short period of occlusion whereas the clinical reports were obtained in patients with persistent ischemia.

In the peripheral MCA territory, the changes in IC\textsubscript{MRgl} of each group was smaller than the corresponding changes observed in the central MCA territory. No significant changes in the non-MCA territory of the ischemic hemisphere were noted. These data indicate that there is a gradient of metabolic derangement surrounding the central MCA region. Histological evaluation showed a similar gradient of damage, with the peripheral territory tending to exhibit less severe damage than the central MCA territory, with no damage in the non-MCA territory.

As shown in figure 9, in which the distribution of pooled IC\textsubscript{MRgl} data from the posts ischemic hemisphere is plotted against the grade of histologic damage, IC\textsubscript{MRgl} that is increased beyond a critical level (approximately 140\% of the sham) appears to be associated with mild to moderate damage, whereas a depressed IC\textsubscript{MRgl} to levels below 70\% of the sham generally leads to moderate or severe damage. Kuhl et al\textsuperscript{38} observed in stroke patients using positron emission tomography (PET), that glucose metabolism is generally a more sensitive indicator of cerebral dysfunction than is CBF. Recently, Baron et al\textsuperscript{39} also reported that a clearly depressed local cerebral oxygen consumption (IC\textsubscript{MRO}_{2}), to a level lower than approximately 70\% of the value in the contralateral hemisphere, seems to indicate evolving ischemic necrosis. Local cerebral blood flow, however, did not appear to be a good indicator of insult. In the present study, IC\textsubscript{BF} measured during the recovery period was rather similar among all three different groups of animals (fig. 4). It is suggested that the recovery of blood flow itself might not be a key factor in determining the restoration of tissue function whereas various metabolic parameters appear to be a better prognostic indicator of tissue damage. Since the noninvasive measurements of IC\textsubscript{BF}, IC\textsubscript{MRgl} and IC\textsubscript{MRO}_{2} in the same brain regions...
have become available in patients by means of PET. It is important to determine the relationship between these parameters and the underlying metabolic rearrangements as well as histologic damage so that the prognosis and therapeutic treatment of stroke patients can be better evaluated.

Acknowledgment

The technical assistance of Mr. Fred Pelzman is greatly appreciated.

References

Phystostigmine Induced Reversal of Ischemia Following Acute Middle Cerebral Artery Occlusion in the Rat

OSCAR U. SCREMIN, M.D., PH.D., AND A.M. ERIKA SCREMIN, M.D.

SUMMARY Cerebral cortical ischemia was induced in anesthetized rats by occlusion of the middle cerebral artery (MCA). Cerebral blood flow (CBF) was measured with the H2 clearance technique in the center and periphery of the ischemic territory. A decrease of CBF to about 50% of pre-occlusion values was observed in both areas. Administration of Physostigmine, a cholinesterase inhibitor, at a dose of 0.15 mg/Kg by intravenous route, induced an increase of CBF in the ischemic cortex. This change in CBF reached 120% of pre-occlusion level in the periphery and 80% of pre-occlusion value in the center of the area of distribution of the occluded artery. Although Physostigmine induced an increase in arterial blood pressure, the cerebral hyperemia observed both in normal and ischemic cortex could still be demonstrated after blockade of the pressor effect by bleeding or Phentolamine administration.


REFERENCES

OCCLUSION of a cerebral vessel such as the middle cerebral artery, leaves a residual blood flow in its distribution field due to the existence of interarterial anastomosis. These anastomoses are known to exist in humans3 as well as in experimental animals.4–6 By simple hemodynamic consideration, decrease in vascular resistance in ischemic areas should lead to enhancement of perfusion from arterial collaterals provided that systemic blood pressure is sustained. Some authors have failed to demonstrate an improvement of blood flow in areas of ischemia25–27 with the use of cerebral vasodilators, suggesting that blood vessels cannot dilate in this situation. This is not an unanimously accepted concept however, since both CO2,28,29 and papaverine30 have been shown to enhance blood flow after middle cerebral artery occlusion in animals. In view of this, it was considered of interest to explore the potential usefulness of Physostigmine, a cholinesterase inhibitor known to induce a redistribution of vascular resistance leading to cerebral vasodilatation and moderate hypertension.8 The increase of cerebral perfusion induced by this drug is not accompanied by metabolic activation and consequently cerebral venous oxygen content rises considerably,9 a fact that might help preserve the integrity of ischemic tissue. An additional feature of the cerebral vasodilatation induced by Physostigmine is that it is potentiated by hypercapnia9,10 and hypoxia.10 This might result in a greater sensitivity to the agent of vessels in the area of ischemia where such conditions are known to prevail.31

From the Rehabilitation Medicine and Research Services, Veterans Administration Medical Center, and the Department of Neurology, University of New Mexico School of Medicine, Albuquerque, New Mexico.

This investigation is supported by funds from the Veterans Administration Research Advisory Group.

Address correspondence to: Oscar U. Scremin, M.D., Ph.D., Research Service (131), VA Medical Center, 2100 Ridgecrest Drive, SE, Albuquerque, New Mexico 87108

Received June 26, 1985; revision #1 accepted January 9, 1986.
Cerebral glucose metabolism during the recovery period after ischemia--its relationship to NADH-fluorescence, blood flow, EcoG and histology.
K Tanaka, E Dora, J H Greenberg and M Reivich

*Stroke*. 1986;17:994-1004
doi: 10.1161/01.STR.17.5.994

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1986 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/17/5/994