Correlation of Local Cerebral Blood Flow, Glucose Utilization, and Tissue pH Following a Middle Cerebral Artery Occlusion in the Rat

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SUMMARY The use of three sets of the double-tracer autoradiographic technique to measure topographical changes of local cerebral blood flow (LCBF), glucose utilization (LCGU), and tissue pH following a 3 h middle cerebral artery (MCA) occlusion in the rat is described.

In a sham-operated group of animals there was 10% reduction of LCBF and 7% reduction of LCGU in the most affected areas as compared to the contralateral homologous regions. However, the ratio of LCGU/LCBF in the affected areas remained within normal limits.

In the MCA-occluded animals, LCGU showed a bimodal response to decreased LCBF. LCGU decreased with reduced LCBF until LCBF fell to 38% of normal. Below this LCBF level LCGU increased, most likely implying anaerobic glycolysis. Decline of tissue pH corresponds to the mismatch of LCBF and LCGU. These results suggest that brain tissue pH change cannot be predicted on the basis of LCBF or LCGU alone.

OCCLUSION of the middle cerebral artery in the rat results in reproducible ischemic brain damage, providing one of the best focal ischemic models in the rat brain. However, both the size and degree of ischemia will vary from animal to animal; this is especially the case in the lateral portion of the caudate nucleus.

The autoradiographic technique for quantitative measurement of local cerebral blood flow (LCBF) and local cerebral glucose utilization (LCGU) allows us to evaluate topographical changes of these two metabolic variables. Tissue pH can be measured by an autoradiographic technique we recently described that uses 14C-labelled 5,5-dimethyl-2,4-oxazolidinedione (14C-DMO). Although single-tracer autoradiographic techniques enable us to measure these metabolic variables in many brain structures only one physiological variable can be measured in each animal. Multitracer autoradiographic methods have accordingly been proposed to overcome these problems and to eliminate animal to animal variation. We therefore investigated the correlation of LCBF, LCGU and tissue pH following a middle cerebral artery occlusion in the rat using three series of a double tracer autoradiographic technique. We found that tissue pH following ischemia is determined by the degree of mismatch between increased LCGU and decreased LCBF.

Materials and Methods

General Procedure

Wistar rats (200–250 g), fasted except for water for 14–16 hours, were used through the experiments. They were anesthetized with 1.5–2.0% halothane during cannulation of the femoral artery, vein and occlusion of the middle cerebral artery. The rats were allowed to awake from anesthesia and the lower half of their body was immobilized with a loose-fitting plaster cast on a lead block. Body temperature was kept around 37°C with a heating pad. Blood pressure and blood gases were serially checked during the experiments.

The animals were divided into 2 groups. Four rats were used for sham operation experiments in which LCBF and LCGU were measured using a double tracer technique. Nine rats, all with occluded left MCA, were divided into 3 groups. In the first group (n = 3), LCBF and LCGU were measured simultaneously using 14C-Iodoantipyrine (14C-IAP) and 18F-fluoro-2-deoxyglucose (18F-FDG). The second group (n = 3) was used for simultaneous measurement of tissue pH and LCBF using 14C-DMO and 18F-4-fluoro-antipyrine (18F-FAP). In the third group (n = 3), the tissue pH and LCGU were measured simultaneously using 14C-DMO and 18F-FDG. It should be noted that none of these methods, strictly speaking, give simultaneous measurements because the tracers need different times for equilibration. The time schedule for the measurement of LCBF, LCGU, and tissue pH is shown below.

<table>
<thead>
<tr>
<th>Time Schedule for Radioisotope Administration</th>
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<tbody>
<tr>
<td>MCA occlusion</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>0 hr</td>
</tr>
<tr>
<td>1 hr</td>
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<tr>
<td>2 hr</td>
</tr>
<tr>
<td>2:15 hr</td>
</tr>
<tr>
<td>2:59 hr</td>
</tr>
<tr>
<td>3 hr</td>
</tr>
</tbody>
</table>

At the termination of each experiment, the animal was decapitated and the brain was quickly removed and frozen in liquid freon-12.

Middle Cerebral Artery Occlusion

Middle cerebral artery (MCA) occlusion was performed according to the method of Tamura et al. The operation was performed through a small subtemporal
cranietomy under 1.5% halothane anesthesia. The stem of the proximal left MCA was occluded by electrocoagulation with a fine-tipped bipolar forceps (Weck, C-15130) under the surgical microscope. For sham operation experiments, after the craniectomy the dura and arachnoid were opened in the same fashion.

Quantitative Double Tracer Autoradiographic Technique

The quantitative double tracer autoradiographic technique using $^{14}$C and $^{18}$F has been described in detail elsewhere.  Briefly, the method is based on the difference in the physical half-life of two tracers, $^{18}$F ($t_{1/2} = 110$ min, $E_{avc} = 240$ KeV) and $^{14}$C ($t_{1/2} = 5730$ yr., $E_{avc} = 45$ KeV).

$^{18}$F standards were prepared in each experiment by preparing 10 to 100 $\mu$Ci of $^{18}$F-FDG (or FAP) in homogenized rat brain. The standard mixture was frozen by immersing it in liquid freon-12. One half of the standard was cut into 20 $\mu$m thick sections in a cryostat and the other half was used for the assay of $^{18}$F radioactivity. About 50 times greater radioactivity of $^{18}$F than that of $^{14}$C was administered to the rat.

The tissue concentration of $^{18}$F was measured using a calibration curve obtained by plotting the optical density of the autoradiogram as a function of the $^{18}$F radioactivity. 20 $\mu$m thick brain sections were cut and the first exposure was done for 2 hours with $^{18}$F standards and $^{14}$C standards. Cross contamination of $^{14}$C in the first exposure was less than 2% under normal conditions and less than 4% in an uncoupling condition. Three days later (39 half-lives of $^{18}$F), the second exposure for 5–6 days was done to obtain the $^{18}$F image. The autoradiographic image for the second exposure was entirely due to the $^{14}$C radioactivity. Blood samples were counted immediately after the experiment to determine $^{18}$F radioactivity using a gamma scintillation counter (Model 810C Baird-Atomic Inc., Cambridge, Mass., USA). $^{14}$C radioactivity in the blood sample was measured 3 days after the $^{18}$F radioactivity had decayed to a negligible level.

Measurement of LCBF

LCBF was measured by the double tracer technique, using $^{14}$C-IAP or $^{18}$F-FAP, 13 2 hours and 59 minutes after MCA occlusion. For the measurement of LCBF and LCGU, 30 $\mu$Ci of $^{14}$C-IAP was injected intravenously for 1 minute with constant infusion. Two to three mCi of $^{18}$F-FAP, evaluated as a diffusible tracer in our laboratory, 13 was injected for the simultaneous measurement of LCBF and tissue pH. LCBF was calculated using the operational equation described by Sakurada et al. 4 The tissue-blood partition coefficients of 0.80 and 0.89 were used for IAP and FAP, respectively. 13

Measurement of LCGU

The LCGU was measured by using the $^{18}$F-FDG autoradiographic technique, which is based on the $^{14}$C-2-deoxyglucose methodology. 9 Three to four mCi of $^{18}$F-FDG (specific activity 300 mCi/mmole at the time of administration) in 1 ml of normal saline was injected intravenously as a bolus 135 min after MCA occlusion. LCGU was calculated according to the equation of Sokoloff et al. 5 In this study we used 0.397 for the FDG lumped constant estimated by Reivich et al., 9 and confirmed by our own experiments (unpublished observation), and the rate constants for DG measured in rat.

Measurement of Tissue pH

The tissue pH was measured by the $^{14}$C-DMO autoradiographic method. 8 One hour after MCA occlusion, 60 $\mu$Ci of $^{14}$C-DMO was injected intravenously. Two hours after $^{14}$C-DMO intravenous injection, a final blood sample was taken and the animal was decapitated. Intracellular pH (pH_i) values were calculated by the following equation: 15

$$\text{pH}_i = 6.13 + \log \left( \frac{(\text{DMO})_e}{(\text{DMO})_i} \right)$$

where $\text{DMO}_e = \frac{(\text{DMO})_i - (\text{DMO})_x \times (% \text{ECS/100})}{(% \text{H}_2\text{O in brain} - % \text{ECS in brain})/100}$

$\text{DMO}_x = \frac{(\text{DMO})_e}{0.93}$

Water content in the plasma was assumed to be 93%, in gray matter 81%, and in white matter 69%. In our calculation we also assumed the extracellular space in the normal rat brain to be 15% of the total water in the gray matter and 5% in white matter. 16, 17 The content of DMO in the extracellular space (DMO)e and the pH of the extracellular space were taken to be equal to those in the plasma. Although such an assumption may be true in the steady state, it may not hold in conditions associated with rapid changes in the acid-base parameter, such as in the initial stage of cerebral ischemia. 15, 16 However, it has been reported that the change in the extracellular pH immediately after ischemic insult becomes acidic, then slowly recovers to the plasma pH level. 15, 16 On the basis of the published data, we estimated the extracellular space in the cortex to be 50% lower than in the normal cortex 3 hr after MCA-occlusion. 19 Extracellular space for the cortical gray matter and caudate nucleus in the ischemic hemisphere was assumed to be 7.5%. This value takes into account a maximum shrinkage of the extracellular space. However, if the extracellular space is larger, the true pH values would be lower than those calculated with the assumptions mentioned above.

Qualitative Measurement of Precursor Pool for FDG

Sixty $\mu$Ci of $^{14}$C-3-O-methyl-D-glucose ($^{14}$C-3-MG) (specific activity 329 mCi/mmole; New England Nuclear Corp., Boston, MA) and 4 mCi of $^{18}$F-FDG was injected 2 hrs and 15 minutes after MCA occlusion to test whether accumulation of $^{18}$F-FDG in severely ischemic areas following MCA-occlusion was due to nonspecific accumulation in the precursor pool rather than to changes in the local metabolism.
Region CBF CGU
A 117 ± 25 90 ± 17
B 135 ± 25 99 ± 25
C 140 ± 29 114 ± 32
D 105 ± 14 102 ± 38
E 119 ± 22 93 ± 16
a 106 ± 13 85 ± 13
b 120 ± 22 99 ± 15
c 127 ± 24 108 ± 20
d 90 ± 13 85 ± 20
e 110 ± 20 88 ± 17

Values are mean ± SD for sham operated rats.

FIGURE 1 (A & B). Double tracer autoradiograms of coronal sections of rat brain using 14C-IAP(A) and 18F-FDG(B) taken 3 h after sham operation. Regional values for LCBF and LCGU are given in ml/100 g/min and μmol/100 g/min respectively.

Results

1. Physiological Parameters

The physiological values of the MCA-occluded and the sham-operated group are given in table 1. No significant differences in the physiological values were observed between these two groups. The MCA-occluded group showed a small increase in the plasma glucose level but the difference was not statistically significant.

2. Relationship Among CBF, CGU, and Tissue pH

a) Sham-operated Group

The regional changes of LCBF and LCGU in sham-operated animals are shown in figure 1 as a mean value for 4 rats. There was a 10% reduction in LCBF and a 7% reduction in LCGU in the most affected area, but the differences were not statistically significant. The LCGU/LCBF ratio remained within the normal range.

b) MCA-occluded Group

Typical 18F-FDG (A-D) and 14C-IAP (E-H) double tracer autoradiograms of the selected anatomical locations in the rats subjected to the left MCA occlusion are shown in figure 2. The relationship between percent change in LCBF and LCGU is shown in figure 3. We would like to point out that the use of the rate constants measured in the normal brain in the calculation of LCGU in ischemic brain will underestimate LCGU in the ischemic regions as shown by Hawkins et al. However, the underestimation will depend on the severity of ischemia, larger underestimation in more severe ischemia and visa versa. This will not have influence on our conclusions. If actual rate constants could be used the data presented in figure 3 would show even sharper division and probably better correlation in the area with severe ischemia. The degree of LCBF reduction varied over a wide range through the occluded cerebral hemisphere, from a slight drop (90% of normal) to a severe decrease (6% of normal) as compared to the contralateral homologous area. The most severely decreased areas were the lower portion of the frontal and sensorimotor cortex and the lateral portion of the caudate nucleus. However, there was a wide variety of LCBF reduction in the lateral portion of the caudate nucleus due to anatomical variation. In the area of mild ischemia, the pattern of regional change in glucose metabolism was similar to that of blood flow change (less than 62% reduction in LCBF). However, in the areas of moderately decreased LCBF (less than 38% of contralateral LCBF), the LCGU was increased. To rule out nonspecific accumulation of FDG, we injected the MCA-occluded rat with 14C-3-MG and 18F-FDG simultaneously (fig. 4). The most severely ischemic area showed an accumulation of FDG but no change in the 14C-3-MG uptake, implying no change in FDG concentration in the precursor pool between ischemic and nonischemic cortex. Fieschi et al and Shigeno et al also compared heterogeneously increased deoxyglucose uptake and 14C-3-MG uptake in different cerebral ischemic animals and also concluded that there was no increase in the precursor pool for deoxyglucose.

The regional pH values of cerebral tissue obtained from the 3-hr MCA occluded hemispheres of rats were plotted as a function of the percent change of the cerebral blood flow (fig. 3). Representative autoradiograms are shown in figure 5. The pH values in the tissue of non-occluded hemispheres were 6.93 ± 0.02. A significant decrease in the tissue pH was observed in the area having LCBF 62% lower than that of the contralateral side.

Representative double tracer autoradiograms obtained with 18F-FDG and 14C-DMO, giving regional values for LCGU and pH, are shown in figure 6.

Table 1. Physiological Data in Sham Operated and MCA Occluded Rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham group (n = 4)</th>
<th>MCA occluded group (n = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP</td>
<td>138 ± 5</td>
<td>135 ± 7</td>
<td>ns</td>
</tr>
<tr>
<td>PaO2</td>
<td>91 ± 6</td>
<td>94 ± 8</td>
<td>ns</td>
</tr>
<tr>
<td>PaCO2</td>
<td>36 ± 1</td>
<td>38 ± 3</td>
<td>ns</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.02</td>
<td>7.41 ± 0.006</td>
<td>ns</td>
</tr>
<tr>
<td>Plasma glucose</td>
<td>142 ± 22</td>
<td>155 ± 24</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
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FIGURE 2. Double tracer autoradiograms 3 h after left MCA occlusion using $^{18}$F-FDG for LCGU (A-D) and $^{14}$C-IAP for LCBF (E-H). A variable reduction of blood flow in different brain regions is shown in autoradiograms E-H. In the mildly ischemic area, the pattern of reduction in LCGU was similar to that of LCBF, but in the moderate to severely ischemic area a heterogeneous increase of LCGU was seen.

Discussion

Experimental studies have suggested that increase in the tissue lactate concentration caused by anaerobic metabolism of glucose and a fall in tissue pH in ischemic brain tissue may be a major damaging agent. Recent clinical data has also supported this hypothesis that the outcome of stroke in patients with hyperglycemia on admission was significantly poorer than that of the age-matched patients with stroke and without hyperglycemia. Excess accumulation of lactate may, therefore, be important in the management of patients. Direct in vivo measurement of the regional concentration of lactate in the tissue is technically difficult. However, we directly measured hydrogen ion activity (pH) in the brain tissue and correlated it to LCBF and LCGU by means of an autoradiographic technique. We were therefore able to relate tissue pH directly to the changes in LCBF and LCGU following MCA occlusion.

Several assumptions were made in our study. The lumped and rate constants of normal brain were used to estimate LCGU in ischemic brain. A partition coefficient obtained in normal brain tissue was used to estimate LCBF in ischemic tissue. In the pH study the assumptions described above about water content and the change in the extracellular space were made. These assumptions may produce certain errors for calculation of the intracellular pH. Despite these assumptions, which are widely used in these measurements, we believe our results are informative.

The fact that the brain can tolerate a 50% reduction in LCBF without any symptoms of oxygen deficiency was reported over 20 years ago. Since the normal brain extracts only one-third of the oxygen from arterial blood, this hypothesis seems theoretically reasonable. On the other hand, since only one-tenth of the glucose is extracted into the brain from arterial blood, glucose delivery is even less critical in ischemia. Our
measurements of LCBF and LCGU in the same rat showed that LCGU has a bimodal response to decreased LCBF (fig. 3). LCGU decreased with the decrease in LCBF until blood flow was reduced to 38% of normal. Below this level an increase in LCGU was observed, suggesting anaerobic glycolysis. This indicates that knowing the deoxyglucose uptake in ischemia without knowing the rate of blood flow or the oxygen metabolism is not very informative, because we do not know if aerobic or anaerobic glucose metabolism is taking place.

In the upper part of figure 3, tissue pH is compared to the percent change of LCBF. The lower part shows the correlation between LCBF and LCGU. In the areas with LCBF lower than 38% of normal, an inverse relationship between LCBF and LCGU was observed. (This was not the case in a few severely ischemic areas where LCBF was less than 10% of the contralateral LCBF). Since blood flow was very low, it is possible that insufficient deoxyglucose reaches this brain tissue. A decline in tissue pH correlates well with a decrease in the CBF in areas with LCBF below 38% of normal contralateral LCBF. It was difficult to show that the tissue pH was relatively high in the severely ischemic area (less than 10% of contralateral LCBF), because the severely ischemic area was very small in our experiments. However, a careful observation of the individual cases provided some interesting results (fig. 6).

A representative autoradiogram showing the relation of glucose metabolism and tissue pH is shown in figure 6. There was an increase of LCGU and decrease of pH in region 5. Although region 3 is more ischemic than region 6, the pH in 3 is higher than in 6. This phenomenon could be explained by the fact that a continuous supply of glucose increases lactate accumulation. In other words, the glucose supply becomes the limiting factor in severely ischemic areas and the lactate does not increase as much as it would in a mild ischemic area. Support for our hypothesis is given in figure 5, which reveals a topographical change in LCBF and tissue pH. The pH values of the severely ischemic area (region 3) were definitely higher than those of mildly ischemic areas (region 5 and 6). These findings are important in the discussion about whether incomplete ischemia is more noxious to brain tissue than complete ischemia.44-47

According to Steen et al,44 a low LCBF (less than 10% of normal) in the ischemic brain is clinically bet-
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Region CBF pH
1 147 6.97
2 36 6.82
3 9 6.85
4 46 6.86
5 16 6.61
6 23 6.59

Figure 5 (A & B). Representative double tracer autoradiograms of $^{18}$F-FAP (A) and $^{14}$C-DMO (B) in the 3 h left MCA occluded rat. Regional change of CBF and pH is shown.

ter than no flow at all. Our results reveal that severely ischemic areas did not show the greatest reduction in tissue pH. This can be seen by comparing regions 5 and 6 to the same regions in figures 5 and 6. Thus, at LCBF below 10% of normal, the tissue is less acidic than when LCBF is reduced 10–20% of normal. This indicates that the blood flow of 10–20% of normal supplies insufficient amounts of glucose to cause lactate accumulation and severe acidosis. A similar observation was reported by Pulsinelli et al., who suggested that the balance between blood glucose and tissue oxygen concentration is critical in determining the severity of ischemic damage to the tissue.

Experiments reported here suggest a CBF threshold below which the tissue pH changes (fig. 3). This threshold was found to be around 38% of control CBF, and very close to the changing point of the glucose metabolism pattern (fig. 3). This threshold is obviously higher than that for functional and morphological integrity. In future, the analysis of the relationship between damage in ischemic tissue and tissue pH could provide us with a critical pH value for which tissue damage in this model is irreversible. According to a concept recently proposed, the threshold for cellular damage should be determined by the severity and duration of ischemia. Tissue pH could accordingly be a more reliable and more sensitive indicator of tissue damage, since changes in tissue pH are derived both from the severity and duration of ischemia.

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