Correlation Between Glucose Utilization and Metabolite Levels During Focal Ischemia in Cat Brain

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SUMMARY Focal ischemia was produced in cat brain by occluding the middle cerebral artery. After 60 min of ischemia the rate of glucose utilization, as measured by the uptake of $[^{14}C]$ deoxyglucose ($[^{14}C]$DG), was correlated with tissue levels of ATP, phosphocreatine, and lactate measured in the same regional samples. Ischemia caused local increases of $[^{14}C]$ DG uptake which were associated with mild to moderate anaerobic perturbations of metabolite levels. Altered metabolite levels also occurred in regions in which the rate of glucose consumption was not markedly different from that of the non-ischemic hemisphere. In addition, there were regions with decreased $[^{14}C]$ DG uptake which invariably were depleted of ATP and phosphocreatine. Thus, suppression of glucose metabolism was restricted to the most severely ischemic areas, where the delivery of glucose may be rate-limiting.

MEASUREMENT of the cerebral rate of glucose consumption using radioactively labeled glucose or glucose analogs has potentially valuable clinical application. These methods, however, do not distinguish between aerobic and anaerobic utilization of glucose. This distinction is particularly important for damaged brain in which the fraction of glucose metabolized anaerobically may be greater than normal. The presence of anaerobiosis can be detected by other methods, such as measurement of tissue levels of lactate. Thus, by combining a measure of tissue metabolite levels with a measure of glucose utilization, it is possible to make a qualitative distinction between aerobic and anaerobic metabolism.

Marked regional variations in the rate of glucose consumption have been demonstrated in experimental models of cerebral ischemia and hypoxia using the $[^{14}C]$ deoxyglucose autoradiographic technique. Thus, occluding the middle cerebral artery in the cat resulted in closely juxtaposed regions of increased and decreased glucose utilization within the ischemic hemisphere. Using the same model for the present study we have measured tissue levels of ATP, phosphocreatine, and lactate in regions with altered glucose consumption. By determining the uptake of $[^{14}C]$ deoxyglucose in the same regional samples analyzed for metabolite content, it was possible to correlate variations in glucose utilization directly with anaerobic perturbations of metabolite levels.

Methods

Cats of either sex weighing between 3.5 and 4.0 kg were anesthetized with pentobarbital (40 mg/kg of body weight), immobilized with gallamine, and ventilated mechanically with 30% O$_2$ and 70% N$_2$. Arterial blood gases were measured at predetermined intervals and arterial pressure was monitored continuously via femoral catheters. Arterial Pco$_2$ was maintained between 31 and 34 torr by adjusting respiratory rate and volume. Rectal temperature was held at 37°C with a servo-controlled heat lamp. The EKG was recorded from 2 pairs of brass electrodes which were screwed into the skull bilaterally over the ectosylvian gyri.

The left middle cerebral artery was exposed via a transorbital approach. The artery was occluded with a miniature Mayfield clip in 8 animals; 2 additional animals were sham-operated without occlusion. In 2 of the 8 experimental animals, arterial pressure was lowered to 90 torr by controlled hemorrhage in order to increase the degree of cerebral ischemia.

At 60 min post-occlusion, 75 $\mu$Ci (1.5 $\mu$mole) per kg animal weight of 2-deoxy-D-[$^{14}$C] glucose ([$^{14}$C]DG) was rapidly injected in 3 ml of isotonic saline through a femoral venous catheter. Arterial blood was sampled initially at 10-30 sec intervals, and later at 2-5 min intervals for determination of plasma [14C] DG and glucose concentrations.

At 90 min post-ischemia, the brain was frozen in situ by pouring liquid N$_2$ into a styrofoam cup placed over the intact calvarium. During 15 min of freezing, mechanical ventilation was continued and arterial pressure remained nearly normal. This technique freezes most regions of the brain without ischemic artifact because deeper layers continue to be perfused until the arrival of the freezing front. The frozen brain was then sectioned in the coronal plane at 1 cm intervals with a pre-cooled saw, taking care to keep the tissue chilled with liquid N$_2$.

In a -30°C chamber, multiple samples were dissected with a small cork borer (see fig. 1) and weighed (2-5 mg) prior to extraction. The samples were acid-extracted, and the extracts analyzed for content of ATP, phosphocreatine, and lactate using enzymatic...
methods. The \[^{14}C\]-content of each extract was determined by liquid scintillation counting. In 3 extracts, the concentrations of \[^{14}C\] DG and \[^{14}C\] DG-6-P were measured individually after separation by ion exchange chromatography. Ion exchange resin (Biorad AG1-X4, 200-400 mesh) was converted to the acetate form, and small columns were prepared in Pasteur pipettes. \[^{14}C\] DG-6-P, which is retained by the resin, was eluted with ammonium acetate. Of the total radioactivity added to the columns, the percentage recovery in the form of \[^{14}C\] DG plus \[^{14}C\] DG-6-P varied from 92% to 111%. Autoradiograms of tissue sections were prepared by slicing the frozen brain at a thickness of 20 microns in a \(-15^\circ C\) cryostat. The sections were dried and exposed for 10 days on Kodak SB-5 x-ray film.

For samples in the non-ischemic hemisphere the rate of glucose consumption was calculated from tissue \[^{14}C\] concentration measured in the sample extract and the arterial curves of plasma \[^{14}C\] DG and glucose, according to the formulation of Sokoloff et al. Values for the lumped constant and the rate constants determined in rat, which are similar to those reported for monkey, were used in the calculations.

For samples in the ischemic hemisphere, absolute rates of glucose utilization were not calculated because 1) the value of the lumped constant is not known for ischemic brain and 2) the mathematical model does not take into account a limitation of glucose delivery which may occur in regions with low blood flow. Preliminary experiments in our laboratory suggest that the lumped constant during ischemia is not the same as in control animals. Consequently, for samples in the ischemic hemisphere, the uptake of \[^{14}C\] DG was expressed as a percentage of the uptake in a homologous sample from the non-ischemic hemisphere (see fig. 6).

**Figure 1.** Regional sample selection. In situ frozen cat brain was sectioned at \(-196^\circ C\) at the level of the caudate nucleus. At \(-30^\circ C\) an elliptical cork borer (2 \(\times\) 3 mm) was pressed into several brain regions and sample cores (1-2 mm in depth) were removed from the brain section for determination of metabolite levels and \[^{14}C\] DG uptake.

**Figure 2.** Effect of focal ischemia on the EEG illustrated by two examples. In animal 1, the initial EEG suppression partially reversed between 5 and 90 min post-occlusion. In contrast, there was no EEG recovery during the 90 min period of ischemia in the second example.
TABLE 2 Metabolite Levels and Glucose Utilization in the Non-Ischemic Cerebral Hemisphere

<table>
<thead>
<tr>
<th>Region</th>
<th>ATP</th>
<th>PCr</th>
<th>Lac</th>
<th>CMR-Glu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex</td>
<td>2.31</td>
<td>4.82</td>
<td>1.28</td>
<td>18.3</td>
</tr>
<tr>
<td>White matter</td>
<td>1.98</td>
<td>3.18</td>
<td>0.74</td>
<td>4.4</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>2.49</td>
<td>6.55</td>
<td>1.46</td>
<td>20.6</td>
</tr>
</tbody>
</table>

Mean values ± standard error for 8 animals.

ATP, phosphocreatine (PCr), and lactate (Lac) values are given in mmol/kg. Cerebral metabolic rate for glucose (CMR-Glu) is given in μmol/100g/min.

Figure 3 shows the autoradiographic image of [14C] DG uptake and the regions sampled for metabolite and 14C content. In this brain there was a gradient of decreasing ATP and phosphocreatine and increasing lactate levels between samples 2 and 6. In the ischemic focus (sample 6), which was depleted of high energy phosphates, the uptake of [14C] DG was markedly reduced. In adjacent regions (samples 5 and 7) with less complete reduction of ATP and phosphocreatine, there was increased uptake of [14C] DG compared to the non-ischemic hemisphere. [14C] DG uptake was also increased in region 3, which showed only mild ischemic perturbations of metabolite levels. However region 4, which had similar metabolite levels, showed no increase of [14C] DG uptake. Finally, in the caudate nucleus (sample 8), depletion of ATP and phosphocreatine again correlated with a suppressed uptake of [14C] DG. It should be noted that the low amounts of radioactivity present in samples 6 and 8 were slightly below the calculated tissue level of non-metabolized [14C] DG (67 μCi/kg), which is derived from the arterial concentration curve.

Figure 4 illustrates a brain in which the ischemic alteration of metabolite levels was restricted to the cortical sulci (regions 4 and 5). ATP and phosphocreatine were markedly reduced but not depleted in these sulci, while lactate and the uptake of [14C] DG were elevated relative to other cortical regions. There were also smaller areas with increased [14C] DG uptake within 2 of the sulci (arrows) ipsilateral to the middle cerebral occlusion. In contrast to the changes within the sulci, the gyri of the ipsilateral cortex (regions 2 and 3) had nearly normal levels of metabolites and only slightly reduced [14C] DG uptake compared to non-ischemic hemisphere (region 1).

Figure 5 shows a brain with more severe alterations of metabolite levels. Samples 3, 4, and 5 were depleted of ATP and phosphocreatine, and lactate was in excess of 35 mmol/kg. Again, there was little or no ac-
Phosphocreatinine
Region ATP PCr Lac
1 2.40 5.06 1.3 245
2 2.18 3.92 16.5 230
3 0.14 0.00 49.6 26
4 0.06 0.00 36.2 5
5 0.13 0.00 42.4 38
6 2.55 6.49 2.4 235

**Figure 5.** Focally decreased $[^{14}\text{C}]$ DG uptake associated with depletion of high energy phosphates. Tissue concentrations of ATP, phosphocreatine (PCr), and lactate (Lac) are given in mmol/kg; $[^{14}\text{C}]$ DG uptake ($u$C) is expressed in $\mu$Ci/kg. The calculated tissue level of non-metabolized $[^{14}\text{C}]$ DG in this brain was 112 $\mu$Ci/kg.

**Figure 6.** Correlation between $[^{14}\text{C}]$ DG uptake and metabolite levels during focal ischemia. Fifty-three samples from cerebral cortex and 6 from caudate nucleus of the ipsilateral hemisphere (8 animals) were analyzed for ATP, phosphocreatine, lactate, and $[^{14}\text{C}]$ DG uptake. For each sample the uptake of $[^{14}\text{C}]$ DG, expressed as a percentage of the uptake in a homologous sample in the non-ischemic hemisphere of the same animal, was plotted against the level of the individual metabolites.

For all 8 experimental animals, the uptake of $[^{14}\text{C}]$ DG was plotted against metabolite levels in gray matter samples from the ischemic hemisphere (fig. 6). Samples in which $[^{14}\text{C}]$ DG uptake was less than 80% of the control hemisphere were nearly devoid of ATP and phosphocreatine, although lactate was greater than 30 mmol/kg. In this brain, the calculated tissue content of non-metabolized $[^{14}\text{C}]$ DG (112 $\mu$Ci/kg) was far in excess of the total radioactivity in samples 3, 4, and 5.

For all 8 experimental animals, the uptake of $[^{14}\text{C}]$ DG was plotted against metabolite levels in gray matter samples from the ischemic hemisphere (fig. 6). Samples in which $[^{14}\text{C}]$ DG uptake was less than 80% of the control hemisphere were nearly devoid of ATP and phosphocreatine, although lactate was greater than 30 mmol/kg. Increased $[^{14}\text{C}]$ DG uptake occurred over a wide range of metabolic alteration. Thus, in several samples with ATP and phosphocreatine levels less than 1.0 mmol/kg, the uptake of $[^{14}\text{C}]$ DG was more than 100% of the non-ischemic hemisphere. However, increased $[^{14}\text{C}]$ DG uptake also occurred in samples with only moderate perturbations of metabolite levels: ATP greater than 1.5 mmol/kg, phosphocreatine greater than 4.0 mmol/kg, and lactate in the range 3.0–7.0 mmol/kg. Finally, there were several samples with altered metabolite levels which took up a normal amount of $[^{14}\text{C}]$ DG compared to the non-ischemic hemisphere.

In 3 samples with increased $[^{14}\text{C}]$ DG uptake (one animal), the tissue concentrations of $[^{14}\text{C}]$ DG and $[^{14}\text{C}]$ DG-6-phosphate were measured separately after isolation by ion exchange chromatography (table 3). In these samples, the phosphorylated metabolite comprised 64%–81% of the total radioactivity. In 2 of the 3 samples, the measured amount of $[^{14}\text{C}]$ DG was slightly lower than that calculated from the arterial curves, but in the third sample the measured value was only 37% of the calculated amount.

**Discussion**

Occlusion of the middle cerebral artery causes a variable reduction of blood flow in different brain regions. In the present study, these gradations of ischemia produced a broad spectrum of changes in tissue metabolite levels, ranging from a 3-fold elevation of lactate to total depletion of ATP and phosphocreatine. There were also pronounced variations in the rate of glucose utilization as measured by the uptake of $[^{14}\text{C}]$ DG. In brain regions with increased $[^{14}\text{C}]$ DG uptake, tissue levels of high energy phosphates were lower than control, but not depleted. In addition, the accumulation of lactate in these regions indicated that anaerobic glycolysis had been activated. However, the uptake of $[^{14}\text{C}]$ DG was not increased in all regions with anaerobic perturbations of metabolite levels. Indeed, in the most severely ischemic areas, there was reduced uptake of $[^{14}\text{C}]$ DG.

Ischemia causes a sudden increase in the rate of

<table>
<thead>
<tr>
<th>Sample #</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[^{14}\text{C}]$ Total</td>
<td>238</td>
<td>263</td>
<td>215</td>
</tr>
<tr>
<td>$[^{14}\text{C}]$ DG-6-P</td>
<td>152</td>
<td>214</td>
<td>172</td>
</tr>
<tr>
<td>$[^{14}\text{C}]$ DG</td>
<td>73</td>
<td>80</td>
<td>25</td>
</tr>
<tr>
<td>Calculated $[^{14}\text{C}]$ DG</td>
<td>67</td>
<td>67</td>
<td>67</td>
</tr>
</tbody>
</table>

Values given in $\mu$Ci/kg. Calculated $[^{14}\text{C}]$ DG value derived from arterial curve.
glucose utilization in brain.\textsuperscript{18} While this activation would explain the regions with a high \([^{14}C]\) DG uptake, it calls into question those regions with normal or reduced uptake. In the present study, \([^{14}C]\) DG was administered 60 min after the onset of ischemia. Thus the uptake of the radioactive tracer will be a function of the rate of glucose utilization after 60 min of ischemia. Regions with a nearly normal uptake of \([^{14}C]\) DG indicate that, in spite of altered metabolite levels, the rate of glucose consumption had returned to control after a 60 min insult. The initial activation of anaerobic glycolysis may subsequently diminish due to a limitation of glucose delivery or to an inhibition of glycolysis by increasing tissue acidosis. However, the present data do not explain the variation of \([^{14}C]\) DG uptake in different regions with comparable perturbations of metabolite levels (see fig. 3, samples 3 and 4).

The uptake of \([^{14}C]\) DG was lower than control only in regions depleted of high energy phosphates. At this severe degree of ischemia, the reduced delivery of glucose may become the rate-limiting step in glucose metabolism. In the extreme case, cessation of blood flow would prevent the entry of the radioactive tracer into the tissue. Furthermore, with a reduction of blood flow sufficient to cause depletion of high energy phosphates, the depletion itself will block metabolism since phosphorylation of glucose depends on ATP.

In the present study, brain freezing was initiated 30 min after the bolus injection of \([^{14}C]\) DG. Therefore, the measurements of metabolite levels and of the rate of \([^{14}C]\) DG uptake were not simultaneous measures. In a steady-state, the lack of simultaneity would not interfere with the regional correlation between the rate of glucose utilization and the level of tissue metabolites. However, it is difficult to rule out changes in steady-state even though the infusion of the \([^{14}C]\) DG was delayed for 60 min in order to avoid the initial transients of flow and metabolism which follow occlusion of the middle cerebral artery.

Quantitation of the rate of glucose utilization, using the \([^{14}C]\) DG technique, requires that the tissue concentration of non-metabolized \([^{14}C]\) DG be known. Non-metabolized \([^{14}C]\) DG (free deoxyglucose) represents that portion of the tissue content of deoxyglucose which was transported from blood to brain but which was not phosphorylated. Normally the quantity of free \([^{14}C]\) DG can be calculated from the arterial concentration curve and the rate constants for transport and phosphorylation of deoxyglucose.\textsuperscript{2} In the present study, there was good agreement between the calculated and measured amounts of non-metabolized \([^{14}C]\) DG in 2 of the 3 extracts analyzed from regions with a higher than normal uptake. However, in regions with decreased uptake, the calculated concentration of non-metabolized \([^{14}C]\) DG overestimated the actual concentration and, indeed, frequently exceeded the total tissue content of radioactivity. Thus, in low flow regions, it is not possible to calculate the tissue content of free \([^{14}C]\) DG using the present methods. However, if flow were measured independently, then such a calculation might become feasible. Alternatively, direct measurement of \([^{14}C]\) DG-6-P would obviate the determination of non-metabolized \([^{14}C]\) DG. Direct measurement of \([^{14}C]\) DG-6-P following separation by ion-exchange chromatography becomes increasingly difficult, however, with smaller regions of brain containing low amounts of radioactivity.

Clinical application of the radio-tracer methods for glucose utilization may prove to be a valuable tool for evaluating brain metabolism and function. The present investigation, as well as previous studies,\textsuperscript{6,7} demonstrates the need for regional measurements of metabolic rate. Thus, within a damaged brain, alterations of brain function and metabolism may be extremely localized as evidenced in the present model of focal ischemia.

Brain function is closely linked to the rate of energy utilization. Normally there is a tight coupling between the rates of glucose and oxygen consumption and the rate of energy utilization. However, anaerobic consumption of glucose, which yields only a fraction of the energy generated by oxidative metabolism, disrupts this normal coupling. Thus a high rate of glucose consumption does not necessarily indicate a high rate of energy use if a significant proportion of the glucose is metabolized anaerobically. At present there is no way to distinguish anaerobic from aerobic metabolism of glucose without a simultaneous measure of a) regional blood flow or b) regional oxygen consumption. Nevertheless, focal hypermetabolism of glucose may be a sensitive clinical indicator for regional ischemia. Furthermore, hypometabolism of glucose would indicate without ambiguity a suppression of energy utilization and brain function.

References


Primary Pontine Hemorrhage: Clinicopathological Correlations

Noboru Goto, M.D., Mitsuo Kaneko, M.D., Yasuaki Hosaka, M.D., and Hiroaki Koga, M.D.

SUMMARY In 18 autopsies from patients with primary pontine hemorrhage we studied the sites of bleeding, the volumes and development of hematomas and clinicopathological correlations. A modular optical electronic planimeter was introduced to measure the size of hematomas. The series of patients can be divided into 2 groups from the viewpoint of bleeding sites, their development and clinical symptomatology. These are 1) the tegmentobasiliar type and 2) the tegmental type. The precise location of the origin of hemorrhage, and the approximate volume of hematomas can now be determined with the help of computerized tomography. This information will be of help in understanding clinical symptoms. Two different typical patient reports, selected from the collection, are presented.

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Primary pontine hemorrhage is a relatively rare condition compared with supratentorial cerebral hemorrhage. It is generally accepted that about 10% of all cerebral hemorrhages occur in the pons, and the symptoms of this kind of hemorrhage have been of interest to clinicians for over a century.

Clinicopathological investigations, however, were rather scarce at the time Környey (1939) reported his clinical and anatomic study of this condition. Epstein (1951) reported clinical and pathological findings of 7 patients with primary pontine hemorrhage studied over a 33-year period. Silverstein (1972) reviewed past research on primary pontine hemorrhage and presented a clinicopathological series of 50 patients.

Calculations of the volume of a pontine hemorrhage and its correlations to clinical symptoms were not included in previous publications. The authors have investigated this correlation and present the results of their findings.

Methods

For the study of primary pontine hemorrhage, 38 clinical observations were available with 19 autopsies. One of these, in which the origin of hemorrhage was easily detected, due to arteriovenous malformation in the midbrain, was excluded. In the remaining 18 patients no evidence of traumatic injury, neoplasm, supratentorial mass effect, or blood dyscrasia, described in previous publications, was found. The condition most likely to lead to primary pontine hemorrhage was hypertension, which affected about two-thirds of the patients.

The brain stems and cerebella, including the diencephali of 18 patients, were cut transversely into sections about 1 cm thick after fixation in a 10% solution of formalin. After macroscopic observations, photographs were taken of the sections containing hematomas with a scale for measurements. Each color transparency was enlarged to exactly the original brain size using a photographic enlarging apparatus, and traced on paper. Then the area of the hematoma on each trace was measured with a modular optical planimeter (Digiplan, Kontron Co.). The volume of the hematomas was calculated from the average measured sectional areas and the length of their axial extensions.

For histological investigations, the sections were fixed in a solution of 5% potassium dichromate and 5% potassium chromate with several changes of solution for 3 weeks after the macroscopic observations. Each color transparency was enlarged to exactly the original brain size using a photographic enlarging apparatus, and traced on paper. Then the area of the hematoma on each trace was measured with a modular optical planimeter (Digiplan, Kontron Co.). The volume of the hematomas was calculated from the average measured sectional areas and the length of their axial extensions.

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