Regional Cerebral Glucose Metabolism During and After Bilateral Cerebral Ischemia in the Gerbil

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SUMMARY Cerebral metabolic rate for glucose (CMRG) was measured using the 14C-deoxyglucose technique in a stroke model of the gerbil produced by bilateral common carotid artery occlusion. During 30 minutes of ischemia, 14C-deoxyglucose uptake in the brain was increased along the border zone between the ischemic and nonischemic area and decreased in the ischemic areas. During the early stage of reperfusion (2 or 3 to 30 minutes), CMRG increased 50 to 150% in the cerebral cortex, caudoputamen and thalamus and 270 to 320% in the hippocampus, globus pallidus and amygdala. During the late stage of reperfusion (15 to 45 minutes), heterogeneity of CMRG appeared in the cerebral cortex, caudoputamen and thalamus. CMRG decreased to less than 50% of the control value in the cerebral cortex but remained at 200 to 300% of control in the hippocampus, globus pallidus and amygdala. The latter structures exhibited a larger and more protracted increase in glucose metabolism than the other structures most probably due to the histological vulnerability to ischemia of these structures. The relationship between the transient increase in the glucose metabolism and cell function is discussed.

CEREBRAL TISSUE OF MAMMALS is highly vulnerable to both anoxia and ischemia. There are reports of reversibility in the neurological, electrical and chemical function during the period of reoxygenation or reperfusion after a certain period of insult.1,4,5,6 The degree of reversibility has been found to depend on a number of factors.

A better understanding of this reversibility may be important in the treatment of cerebral anoxia or ischemia since there is frequently reoxygenation or reperfusion of the brain following cerebral embolism, cardiac arrest or intoxication by various poisons.

Prior to the introduction of the 2-deoxyglucose technique for the measurement of regional cerebral glucose consumption by Sokoloff, et al,7 only global techniques existed for measuring glucose metabolism. Consequently, regional alterations in metabolism could not be seen. The employment of the 2-deoxyglucose technique in the study of cerebral ischemia or anoxia may lead to an extended knowledge of cerebral damage following this type of insult.

Our laboratory has previously reported measurements of regional cerebral glucose metabolism following focal cerebral ischemia in cats.8 Diemer, et al9 have recently examined glucose utilization following total ischemia in rats. There are few studies in the literature, however, dealing with the reperfusion period following a stroke in an experimental animal.2

In the present studies a stroke model in the gerbil utilizing bilateral carotid occlusion has been utilized. In this model, all animals develop severe neurological deficits and the severity of cerebral ischemia is rather uniform from animal to animal.10,11 This uniformity allows for the precise evaluation of the course following reperfusion.

Methods

Male mongolian gerbils (60–100 gm) were anesthetized with ketamine (5 mg/100 gm body weight, intraperitoneally) followed by the inhalation of 80% nitrous oxide and 20% oxygen. Two additional doses (2.5 mg/100 gm body weight) of ketamine were administered as required during the surgical preparation. Small polyethylene catheters (PE10) were inserted into the left femoral vein and the tail artery with the aid of an operating microscope. Both common carotid arteries were separated from the surrounding tissue and vascular occluders consisting of small rubber sutures attached to plastic tubes with a blind end were placed over the arteries. The rubber suture and the blind end of the plastic tube make a small noose and occlude the vessel when the rubber suture is pulled. This device allows for the occlusion of the artery with minimal damage to the vessel wall, as well as the ability to reopen the vessel when the suture has been released. To decrease mortality of the animals following carotid occlusion, some of the latter animals received a tracheostomy (PE160 polyethylene tube) but those animals were allowed to breathe spontaneously. Following the surgical preparation, the animal was loosely taped onto a plastic stage and allowed to breathe room air. Body temperature was maintained at 37°C by means of a servocontrolled heat lamp and blood pressure was recorded from the tail artery catheter utilizing a blood pressure transducer and a Hewlett-Packard polygraph. All animals recovered from the anesthetic rather quickly and exhibited a full response to visual, auditory, and painful stimuli. In the control group, not subjected to carotid occlusion (6 animals), the measurement of regional cerebral glucose metabolism was initiated after the animal was allowed to recover from the anesthesia for at least one hour. Thirteen animals were exposed to bilateral cerebral ischemia by tightly pulling the rubber sutures on the carotid arteries. In five of these animals

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the metabolic measurement was obtained during the cerebral ischemia (ischemic group) while the remainder of the studies were performed during the reperfusion period following 30 minutes of ischemia (reperfused group). In the reperfused group, blood pressure frequently dropped following the release of the sutures, and the infusion of low molecular weight dextran was necessary to keep blood pressure stable and to prevent the deterioration of the animals. All animals were heparinized (40 units) before the carotid occlusion.

The cerebral metabolic rate for glucose (CMRG) was measured using the autoradiographic technique of Sokoloff, et al. as modified for changing plasma glucose concentration by Savaki, et al. Fifteen μCi of 14C-2-deoxyglucose (14C-2-DG) (New England Nuclear) was injected into the femoral venous catheter and 50 arterial blood samples (initially at 20 second intervals and later at 2–10 minute intervals) were taken for the determination of the arterial plasma concentration of 14C-2DG. These samples were obtained at times following the injection of the 2DG to accurately define the plasma 14C-2DG concentration time course. To minimize blood loss, only 25 μl of blood was taken in a microcentrifuge tube which was centrifuged to produce at least 10 μl of plasma. Larger samples were taken at the 10 minute intervals for the determination of the arterial glucose concentration (approximately 80 min).

Total blood loss for arterial sampling was approximately 0.6 ml. In the ischemic group, the tracer was given 2–3 minutes following the bilateral occlusion of the carotid artery and in the reperfused group, three animals obtained the tracer 2 or 3 minutes following the reflow (early stage group) and five animals at 15 minutes following the reflow (late stage group). In all animals, 30 minutes following the injection of 14C-2DG, the animal was killed by decapitation and the brain rapidly removed and frozen in freon at −55°C (Dupont). The frozen brain was sectioned (20 micron thick sections) in a cryostat (American Optical) and the sections placed on glass coverslips and dried on a hotplate (60°C). The dried brain sections were placed on x-ray film (SB5-Kodak) for 10 days.

Densitometric readings of the autoradiograms were obtained from the right side of the brain using a densitometer (Gamma Scientific) equipped with a 230 micrometer aperture. The identification of the structures in the brain is based on the 14C-2DG atlas of the rat brain, since the appearance of 14C-2DG autoradiograms of the gerbil is very similar to that of the rat. For the calculation of CMRG, a lumped constant of 0.483 (normal rat value) was used for the control group and for the reperfused group, since there is no evidence of a difference in lump constant between normal brain and postischemic reperfused brain. The similarity of the lumped constant between cat, monkey and rat suggests that the lumped constant in the gerbil will be similar. In the ischemic region of the brain of the ischemic group, only the 14C concentration was measured since the uptake of 14C-2DG is limited in these regions by the decreased delivery of isotope. Additionally, an alteration of the lumped constant in these regions may be expected.

### Results

Mean arterial blood pressure (MABP) remained above 70 mm Hg in all animals of the control group throughout the study in spite of the relatively large amount of blood loss by arterial sampling. As can be seen in figure 1, activated neuronal columns and a highly localized uptake of 14C-2DG in lamina IV were observed in some regions of the cerebral cortex. The auditory cortex and medial geniculate body showed a lower CMRG value than that obtained in the rats, although the inferior colliculus showed a similar glucose utilization rate.

In the ischemic group, blood pressure increased transiently following carotid occlusion but stabilized between 100 and 140 mm Hg (MABP). It stayed above 80 mm Hg throughout the study, except in one animal whose blood pressure fell to 64 mm Hg by the end of the study. The neurological state of the animals deteriorated rapidly to a coma following the bilateral carotid occlusion with only a weak pain reflex (response to the painful stimuli on the back and legs of the animals) being present in some of the animals. Pupils were dilated and corneal reflexes were lost in the latter part of the ischemic period. Generalized tonic or clonic convulsive seizures were also observed in all animals. The severity of the neurological deficit was rather uniform from animal to animal. Glucose metabolism in structures fed by the vertebral basilar arterial system remained at the same level as in the control animals. An increased 14C activity was seen along the border zone separating the two arterial systems. This zonal increase of 14C-2DG uptake was seen in the midbrain (inferior colliculus, substantia nigra, as well as the medial caudal thalamus) (figs. 2, 3).

14C-2DG autoradiograms in the carotid arterial system looked quite different from those of control animals. Due to the error of the 2DG technique (see Discussion) to measure glucose utilization in severe

![Figure 1](attachment:figure1.png)
ischemia, only $^{14}$C-2DG concentration was shown in table 1 with comparison to structures in the vertebrobasilar arterial system. Severely decreased $^{14}$C-2DG uptake in the carotid arterial system shown in figure 4 suggests limited $^{14}$C-2DG and glucose delivery to this region, but $^{14}$C-2DG uptake was not zero in all structures in the ischemic region.

All reperfused animals showed a sudden decrease in blood pressure following the release of the occluding device from the carotid artery. The amount of low molecular weight dextran required to keep arterial blood pressure at a normal level was between 0.3 to 0.8 ml. After arterial blood pressure was stabilized, no additional dextran was required and MABP remained above 60 mm Hg throughout the study.

Three animals were studied with the 2-deoxyglucose technique immediately following the reflow (early stage group) and five were studied 15 minutes following the beginning of the reflow (late stage group). All animals of the reperfused group exhibited severe neurological deficits similar to the ischemic group during 30 minutes of ischemia. During the reflow, some neurological functions recovered (corneal reflex, pain reflex and pupillary size), although all animals remained in a coma. The recovery of the pain reflex, however, was not complete and the pupillary reflex to light did not recover. Convulsive seizures were present in all reperfused animals during the ischemic period but no seizure was observed during the reflow except in two animals with equivocal seizure activity.

In the early stage group, CMRG showed a marked increase over the control group in regions where a severe decrease in metabolism was observed in the ischemic group. The difference from the control group was statistically significant in almost all structures. No decrease in CMRG was seen in the cerebral cortex and the increase in CMRG was homogeneous in all structures.

In the late stage group, following 15 minutes of reperfusion, heterogeneity of CMRG within structures was apparent. Additionally some structures showed a decrease in CMRG while others exhibited an increase in CMRG, so that the difference in CMRG between structures became very large. In the cerebral cortex, caudoputamen and thalamus, CMRG was lower than in the control group throughout the structure with higher CMRG values obtained in small regions within each of these structures. CMRG in the hippocampus, amygdala, globus pallidus and hypothalamus remained high compared with early stage group. In the late stage group most structures lost their normal autoradiographic appearance. This was particularly apparent in the cerebral cortex and in the hippocampus (fig. 5). Note the increase in CMRG in the pyramidal layer of the hippocampus.

Mean values for cerebral metabolic rate for glucose of the four groups are summarized in table 1. The $^{14}$C concentration in the ischemic regions of the ischemic group ranged from 21 to 110 nCi per gram, except in the medial geniculate body and the superior colliculus. Although it was low, it still remained at a level of 8% to 50% of the $^{14}$C concentration in the cerebellum. In the early stage group the CMRG values were approximately 1.5 to 2.5 times those of the control group in the cerebral cortex, caudoputamen and the thalamus and 3.7 to 4.2 times the control values in the hippocampus, globus pallidus and amygdala. The latter structures appeared to have a higher glucose metabolism than the former structures. In the late stage group, CMRG of the cerebral cortex was less than one half of the control value. The hippocampus, amygdala, and globus pallidus, however, still had CMRG values 2 to 3 times those of the control group.

In each animal of the control group, the plasma glucose concentration was fairly constant throughout the time course of the 2DG study. In the ischemic and reperfused group, however, it changed in 5 animals and remained constant in 8 animals. There were also
TABLE  Regional Cerebral Glucose Metabolism in Control, Ischemic and Reperfused Group (µM/100gm/min)

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control group (n = 6)</th>
<th>Ischemic group (n = 5)</th>
<th>Reperfusion Early stage group (n = 3)</th>
<th>Reperfusion Late stage group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.D.</td>
<td>Mean</td>
<td>S.D.</td>
</tr>
<tr>
<td>Sensory-motor cortex</td>
<td>102</td>
<td>32</td>
<td>(24)</td>
<td></td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>107</td>
<td>24</td>
<td>(21)</td>
<td></td>
</tr>
<tr>
<td>Visual cortex</td>
<td>123</td>
<td>38</td>
<td>(32)</td>
<td></td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>79</td>
<td>12</td>
<td>(32)</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>69</td>
<td>19</td>
<td>(77)</td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>51</td>
<td>8</td>
<td>(75)</td>
<td></td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>110</td>
<td>19</td>
<td>(37)</td>
<td></td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>53</td>
<td>12</td>
<td>(26)</td>
<td></td>
</tr>
<tr>
<td>Amygdala</td>
<td>66</td>
<td>3</td>
<td>(110)</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>107</td>
<td>14</td>
<td>(86)</td>
<td></td>
</tr>
<tr>
<td>Mediastrial geniculate body</td>
<td>91</td>
<td>16</td>
<td>(205)</td>
<td></td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>81</td>
<td>20</td>
<td>(265)</td>
<td></td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>87</td>
<td>24</td>
<td>131(589)</td>
<td>93</td>
</tr>
<tr>
<td>Reticulocerebral formation</td>
<td>93</td>
<td>28</td>
<td>94(460)</td>
<td>12</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>67</td>
<td>25</td>
<td>69(347)</td>
<td>13</td>
</tr>
<tr>
<td>Vestibular nucleus</td>
<td>218</td>
<td>35</td>
<td>180(784)</td>
<td>81</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>112</td>
<td>26</td>
<td>144(658)</td>
<td>36</td>
</tr>
<tr>
<td>Medulla nucleus</td>
<td>56</td>
<td>23</td>
<td>47(244)</td>
<td>11</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>110</td>
<td>41</td>
<td>116(558)</td>
<td>18</td>
</tr>
</tbody>
</table>

Significant difference from control group (Bonferroni test) *p < 0.05, ‡p < 0.01, §p < 0.001
L: value from regions with low 2DG uptake. H: value from regions with high 2DG uptake.
Values in parentheses are the concentration of 14C (nCi/g).

In structures which are considered to be ischemic, CMRG was not calculated for reasons described in the text. The number of animals of stable stage group is 5 unless otherwise noted.

Large changes in plasma glucose level between animals (from 83 to 217 mg/dl), although there does not appear to be a correlation between plasma glucose level and the glucose metabolic distribution in any of the experimental groups. The maximum change in plasma glucose within an animal was 158 mg/dl whereas the average change was 41 mg/dl.

Discussion

The quantitative measurement of CMRG in a small animal such as the gerbil is complicated by the difficulty in obtaining adequate blood samples for the counting of radioactivity. Special attention was paid to minimize the blood loss due to arterial sampling and in the control group blood pressure remained stable throughout the study. Although a stable glucose level is desirable throughout the study, this was not always possible. Consequently, the equation of Savaki, et al was utilized in the calculation of metabolic rates when the plasma glucose level varied throughout the study. The comparable values in CMRG in the structures of the vertebral basilar arterial system in all experimental groups suggests that the variable glucose concentration did not cause a serious problem. Cerebral blood flow in the carotid arterial system of this ischemic model of the gerbil was between 1 and 5 ml/100gm/min during...
ischemic period (unpublished data in our laboratory, Nadasy et al). In order to assess the error introduced by a flow limitation in the 2-deoxyglucose model, we have modified this model to include a flow term. The error introduced in these flow states is greater than 20%. Due to this larger error we have refrained from calculating CMRG in ischemic regions. CMRG in the vertebrobasilar arterial system has little error since cerebral blood flow in this region stayed above 70% of the control value during the ischemic period and also during the reperfusion period (unpublished data in our laboratory, Nadasy et al).

CMRG in structures in the carotid arterial system of the two reperfusion groups may be in error if, as has been suggested, the rate constants for 14C-2DG are changed in ischemia. Hawkins et al measured rate constants for fluorodeoxyglucose in ischemic brain of stroke patients and observed rather large differences from those measured in normal brain. We estimated the error of CMRG caused by the alterations in the rate constants by correcting these rate constants for normal rats, which were used in this study, proportionally to the ratio of rate constants between normal and ischemic human brain. The understimulation was from 1.7 to 30.0% (mean 10.7%). This estimate is not ideal but we expect that the change in rate constants due to ischemia in this study are smaller than that measured in normal brain. The area of high CMRG is larger in the right cerebral cortex than in the left.

2DG. In the early stage reperfusion group error due to the unfilled free glucose pool can be estimated by assuming that the pool is totally empty at the time of 14C-2DG injection. This was done by setting plasma glucose to zero at the time of 14C-2DG injection and then allowing the plasma glucose to quickly rise to the measured value. Using the modified equation of Savaiki et al this error was found to be a 10% overestimation. Since the 14C-2DG was injected 2–3 minutes following reperfusion in the early stage group, the values in Table 1 will be less than 10% too high due to the unfilled glucose pool. Although there is some evidence from animal studies that the lumped constant is altered during severe ischemia, it returns toward normal during reperfusion. In addition, estimates of the lumped constant in stroke patients have suggested that there is no change.

It has been shown that seizure activity may cause large increases in glucose metabolism. If the seizures are caused by the depolarization of the cerebral cortex, this will influence the metabolic rates obtained in this study. However, by recording electrical activities on the brain and spinal cord simultaneously, Cohn showed that convulsive activity in the gerbil caused by bilateral carotid occlusion has extracranial origin (i.e.: the electrical activity on the spinal cord exhibited spikes while that on the cortex was depressed). In the present study, seizure occurred almost always during the ischemic period and was rarely observed during the reperfusion period. Although EEG was not monitored in this study, in other animals in which EEG and brain extracellular potassium were measured, little paroxysmal discharge of EEG was recorded during the reperfusion period. However, further studies which correlate electrocorticogram to local glucose metabolism are needed to evaluate the effect of seizure on glucose metabolism in postischemic brain.

The stroke model of the mongolian gerbil is a very useful and widely accepted model for the study of cerebral ischemia. Although a number of the studies in the literature utilize a unilateral occlusion of the common carotid artery model, a bilateral occlusion model also has significant merit in the study of reperfusion. Low molecular weight dextran given to animals of the reperfusion groups may alter the pattern of glucose metabolism in postischemic brain through its effect on cerebral blood flow. But it seems that the postischemic brain gets better reperfusion with the infusion of dextran than without since arterial blood pressure was stabilized with the addition of this fluid and it does not cause any decrement in blood flow. Thus observed changes in CMRG are if anything an underestimation of true metabolic alterations during the reperfusion period.

During the ischemic period, 14C-2DG uptake in the ischemic region was very low compared to the nonischemic region in the vertebrobasilar arterial system, although nonzero, and in some structures rather high values of 14C-2DG uptake were obtained. During severe ischemia, indicated by the depletion of high energy phosphates, glucose consumption in the brain is
The nonzero $^{14}$C-2DG uptake found in this study during ischemia can be expected since cerebral glucose utilization will drop only in very marked ischemia. The zonal increase of $^{14}$C-2DG uptake between the ischemic and the nonischemic region has already been reported and is hypothesized to be caused by anaerobic glycolysis.8

The most striking finding in this study was the increase of glucose metabolism in the postischemic brain just after reperfusion. This increase in metabolism appears to spread throughout the region that had been ischemic prior to the reperfusion and appears rather homogeneous, although the increase in the metabolism is greater in the hippocampus, globus pallidus, and amygdala than in the cerebral cortex and caudoputamen. There are three possible explanations for the observed increase in glucose metabolism during the reperfusion period. Firstly, the blood flow perfusing the previously ischemic region may still be low resulting in anaerobic glycolysis. Secondly, there may be a uncoupling between the oxygenation and energy production in the tissue. This uncoupling may be caused by damage to the mitochondria during the ischemiap. Thirdly, the damaged neuronal tissue may require much more energy for repair (especially the sodium-potassium transmembrane pump). Crowe, et al.9 measured extracellular potassium in the gerbil brain, and showed a more than 90% recovery during the reperfusion period even after severe ischemia caused by bilateral carotid occlusion. The recovery is prolonged, however, for longer durations of occlusion. They also showed full recovery of the potassium pump mechanism and an intact plasma membrane indicated by normal potassium uptake kinetics within minutes during the reperfusion period. It is quite likely that the postischemic brain requires a great deal of energy during the early period of reperfusion in order to restore the gradient of sodium and potassium across the cellular membrane. The first mechanism does not adequately explain the increase in CMRG, since blood flow as measured by $^{14}$C-iodoantipyrine recovers to greater than 50% of the control level 5 minutes into reperfusion in the bilateral common carotid occlusion model of the gerbil (Nadas et al., in our laboratory, unpublished data).

In the late stage group, the 2-deoxyglucose was infused 15 minutes into the reperfusion and in this group CMRG went down below the control value in both the cerebral cortex and the caudoputamen. This suggests that extra energy is not required at this period of reperfusion. Alternatively, oxygenation and energy production may be coupled again so that less glucose is required for the production of energy for cellular survival.

The hippocampus, amygdala and globus pallidus showed a tendency for higher CMRG values after the reperfusion than the cerebral cortex or the caudoputamen. These structures have already been shown to be particularly susceptible to ischemia and anoxia.30, 31 Diemer, et al.6 found a local increase in glucose consumption in the hippocampus, globus pallidus, and substantia nigra in a model of total cerebral ischemia in rats that they relate to the regional selectivity of these structures to ischemic damage. Recently, Pulsinelli et al.32 observed a depressed CMRG in the postischemic brain of the rat but a relatively increased CMRG in the striatum and hippocampus compared to adjacent structures during latter period of reperfusion. Levy and Duffy1 showed a decrease in high energy phosphate utilization in the brain 24 hours after reperfusion following a unilateral carotid occlusion in the gerbil while at 4 hours after reperfusion high energy phosphate utilization was increased by more than 50%. They also noted a regionally concentrated $^{14}$C-2DG uptake in the hippocampus, cortex and thalamus four hours after reperfusion. They suggest that the increase in glucose consumption combined with the decline in cerebral blood flow may play a major role in the continuing damage to the brain during reperfusion.

In this study, glucose consumption in the brain definitely increases during the early stage of reperfusion following a period of ischemia and decreases 15 minutes into the reperfusion. It is unclear whether the increase in cerebral glucose consumption seen during reperfusion following a period of ischemia causes further tissue damage as suggested by Levy, et al. To answer these questions, further studies that relate glucose metabolism to other variables such as pH, oxygen consumption or electrical activity are required. It would also be most useful to measure cerebral blood flow and relate it to the glucose metabolism in the postischemic brain.

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