Correlation Between Brain Surface Potassium and Glucose Utilization After Bilateral Cerebral Ischemia in the Gerbil

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SUMMARY The correlation between cerebral glucose utilization and brain surface potassium concentration (BS-K\(^+\)) was studied during reperfusion following bilateral cerebral ischemia in the gerbil. Cerebral glucose utilization rate was measured by the \(^{14}\)C-2-deoxyglucose method and BS-K\(^+\) was continuously monitored by a potassium sensitive membrane electrode. BS-K\(^+\) increased from 3.0 ± 0.6 mM (mean ± S.D.) before ischemia to 58.7 ± 17.3 mM 30 minutes after the occlusion of both common carotid arteries. The rate of decline of BS-K\(^+\) after release of occlusion differed between animals. Glucose utilization rate in the cerebral cortex immediately under the potassium electrode was low but homogeneous in 7 animals while in 5 animals the metabolic pattern was heterogeneous with areas of both low and high glucose metabolism. The former animals exhibited a fast recovery of potassium flux while the latter animals showed a slow recovery. Glucose utilization rate and potassium half recovery time were linearly correlated.

These studies suggest that the reason that potassium flux may not recover rapidly in postischemic brain tissue is due to the lack of sufficient energy for a rapid re-establishment of the ion gradient across the cell due to the inefficient energy production of anaerobic glycolysis.

EXTRACELLULAR POTASSIUM CONCENTRATION of the brain (EC-K\(^+\)) is regulated by several factors including neuronal activity, energy production by the cells, clearance by circulating blood, as well as redistribution of potassium in the extracellular space. The former two are the main factors which control EC-K\(^+\) in physiologically functioning brain. During cerebral ischemia, failure of the membrane ion pump due to lack of adequate energy production causes EC-K\(^+\) to increase. Following restoration of cerebral blood flow EC-K\(^+\) can be brought back to preischemic levels.

Cerebral glucose utilization and cerebral blood flow are tightly coupled in normal brain. In cerebral ischemia, cerebral glucose metabolism switches from oxidative metabolism to anaerobic glycolysis at a certain threshold of cerebral blood flow. But the relationship between cerebral glucose utilization, cerebral blood flow and EC-K\(^+\) in postischemic recirculated brain is still obscure.

In the present study, brain surface potassium concentration (BS-K\(^+\)), electroencephalogram (EEG) and regional glucose utilization of the cerebral cortex were studied. A membrane type surface electrode was used for the potassium measurements to minimize damage to the brain.

Methods

Male mongolian gerbils (55–75 gm) were anesthetized by inhalation of halothane (initially 4%) followed by 1–2%) in 30% oxygen, balance nitrous oxide. Small polyethylene catheters (PE10) were inserted into the left femoral vein and artery with the aid of an operating microscope. Specially designed occluders (small nooses consisting of a rubber suture and small plastic tube) were implanted on both common carotid arteries for transient occlusion and subsequent reperfusion. The animal was then secured on a stereotaxic head holder, the skin over the skull was excised and a round hole of 4 mm diameter was drilled on the left side of the skull over the parietal cortex. The edge of the hole was placed 1 mm lateral to the sagital suture and 1 mm posterior to the coronal suture. To find the topographic relationship between this hole and cerebral structures, a fine platinum wire was inserted into the brain of an animal not undergoing a glucose utilization measurement. This platinum wire was placed at the center of the hole in the skull and parallel to the plane of section using a stereotaxic apparatus. After fixing the brain with formalin, a sagital cut was made at the level of this wire and the brain section carefully examined. At this level, the hippocampus assumed a horse shoe shape and this shape was used as a mark in the subsequent animals to find the appropriate sections under the potassium electrode. With the aid of an operating microscope the dura was incised using a 27 gauge needle and a fine forceps. A plastic cannula with a 4 mm outer diameter holding a potassium sensitive membrane electrode was placed on the surface of the brain using a micromanipulator and fixed in place by dental cement. Two small screws which were fixed on the left side of the skull to help secure the dental cement to the skull, were also used for recording of the EEG. After the surgical preparation, the halothane was discontinued and the 70% nitrous oxide/30% oxygen mixture was continued since the animal was kept in the stereotaxic apparatus to facilitate the potassium measurements. Body temperature was kept at 37°C by means of servo-controlled heat lamp and blood pressure was monitored from the femoral artery catheter
utilizing a blood pressure transducer and a polygraph. In 12 animals, thirty minutes after the halothane was discontinued, bilateral cerebral ischemia was induced by occlusion of both common carotid arteries. After 30 minutes, the occlusion was released and the tissue was allowed to reperfuse (experimental animals). In three animals, BS-K + was monitored without carotid occlusion (control animals). In the experimental animals, regional cerebral glucose utilization was measured from 15–45 minutes after the start of reperfusion (i.e. 75 to 105 minutes after the halothane was discontinued) and in control animals, the glucose utilization measurements were made 75 to 105 minutes after halothane was discontinued.

Glucose utilization in the brain was measured by the 14C-2-deoxyglucose (14C-2DG) method7 and quantitative autoradiography.8 Fifteen μCi of 14C-2DG in 0.3 ml physiological saline was injected intravenously, following which 10 arterial samples of about 25 μl were obtained for the measurement of plasma 14C activity. Every ten minutes, a larger volume of blood (about 80 μl) was obtained in which plasma glucose concentration was measured. Total blood loss by arterial sampling was 0.6 ml and mean arterial blood pressure was kept above 81 mm Hg in all animals during the measurement of glucose utilization. Thirty minutes following injection of 14C-2DG the animal was killed by either decapitation or rapid intravenous injection of an overdose of pentobarbital. Twenty micron thick sections of the brain were cut, autoradiograms prepared and analyzed by computer assisted densitometry. For comparison with the BS-K +, an average of 10 sections (200 μm apart) considered to be under the potassium electrode was used. The shape of the hippocampus on the coronal sections was used as a marker to find these sections. A cortical band 2 mm wide was read in these sections (fig. 1) using a densitometer (Gamma Scientific) interfaced to a PDP11 computer. The aperture size was 240 μm and the program allowed 200 readings to be made over a period of 20 seconds while the film was moved relative to the densitometer light spot. For the comparison with EEG, a simple average of the glucose utilization rates of all measured structures in the cerebral cortex was used. For the calculation of glucose utilization, the deoxyglucose equation for changing plasma glucose concentration was used.9 In both the control and the experimental animals, the rate constants for 14C-2DG of normal rats were used, although changes in rate constants might be expected in the postischemic brain (see Discussion). The value of the lumped constant for normal rats (0.483) was used in all animals except one, which had a plasma glucose concentration of 19.4 mM/L. In this animal, a value appropriate to this degree of hyperglycemia (0.4) was used (Kennedy, personal communication).

Brain surface potassium concentration was measured by a potassium sensitive membrane electrode as described by Crowe et al.6 The electrode is made of Pyrex glass tubing (1.0 mmID, 1.5 mmOD), with a valinomycin-silicone rubber membrane fixed to the end of the tubing and a Ag/AgCl wire inside the tubing. The glass tubing was filled with 3 mM KCl and 150 mM NaCl. This electrode was fixed in a plastic cannula which was implanted on the surface of the brain of the animal. Another Ag/AgCl wire for the measurement of DC potential was placed in the plastic cannula and connected to the brain by a salt bridge of physiological saline. A reference electrode was placed subcutaneously on the back of the animal. DC potential of the brain surface, uncorrected potassium and potassium corrected by DC potential were monitored using an electrometer with high impedance inputs and DC amplifiers. Calibration was done in two solutions of 3 mM KCl + 150 mM NaCl and 30 mM KCl + 123 mM NaCl respectively before and after the study. To evaluate the speed of potassium decline after reperfusion, the time from the release of occlusion to the point where the potassium level was half way between the maximum value and the preischemic base line value (potassium half recovery time) was obtained from the corrected potassium recording.

EEG was recorded bipolarly from two screws. One of the screws was placed over the left frontal region of the brain while the other was over the left occipital region, so that the recorded EEG was generated by the majority of the left cerebral hemisphere. The distance between the screws was approximately 1 cm. The percent of postischemic to preischemic EEG amplitude was calculated and values averaged over the entire reperfusion period (i.e. 45 minutes) or during the 2DG study (i.e. 30 minutes) were correlated to the recovery of extracellular potassium concentration or glucose utilization, respectively.

A polygraph (Grass-Model 7D) was used for the recording of blood pressure, potassium concentrations and EEG.

**Figure 1.** A 14C-2DG autoradiogram from a control animal. Although there is a thin layer of high glucose utilization on the surface of the left cerebral cortex where the potassium electrode was implanted (marked with an arrow), the distribution of glucose utilization in left cerebral hemisphere is not different from that in the right hemisphere. Shows the region of the cortical band used for the comparison between glucose utilization rate and BS-K +.
Continuous slow (3—4 Hz) EEG activity returned in 10 animals after reperfusion. In one animal, no continuous EEG activity was observed during the reperfusion period but paroxysmal EEG activity was observed. This animal was excluded from the correlation calculations between glucose utilization, BS-K⁺ and recovery of EEG. BS-K⁺ was 3.0 ± 0.6 mM before the ischemia and increased to 58.7 ± 17.3 mM after 30 minutes of ischemia. After the release of the occlusion it decreased with a rate of decline differing among animals. Typical recordings of potassium and EEG in two animals are shown in figures 2 and 3. A transient drop in arterial blood pressure following the release of the occlusion necessitated the infusion of a small amount of (0.2—0.6 ml) of low molecular weight dextran (Macrodex®) in some animals. After this infusion mean blood pressure remained above 81 mmHg in all animals. The 2DG autoradiograms showed a homogeneous pattern of low glucose utilization in some regions of the cerebral cortex with a heterogeneous pattern of high and low values in other regions of the cortex (fig. 4 and 5). The 2DG distribution under the potassium electrode showed a homogeneously low pattern in 7 animals with a heterogeneous pattern in the remaining 5 experimental animals. The mean glucose utilization rate was 63.9 ± 37.4 µM/100g/min under the potassium electrode and 43.1 ± 35.6 in the homologous region in the contralateral cortex. The difference was not statistically significant. The mean values of glucose utilization are shown in table 1. The large deviations (especially in the experimental animals) and the small number of control animals makes detection of significant differences difficult. Only in the caudate-putamen, thalamus and cerebellar cortex were significant differences found, although there appears to be an appreciable metabolic effect in the hippocampus.

**Correlation Between BS-K⁺ and Glucose Utilization**

Figure 6 shows the changes of BS-K⁺ in animals with a homogeneously low pattern of glucose metabolism as well as in animals in which the glucose metabolism exhibits a heterogenous distribution. Both groups show a similar potassium increase during the ischemic period (55.6 ± 12.5 mM and 63.1 ± 25.0 mM respectively, at 30 minutes after the occlusion), but exhibit a quite different response of BS-K⁺ after the release of the occlusion. The rate of decline of the elevated potassium level was slower in the animals in which glucose metabolism was heterogeneous compared to those with a relatively homogeneous metabolic distribution at the electrode site. There was a statistically

**Results**

BS-K⁺ was 3.1 ± 0.3 mM (mean ± S.D.) at the start of the 2DG study and remained constant throughout the metabolic measurement. In one animal a transient increase of BS-K⁺ of unknown cause was observed 3 times. The base line of BS-K⁺ between these transients, however, was not different from the other two animals. Mean values of cerebral glucose utilization are shown in table 1. One animal had a thin dark layer on the surface of the cerebral cortex beneath the electrode (fig. 1), but this area of high glucose utilization was so small that it did not appreciably effect the calculation of mean glucose metabolism under the electrode. The other control animals did not exhibit any such heterogeneities. The mean glucose utilization rate under the electrode was 73.9 ± 20.3 µM/100gm/min while in the homologous area of the contralateral cortex the utilization rate was 82.2 ± 17.2 µM/100gm/min. Except for this dark layer in one animal, no effect from the electrode implantation was found on the autoradiograms of these three control animals.

**Experimental Animals**

In the 11 experimental animals in which the EEG was recorded successfully, the EEG became isoelectric soon after the occlusion of both carotid arteries. Continuous slow (3—4 Hz) EEG activity returned in 10 experimental animal with a fast recovery of potassium flux. Recording of blood pressure was interrupted by blood sampling from the femoral artery catheter. The EEG tracing has some artifacts from the seizure like movement of the animal during ischemia.

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**Table 1: Local Cerebral Glucose Utilization in Control and Experimental Animals (µM/100 g/min)**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Control animals (n = 3) Mean ± SD</th>
<th>Experimental animals (n = 12) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>67.8 ± 15.0</td>
<td>66.1 ± 36.7</td>
</tr>
<tr>
<td>Sensory-motor cortex</td>
<td>67.2 ± 16.7</td>
<td>63.9 ± 40.0</td>
</tr>
<tr>
<td>Visual cortex</td>
<td>69.4 ± 20.6</td>
<td>46.7 ± 25.6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>38.9 ± 13.9</td>
<td>96.1 ± 53.3</td>
</tr>
<tr>
<td>Amygdala</td>
<td>31.1 ± 6.1</td>
<td>38.3 ± 19.4</td>
</tr>
<tr>
<td>Caudate-putamen</td>
<td>87.8 ± 16.7</td>
<td>50.6 ± 17.7</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>35.0 ± 14.4</td>
<td>48.9 ± 19.4</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>44.4 ± 11.7</td>
<td>52.2 ± 27.8</td>
</tr>
<tr>
<td>Thalamus</td>
<td>78.3 ± 15.6</td>
<td>52.7 ± 16.7</td>
</tr>
<tr>
<td>Reticular formation</td>
<td>51.7 ± 13.9</td>
<td>51.7 ± 9.4</td>
</tr>
<tr>
<td>Cerebellar cortex</td>
<td>44.4 ± 14.4</td>
<td>32.2 ± 7.2*</td>
</tr>
<tr>
<td>Dentate nucleus</td>
<td>88.3 ± 16.7</td>
<td>79.4 ± 11.7</td>
</tr>
</tbody>
</table>

*p < 0.05
†p < 0.01 (students t test)
Correlation Between Glucose Utilization, BS-K⁺ and Recovery of EEG

The recovery of EEG during the reperfusion period and the mean brain glucose utilization rate in the cerebral cortex exhibited a negative linear correlation but the correlation did not reach a statistical significance (fig. 8). There was, however, a significant negative linear correlation between the potassium half recovery time and the EEG amplitude relative to the preischemic level (fig. 9).

Discussion

Occlusion of both common carotid arteries in the gerbil produces severe ischemia in the forebrain since the gerbil lacks communication between the vertebrobasilar and carotid arterial systems. Although glucose metabolism was not measured during cerebral ischemia in the present study, the rapid efflux of intracellular potassium accompanied by an isoelectric EEG following occlusion of the carotid arteries indicates that the ischemia was severe in all experimental animals. Immediately after the release of occlusion of the carotid arteries there was a sudden drop in blood pressure due to the carotid baroreceptor reflex. This occurred in all experimental animals indicating that flow was restored in the previously occluded carotid artery very rapidly following release of the occlusion. However, even though the occluded carotid arteries are patent, there is a possibility of regional defects in perfusion of the brain. In measurements in this ischemia model of regional cerebral blood flow using ¹⁴C-io-doantipyrine, we have observed an altered blood flow distribution with definite perfusion defects in the early state (5 minutes) of reperfusion (Nadasy et al, unpublished data). These flow defects tended to disappear after 30 minutes of reperfusion. The flow distribution, however, was quite different from that of glucose utilization in the cerebral cortex observed in the present study.

The lumped constant for the calculation of quantitative glucose metabolism is lower when plasma glucose is elevated. Since the lumped constant has not been evaluated in the gerbil, except in one animal which had a very high plasma glucose level (350 mg/dl), the value for the lumped constant that was used in this study was that from normoglycemic rats (0.483). Since relative values of glucose utilization rates can be used in the correlation with BS-K⁺, this in no way changes any conclusions. Values of glucose utilization in the posts ischemic recirculated brain may also be in error if, as has been suggested, the rate constants for ¹⁴C-2DG are altered in ischemia. Assuming that the rate constants in the postischemic brain of the gerbil are changed in the same direction and by a similar degree as determined by Hawkins et al for fluorodeoxyglucose in ischemic brain of stroke patients, then glucose utilization may be underestimated by as much as 30%. The error will be small in the animals with high glu-
cose utilization rates and large in animals with a low rate of glucose utilization. This error will also not appreciably alter the relationship observed between potassium half recovery time, EEG recovery and glucose utilization in the cerebral cortex.

There does not appear to be any appreciable damage resulting from the potassium electrode implantation since the 2DG autoradiogram in the area beneath the electrode in the control animals appears normal. Additionally, the preischemic level of BS-K$^+$ is normal. The difference in glucose metabolism between the cortex under the electrode and the homologous contralateral cortex in the control animals was 11.2% and not statistically significant. In the experimental animals this difference was larger (and in the opposite direction), but the difference was still not significant due to the large range in the post release glucose utilization rate data.

The measurement of potassium efflux from the cells during the ischemic insult and its decline following reperfusion was made with a membrane type electrode instead of a microelectrode which measures potassium concentration in the extracellular space. A surface electrode was utilized primarily to minimize trauma as well as to allow stable measurements in an awake preparation. Control measurements with the membrane type potassium electrode yield values of potassium concentration similar to those obtained with a microelectrode. Both the present study as well as other investigations show a similar response to ischemia of BS-K$^+$ and EC-K$^+$ as measured with a microelectrode. A similarity of response of BS-K$^+$ and EC-K$^+$ has also been shown in cortical spreading depression. It thus appears, as has been suggested by Crowe et al, that BS-K$^+$ is primarily determined by EC-K$^+$.

As cortical blood flow is reduced to 12–16 ml/100g/min during progressive ischemia in the baboon, the cortical evoked potential is abolished although EC-K$^+$ is only slightly elevated. When the flow falls further to 8–11 ml/100g/min there is a large increase in potas-
sium concentration. Most of the efflux of potassium from the cells to the extracellular space is reversed during reperfusion. Astrup et al have measured cerebral glucose consumption in a barbiturate anesthetized perfused brain model in the dog before and after the administration of ouabain and found a 40% decrease in glucose metabolism. The amount of energy consumed by the Na⁺-K⁺ pump in the awake brain is probably even greater than in the anesthetized brain, since potassium flux accompanies neuronal activity. Consequently, Na⁺-K⁺ transport and brain metabolism should be closely correlated. If the amount of energy consumed by the Na⁺-K⁺ pump increases, brain metabolism and hence glucose utilization must increase. Conversely, if the production of energy is disturbed, the Na⁺-K⁺ pump will be affected.

In the present study, the cerebral glucose utilization rate and half recovery time of potassium concentration were linearly correlated after 30 minutes of ischemia. Figure 7 indicates that the animals showing the slowest recovery of potassium concentration following the ischemia had the highest rates of glucose utilization. Since the function of the Na⁺-K⁺ pump and brain metabolism are closely correlated, the rate of recovery of potassium concentration in any region must be directly proportional to the amount of energy (ATP) available to the tissue in this region. This would mean that the region in which we observed a slow recovery of potassium concentration are regions not producing sufficient energy for the Na⁺-K⁺ pump. Since the glucose metabolism was found to be high in these regions, it suggests that the energy is most likely produced by anaerobic metabolism which is significantly less effective than the anaerobic production of energy.

It could be argued that enough of the glucose was still being metabolized aerobically so that energy production remained normal. There is evidence in the literature, however, that in spite of an increased glucose consumption, presumably due at least in part to anaerobic glycolysis, energy production is reduced below normal. Therefore, it is certainly possible that energy production in the present study was impaired in the regions exhibiting an increased glucose consumption, leading to a slower normalization of extracellular potassium levels.

Nemoto et al observed that during recirculation following 16 minutes of complete ischemia in the rat, there was a transient fall of brain pH to a level below that during the ischemia. In studies in the cat in which cerebral blood flow, and arteriovenous extraction of oxygen and glucose have been measured following 16 minutes of global ischemia, a similar post-ischemic hypermetabolism has been noted. The ratio of arteriovenous difference of O₂ to that of glucose, used as an indicator of oxidative glucose utilization, decreased during reperfusion although oxygen consumption itself increased in the same period. These results suggest that presence of both aerobic and anaerobic glycolysis in recirculated brain. Studies in our laboratory utilizing the same model of ischemia in the gerbil (Nadasy et al, unpublished observations) showed that flow recovers during reperfusion, indicating that sufficient oxygen is being delivered to the tissue. This is not inconsistent with the hypothesis of anaerobic glycolysis. Duckrow et al observed a hyperoxydation of cytochrome aa₃ during the first 20 minutes of reperfusion after 10 minutes of ischemia in the rat. In another model of reperfusion it was found that although the supply of oxygen, as indicated by the oxidized redox states of cytochrome aa₃, is sufficient during this period of reperfusion, the concentration of ATP in the brain is still decreased. Accordingly, oxidative metabolism must be uncoupled in spite of a sufficient supply of oxygen. Duckrow et al hypothesized that this inhibition is due to a block of reducing equivalent flow to the electron transport chain occurring between the phosphorylation of glucose and the tricarboxylic acid cycle. Our hypothesis of high glucose metabolism with low oxygen metabolism is also consistent with data in the monkey where red venous blood is observed after the release of a clip on a middle cerebral artery without an associated hyperperfusion.

If the glucose utilization in the experimental animals is mainly due to anaerobic glycolysis, which is a less efficient source of energy production, than a negative linear correlation would be expected between glucose utilization rates and EEG recovery (Fig. 8). We observed a significant negative correlation between the half recovery time of potassium concentration and EEG recovery. This lends credence to the assumption that the BS-K⁺ measured by the electrode reflects the behavior of hemispheric potassium. Although the EEG has been measured from screws inserted on either side of the potassium electrode the EEG signal emanates from a region larger than does the K⁺ measurement. If we could have measured the EEG from the exact vol-
volume as was seen by the K⁺ electrode we might expect an even better correlation than was found in this study. EEG activity in the cortex disappears at a slightly higher level of cerebral blood flow than the level where the increase in extracellular potassium concentration due to ischemia occurs and recovers following the decline of elevated potassium during reperfusion. This correlation is to be expected since the neuronal activity is dependent on the recovery of the ion balance across the cell membrane.

These data demonstrate both a heterogeneity of glucose utilization rates during reperfusion following global ischemia in the gerbil as well as a correlation between glucose metabolism and recovery of potassium concentration. This correlation indicates that glucose is not utilized efficiently everywhere in the brain suggesting the existence of anaerobic glycolysis. This heterogeneity may have a relationship to the regional differences in vulnerability of the brain to ischemia.

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