Deleterious Effect of Glucose Pretreatment on Recovery from Diffuse Cerebral Ischemia in the Cat

II. Regional Metabolite Levels

Frank A. Welsh, Ph.D., Myron D. Ginsberg, M.D., Wendy Rieder, B.S., and William W. Budd, B.S.

SUMMARY Glucose was infused intravenously into cats prior to cerebral ischemia. Brain concentrations of glucose, measured in 7 regions, were elevated 2.5-fold compared to those of non-infused animals. Ischemia of 15 or 30 minutes duration caused a greater accumulation of lactic acid in the brain of glucose-infused animals. Post-ischemic restitution of cerebral ATP, phosphocreatine, and lactate during 90 minutes of recirculation was severely impaired in the brain of animals pretreated with glucose compared to untreated animals. Thus, excess lactic acidosis may be a major factor interfering with metabolic restitution following cerebral ischemia.

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CEREBRAL ISCHEMIA causes rapid depletion of the brain's stores of high energy phosphates — ATP and phosphocreatine. Recirculation following defined periods of ischemia leads, in many models, to nearly complete recovery of brain levels of ATP and phosphocreatine. Recently, however, we have described regionally heterogeneous recovery of brain metabolites in glucose-treated versus untreated animals which have been described previously.

In the present study we have examined the effect of pre-ischemic administration of glucose on restitution of metabolite levels following 15 or 30 minutes of ischemia. Glucose administration has previously been shown to increase the neurologic deficit and degree of neuropathologic damage caused by cardiac arrest in the monkey. Since post-ischemic recovery of brain function depends upon restoration of an adequate energy state, we have compared restitution of energy metabolites in glucose-treated versus untreated animals which have been described previously. In the accompanying report we have examined the effect of glucose infusion on post-ischemic cerebral blood flow.

Methods

Cats of either sex, weighing 3-4 kg, were anesthetized with sodium pentobarbital, 40 mg/kg intraperitoneally, and were prepared as previously described. The common carotid arteries were isolated and encircled with loose-fitting ligatures, and the basilar artery was exposed through the clivus. One hour prior to the ischemic insult, a solution of 50% dextrose in normal saline was infused intravenously over a period of 60 minutes at a rate of 50 μl per minute per kg body weight, yielding a total dose of 6 g of glucose for a 4 kg animal. Upon termination of the glucose infusion, cerebral ischemia was produced by occluding the carotid and basilar arteries with miniature Mayfield clips. Following occlusion of the arteries, the animal was bled to a mean arterial pressure of 64 torr by manual hemorrhage into a heparinized syringe. After 15 or 30 minutes of ischemia, normotensive recirculation was initiated by releasing the arterial clips and reinfusing shed blood.

Five groups of animals were studied. In the control group (Group I, n = 4), 2 animals were sacrificed at the end of the period of glucose infusion. Two additional control animals were sacrificed 2 hours after termination of glucose infusion. Group II animals (n = 3) received a 15-minute period of ischemia without recirculation, and Group III (n = 3), 30 minutes of ischemia without recirculation. Group IV animals (n = 5) received a 15-minute ischemic insult followed by 90 minutes of recirculation, and Group V (n = 3), 30 minutes of ischemia plus 90 minutes of recirculation.

At the termination of each experiment, the brain was frozen in situ by pouring liquid N₂ into a styrofoam cup fixed to the exposed calvarium. During the period of freezing, mechanical ventilation of the animals was continued, and the arterial pressure remained steady for 8-12 minutes. This freezing technique has previously been shown to preserve metabolite levels even in deep structures of cat brain without ischemic alteration (see Table 3 and ref. 14). The frozen brain was sectioned in the coronal plane into slices 1 cm in thickness with a pre-cooled saw. In a liquid N₂ bath the slices were illuminated with ultraviolet light (λmax = 366 nm) using a 200 watt mercury arc lamp fitted with Corning filter 5840. Reflected 366 nm light was photographed on Polaroid high contrast film (type 51) using a Nikon macro-photographic camera fitted with Corning filter 5840. Tissue fluorescence (λmax = 450 nm), which in gray matter is derived primarily from NADH, was
photographed through Corning filters 3389 and 5562 on high contrast film.

At -30°C the brain slice was sampled bilaterally at 7 predetermined areas indicated in figure 1. The samples were weighed (2-5 mg) and extracted with 0.1 N NaOH in methanol. One portion of the extract was made 1 mM in cysteine, heated 10 minutes at 60°C to destroy NAD+, and analyzed for NADH using enzymatic cycling. A second portion of the extract was acidified with perchloric acid, centrifuged, and the supernate was neutralized and analyzed for ATP, phosphocreatine, lactate, and glucose using enzymatic, fluorometric techniques.

Tissue specific gravity was determined on a density gradient column. Samples from 4 cortical regions (the lateral and medial gyri of each hemisphere, see fig. 1, LAT and MED) were dissected under kerosene at -30°C. Two additional samples were dissected from subcortical white matter. The samples were placed on a kerosene-bromobenzene density gradient which had been standardized with K2SO4 solutions of known specific gravity. Ten minutes after placement on the gradient, the column position of the sample was recorded and the specific gravity calculated from the standard curve.

In 2 of the Group IV animals, 250 μCi of 14C-antipyrine was infused intravenously over a 3-minute period, beginning 2 minutes before brain freezing. Autoradiograms were made from thin sections of brain (20 μ) which were dried and placed in contact with Kodak SB-5 x-ray film for a 10-day exposure.

Results

The arterial variables, blood pressure and blood gases (table 1) showed no major difference among groups of glucose-infused animals, nor between the series of glucose-treated and the previously reported series of untreated animals. Infusion of glucose caused plasma levels of glucose to rise 4-5-fold in all treated groups (table 2). A further increase of plasma

<table>
<thead>
<tr>
<th>Table 1 Arterial Variables of Glucose-Infused Animals</th>
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<tbody>
<tr>
<td>Group number</td>
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<tr>
<td>Pre-insult period</td>
</tr>
<tr>
<td>MABP</td>
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<tr>
<td>Po2</td>
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<td>Pco2</td>
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<td>Po2</td>
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<tr>
<td>Pco2</td>
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<tr>
<td>pH</td>
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</tbody>
</table>

Group I, control animals, n = 4.
Group II, 15 minutes of ischemia, no recirculation, n = 3.
Group III, 30 minutes of ischemia, no recirculation, n = 3.
Group IV, 15 minutes of ischemia, 90 minutes recirculation, n = 5.
Group V, 30 minutes of ischemia, 90 minutes recirculation, n = 3.
Values for MABP, Po2, and Pco2 expressed in Torr (mm Hg).
Mean arterial blood pressure (MABP) = Diastolic BP + 0.33 (Systolic BP - Diastolic BP).
*Significantly different from value of non-infused group. (See reference 8 for values of non-infused groups), p < 0.05, Student's t-test.
TABLE 2  Plasma Glucose Levels (mg/100 ml)

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Period during experiment</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No infusion Control (n = 3)</td>
<td>170 ± 54</td>
<td>210 ± 81</td>
<td>—</td>
<td>226 ± 60</td>
<td></td>
</tr>
<tr>
<td>Glucose-infused Control (n = 4)</td>
<td>176 ± 38</td>
<td>761 ± 52*</td>
<td>—</td>
<td>540 ± 52*</td>
<td></td>
</tr>
<tr>
<td>No infusion 15 min ischemia no recirculation (n = 3)</td>
<td>166 ± 24</td>
<td>218 ± 25</td>
<td>738 ± 358</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Glucose-infused 15 min ischemia no recirculation (n = 3)</td>
<td>136 ± 18</td>
<td>673 ± 42*</td>
<td>812 ± 75</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>No infusion 30 min ischemia no recirculation (n = 3)</td>
<td>154 ± 10</td>
<td>224 ± 39</td>
<td>968 ± 58</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Glucose-infused 30 min ischemia no recirculation (n = 3)</td>
<td>163 ± 27</td>
<td>860 ± 128*</td>
<td>1197 ± 16*</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Glucose-infused 15 min ischemia 90 min recirculation (n = 3)</td>
<td>138 ± 21</td>
<td>629 ± 135</td>
<td>1102 ± 213</td>
<td>600 ± 175</td>
<td></td>
</tr>
<tr>
<td>Glucose-infused 30 min ischemia 90 min recirculation (n = 3)</td>
<td>175 ± 19</td>
<td>627 ± 23</td>
<td>897 ± 34</td>
<td>631 ± 189</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean ± standard error.
Experimental Period A, before infusion of glucose.
Experimental Period B, after infusion of glucose.
Experimental Period C, after 15 minutes of ischemia.
Experimental Period D, after 90 minutes of recirculation.
*Significantly different from non-infused group, p < 0.05, Student's t-test.

Glucose occurred during the ischemic insult, presumably as a result of arterial hypotension, in both infused and non-infused animals, as described in detail elsewhere. Brain levels of glucose prior to cerebral ischemia were 2.5 times higher in glucose-infused animals (table 3). However, regional levels of ATP, phosphocreatine, lactate, and NADH were not different from those of untreated animals. The
glucose-treated animals did show a reduction of specific gravity both in cortex and white matter when compared to non-infused animals, although the difference in white matter was not statistically significant (fig. 2, Control Groups).

Ischemia of 15 or 30 minutes duration depleted tissue levels of phosphocreatine to 0.5 mmol/kg or lower in all brain regions of both treated and untreated animals (figs. 3 and 4). Regional levels of ATP were lower in glucose-infused animals although the difference was significant only in the 2 white matter regions after 30 minutes of ischemia (fig. 4, WHITE and INT). Lactate levels were higher in all regions of the glucose-treated animals after both durations of ischemia. The increment of lactate ranged from 8-10 mmol/kg in cortex after 15 minutes (fig. 3, LAT, MED, SULC, and CING) and from 10-13 mmol/kg after 30 minutes of ischemia (fig. 4).

Recirculation following 15 minutes of ischemia led to substantial resynthesis of ATP and phosphocreatine in both gray matter and white matter (fig. 5). Phosphocreatine levels were lower in all 7 regions of glucose-infused animals, but this difference was statistically significant only in the cingulate gyrus (fig. 5, CING). In addition, lactate levels ranged from 20-36 mmol/kg in the cortex of glucose-treated animals compared to values of 6 mmol/kg or less in untreated animals (fig. 5, LAT, MED, SULC, and CING). There was also a fundamental difference in the regional pattern of metabolite recovery. In untreated animals, metabolite levels were restored in a homogeneous fashion throughout the cerebral cortex, white matter, and caudate nucleus. By contrast, glucose pretreatment caused a regionally heterogeneous pattern of metabolite recovery (see fig. 7) in which zones depleted of high energy phosphates coexisted adjacent to areas with nearly normal values. Since the precise location of the derangements of tissue metabolites varied in different animals, the resulting average values have large standard errors which obscure the significance of the difference between the two groups of animals.

A similar distinction in the regional pattern of metabolic recovery was noted after 30 minutes of ischemia. In this case, however, regional heterogeneity occurred in the untreated animals as reported previously. By contrast, in the glucose-treated animals, there was a homogeneous absence of high energy phosphates in the cerebral gray matter (fig. 6, LAT, MED, SULC, CING, and CAUD). In the white matter and internal capsule of treated animals, ATP returned to 70% and 88% of control levels, respectively, while phosphocreatine was restored to 45% and 33% of control. Tissue levels of lactate were higher in all regions of glucose-treated than in non-treated animals.
Ischemia caused a fall in the specific gravity of cortex and white matter in both glucose-infused and non-infused animals ($p < 0.05$), with the exception of the white matter of infused animals (fig. 2). Recirculation following a 30 minute insult led to a further decline of specific gravity in cortex but not in white matter. After 15 minutes of ischemia and 90 minutes of recirculation, specific gravity was significantly lower than control values only in the cortex of non-infused animals.

Figure 7 illustrates one example of the regional heterogeneity in restitution of metabolite levels and blood flow. The location of regions sampled is indicated in figure 7a, which also shows regional variations in the intensity of 366 nm light reflected from the frozen brain surface. Areas with higher reflectance intensity (for example, sample H) indicate regions with decreased blood volume. Figure 7b pictures the NADH fluorescent image from the same brain section. Note that the lowest NADH fluorescence intensity was often associated with regions of lower blood volume (samples A, F, G, and H). Furthermore, the areas of decreased reflectance and fluorescence exhibited the lowest levels of ATP and phosphocreatine and highest levels of lactate. In this and many other of the recirculated brains in the present study, regions with unusually low intensity of NADH fluorescence invariably contained high levels of lactate and markedly diminished levels of high energy phosphates. This severe energy failure was not restricted to areas of relatively low perfusion as evidenced by the uptake of $^{14}$C-antipyrine (fig. 7c, samples E, G, and H). In these areas, $^{14}$C-antipyrine uptake was either high (sample H), or exhibited a laminar pattern within the cortex with relatively greater perfusion in the deeper cortical layers (samples E and G). By contrast, the tissue represented by samples B, C, and D showed a regionally uniform antipyrine uptake and also contained the highest levels of ATP and phosphocreatine and lowest levels of lactate.

Table 4 summarizes the effect of ischemia and recirculation on NADH concentration in cerebral cortex of glucose-infused animals. NADH was elevated 3-fold during ischemia and returned to control levels or below during recirculation following 15 and 30 minutes of ischemia, respectively. By comparison, complete cerebral ischemia due to cardiac arrest caused NADH levels to rise 6-fold by 5 minutes and remain increased for 120 minutes.
Table 4  

NADH Levels in Cerebral Cortex of Glucose-Infused Animals During Ischemia and Recirculation

<table>
<thead>
<tr>
<th>Animal group</th>
<th>NADH (μmol/kg)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>13.5 ± 0.7 (n = 4)</td>
</tr>
<tr>
<td>15 Min ischemia</td>
<td>37.3 ± 9.8 (n = 3)</td>
</tr>
<tr>
<td>30 Min ischemia</td>
<td>37.3 ± 6.6* (n = 3)</td>
</tr>
<tr>
<td>15 Min ischemia plus</td>
<td>16.6 ± 3.5 (n = 5)</td>
</tr>
<tr>
<td>90 Min recirculation</td>
<td>8.3 ± 0.7* (n = 3)</td>
</tr>
<tr>
<td>5 Min cardiac arrest</td>
<td>87.3 ± 9.2* (n = 3)</td>
</tr>
<tr>
<td>120 Min cardiac arrest</td>
<td>75.5 ± 4.5* (n = 2)</td>
</tr>
</tbody>
</table>

For each animal, the NADH concentration was calculated from an average of 8 cortical samples.
Values are means ± standard errors.
*Significantly different from control group, p < 0.05, Student's t-test.
The cardiac arrest animals were not pretreated with glucose.
deleterious effect of glucose. As discussed previously,\textsuperscript{13} the use of insulin to curtail the hyperglycemia which occurs during ischemia did not alter the metabolic outcome. In order to test whether administration of glucose following ischemia was as deleterious as pretreatment, glucose was infused during recirculation following 15 minutes of ischemia in 3 animals. Compared to non-infused animals (mean cortical ATP = 1.66 ± 0.03, lactate = 5.1 ± 1.1 mmol/kg), post-ischemic treatment with glucose interfered with restitution of lactate (ATP = 1.63 ± 0.28, lactate = 21.1 ± 10.0 mmol/kg), but the overall impairment of metabolic recovery was not as pronounced as with pre-ischemic treatment with glucose (ATP = 1.41 ± 0.25, lactate = 27.4 ± 7.0 mmol/kg).

Similar results were obtained from a series of 5 animals in which mannitol was substituted for glucose in order to test for non-specific osmotic effects of glucose pretreatment. Again, pretreatment with mannitol impaired metabolic recovery (ATP = 1.55 ± 0.38, lactate = 17.1 ± 7.9 mmol/kg), but the effect was not as great as pretreatment with glucose. Consequently, the deleterious action of glucose may only in part be related to osmotic effects.

The regional heterogeneity of post-ischemic restitution of metabolite levels and of flow (fig. 7) contrasts strongly with the regionally uniform alteration of metabolites and flow\textsuperscript{13} caused by the ischemic insult itself (figs. 3 and 4). Thus, although the accumulation of lactate was the same in all cortical areas during ischemia, there were nevertheless remarkable regional differences in metabolite levels at the end of the 90-minute recirculation period (fig. 7). Therefore, lactate accumulation cannot be the sole determinant of metabolic restitution; rather, there are undoubtedly a myriad of additional critical factors which play a role in the recovery or ultimate death of the tissue.

Within recirculated brain, regions with the greatest
15 Minutes of Ischemia  
plus  
90 Minutes of Recirculation  
No Glucose  
Glucose-infused  

30 Minutes of Ischemia  
plus  
90 Minutes of Recirculation  
No Glucose  
Glucose-infused

**FIGURE 8. Regional energy failure increased by pretreatment with glucose.** Representative diagrams of the regional extent of energy failure (shaded areas) as evidenced by decreased tissue fluorescence intensity and low levels of ATP and phosphocreatine (less than 0.5 mmol/kg).

restoration of ATP, phosphocreatine, and lactate often exhibited the least recovery of NADH levels (sample D, fig. 7). While such samples from cortical sulci may have undergone an artifactual doubling of NADH content during brain freezing (table 3 and ref. 14), it was nevertheless true that NADH varied inversely with lactate levels in post-ischemic brain. This relationship is opposite that which occurs during cerebral ischemia, which causes a rapid increase of both NADH and lactate. Since total cerebral ischemia resulting from cardiac arrest produced a high level of NADH, which remained elevated for at least two hours, the diminution of NADH observed during recirculation in the present model (table 4) must in some way have been triggered by reperfusion of the tissue. The steady-state level of tissue NADH is determined by the balance between the generation of reducing equivalents and their reoxidation in both the cytoplasmic and mitochondrial compartments. During ischemia, the supply of oxygen and substrate is cut off, but since carbohydrate reserves are greater than those for oxygen, the tissue redox state becomes reduced. Upon reperfusion, the fresh supplies of oxygen and glucose lead to a new steady-state for NADH which again is determined by the relative rates of NAD+ reduction and NADH reoxidation. If the cellular damage produced by ischemia were to selectively interfere with the enzymes involved in generating reducing equivalents, then recirculation would lead to reoxidation of NADH in the absence of substrate utilization and without resynthesis of high energy phosphates. In the same brain, other regions with a preserved and perhaps higher than normal rate of generation of reducing equivalents could use both oxygen and glucose for ATP production while remaining in a redox state which was reduced relative to control tissue. Admittedly, the above considerations are speculative, but they would also explain the extreme case in which a total paralysis of NAD+ reduction could lead to subnormal NADH values in post-ischemic brain having little or no blood flow.13

Finally, although the present model of ischemia produced irreversible impairment of both flow and metabolism, there were regional examples of metabolic failure in apparently well-perfused tissue (fig. 7). These examples raise the questions whether the failure to resynthesize and maintain levels of high energy phosphates is flow-limited during the post-ischemic period or whether, quite independent of post-ischemic blood flow, an intrinsic lesion within the metabolic machinery is responsible for the cellular energy failure.

**References**

Cerebral Arterial Constrictions Induced by Human and Bovine Thrombin

RICHARD P. WHITE, PH.D., CHARLES E. CHAPLEAU, M.D., MARION DUGDALE, M.D., AND JAMES T. ROBERTSON, M.D.

SUMMARY

Purified human and bovine thrombin produced comparable tonic contractions in isolated canine basilar arteries. The magnitude of the contractions was closely related to the number of thrombin Units studied rather than to the amount of protein added to the isolation bath. Thrombin had a much slower onset of action, but was more potent in generating sustained contractions than either serotonin or prostaglandin F$_2$-$\alpha$. Moreover, in contrast to serotonin and prostaglandin F$_2$-$\alpha$, the contractions caused by thrombin were not terminated by equivalent washing. The thrombin-induced contractions were significantly inhibited by prostacyclin, medofenamic acid, pbenoxybenzamine and glycerol. Prostacyclin was the most potent of these inhibitors. The results suggest that thrombin in a "free" form may cause vasoconstriction, in addition to platelet aggregation, in hemostasis and could contribute to the genesis of cerebral vasospasm associated with subarachnoid hemorrhage.

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THROMBIN IS ONE OF MANY naturally occurring substances which produce cerebral vasospasm experimentally. Vasospasm persists for hours if thrombin is injected into the subarachnoid space. It might, therefore, be a factor in the genesis of cerebral vasospasm observed clinically. The quantities of thrombin used to produce this experimental phenomenon were approximately equivalent to 0.35 to 4 ml of blood. It was noted that the cerebral vasospasm produced was generally much slower in onset than that induced by many other agents such as serotonin and prostaglandins. This led to the hypothesis that such vasoconstriction was due to an indirect action of thrombin. Subsequent findings supported this view: the intracisternal injection of thrombin markedly elevated the levels of prostaglandins F$_2$-$\alpha$ and E$_{9}$ in cerebrospinal fluid (CSF) and also caused a pleocytosis. Since the synthesis of prostaglandins by fibroblasts is stimulated by thrombin, it was assumed that such synthesis might account for the elevated CSF levels of prostaglandin and the delayed appearance of vasospasm. Brain, blood vessels and other tissue were considered possible sources of vasoactive substances released in response to thrombin, but the mechanisms responsible for the delayed constriction of the cerebral arteries in vivo was believed likely to be an indirect one.

Recently Linder and Alksne demonstrated that the constriction of isolated cerebral arteries caused by the addition of whole blood to the tissue bath was enhanced by thrombin. They attributed this enhancement to an increase in the synthesis of prostaglandins by platelets — an indirect effect. However, cerebral arteries also synthesize a number of prostaglandins which are known to cause vasoconstriction of these vessels and it is possible that thrombin might produce constriction directly by stimulating this synthesis within the arterial wall.

The present study was performed to 1) determine whether thrombin exerted a direct action on cerebral arteries, 2) obtain information of its potency in relation to other agonists, and 3) to ascertain whether certain drugs would modify this action. The results indicate that thrombin could be involved in pathophysiological phenomena in ways heretofore unsuspected.

Methods

Mongrel adult dogs of either sex weighing 17 to 26 kg were anesthetized with sodium pentobarbital (30
Deleterious effect of glucose pretreatment on recovery from diffuse cerebral ischemia in the cat. II. Regional metabolite levels.
F A Welsh, M D Ginsberg, W Rieder and W W Budd

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