Early reversal of acidosis and metabolic recovery following ischemia

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Tissue acidosis is believed to be a key element in ischemic injury of neural tissue. The goal of this study was to determine whether persisting postischemic acidosis or the extent of acidosis would affect metabolic recovery following an ischemic event.

Intracellular pH (pHi), adenosine triphosphate, phosphocreatine, and lactate levels were measured in the cerebral cortex during the early stages of reperfusion, following either 5 or 10 minutes of global ischemia in both normo- and hyperglycemic gerbils. A total of 130 gerbils were injected with a solution containing 1.5 ml Neutral Red (1%) (± 2.5 gm/kg glucose); 30 minutes later, the gerbils were placed under halothane anesthesia, and the carotid arteries were occluded for either 5 or 10 minutes. The brains were frozen in liquid nitrogen at 0, 15, 30, 60, and 120 seconds after reperfusion; they were sectioned and the block face was photographed to determine the pHi by using Neutral Red histophotometry.

At the conclusion of the ischemia, the pHi in all groups had decreased significantly from a control value of 7.05 ± 0.03 (mean ± standard error of the mean). In normoglycemic brains, the pHi values fell to 6.71 ± 0.04 and 6.68 ± 0.11 after 5 and 10 minutes of ischemia, respectively. Hyperglycemic brains were more acidic; values fell to 6.57 ± 0.10 and 6.52 ± 0.24 after 5 and 10 minutes of ischemia, respectively. Lactate levels were approximately fivefold greater than those of control tissue in normoglycemic brains, while lactate levels in hyperglycemic brains were increased eightfold. The adenosine triphosphate and phosphocreatine levels were depleted at the end of ischemia in all groups. After 2 minutes of reflow activity, the pHi levels in both normo- and hyperglycemic brains were restored to those of control values in the 5-minute ischemic group, while the pHi levels remained significantly depressed in the 10-minute ischemic group. Restoration of high-energy phosphates was similar in normoglycemic brains regardless of ischemic duration, recovering to only 20% of the restoration obtained in control tissue at 2 minutes. In hyperglycemic brains, however, there was complete recovery of high-energy phosphates by 2 minutes of reflow activity following 5 minutes of ischemia. Extending the ischemic period to 10 minutes in hyperglycemic brains slowed the rate of metabolic recovery to that observed in normoglycemic brains.

The results indicate that the reflow period permits the rapid restoration of pHi levels substantially before the normalization of primary energetic compounds. In addition, hyperglycemia appears to be transiently beneficial in the initial critical moments of reflow activity following short-term ischemia, but provides no immediate benefit in terms of energy stores when ischemic duration is prolonged. The lack of a prolonged benefit to energy status and the well-known deleterious effects of increased acidosis support the concept that hyperglycemic conditions should be avoided during temporary ischemia.

KEY WORDS • cerebral ischemia • acidosis • intracellular pH • hyperglycemia • gerbil

THE loss of normal oxidative energy metabolism and the development of lactacidosis is central to the myriad metabolic and biochemical perturbations elicited by ischemia. The entire pathological cascade that evolves during ischemia can be directly or indirectly attributed to a rapid and massive energy fail-
by pretreatment with glucose has been shown to increase the extent of brain damage following an ischemic insult. However, the role of lactacidosis in the evolution of brain damage is not unifactorial, because there is evidence that elevated lactate or acidosis alone do not lead to brain damage unless either condition occurs in conjunction with other insults such as ischemia.

There are many reports that have measured energy failure and acidosis during ischemia and the long-term recovery of these functions during reperfusion. However, the time course of intracellular pH (pH) recovery during the early stages of the reflow period has not been well studied, and it is during this post-ischemic period that a host of unique secondary pathological events occur. The purpose of this research was to determine the relationship between acidosis and energy metabolism during this early, dynamic period following the reintroduction of glucose and oxygen and to ascertain whether the duration of ischemia or degree of acidosis would affect the recovery of energy metabolism. This paper focuses on the metabolic recovery and restoration of pH during the first 2 minutes of reflow activity following either 5 or 10 minutes of global ischemia.

Materials and Methods

A total of 130 adult male Mongolian gerbils received an intraperitoneal injection of a saline solution (1.5 ml) containing 1% Neutral Red 30 minutes prior to the ischemic insult. Hyperglycemia was achieved by intraperitoneal injection of glucose (2.5 g/kg) in the Neutral Red vehicle. The gerbils were anesthetized with 2% halothane and 55% nitrous oxide (balance oxygen) during exposure of the carotid arteries. Both carotid arteries were occluded for either 5 or 10 minutes and then reperfusion was initiated. The brains of the 112 gerbils that survived both the surgery and the ischemic insult were fixed in situ by immersion in liquid nitrogen, and subsequently removed in a −30°C glove box. Each brain was sectioned at a thickness of 20 μm in a cryomicrotome maintained at −20°C glove box. The tissue sections were freeze-dried, and regions of the hypoleuclar cerebral cortex were dissected at room temperature. The tissue samples were assayed for ATP, phosphocreatine, glucose, and lactate by standard fluorometric and bioluminescent procedures.

Intracellular pH was determined by histophotometry using color slide film of the tissue block surface during the sectioning process. Each photographic exposure contained the Neutral Red-stained brain and an unstained brain, which served as a spectrophotometric "blank." The photographic slides were imaged using low magnification on an Olympus microscope with an STT camera and analog processor while alternating 450- and 550-nm band-pass (Abs490 and Abs590) filters in the light path. The optical density of the stained brain was calculated with respect to the unstained blank at each of the two wavelengths. A final image was constructed from the ratio image by transformation to pH, according to the empirically derived standard curve, in which the constants were determined from calibrated brain paste standards:

\[ pH = -10.5 - (Abs490/Abs590) / -1.3. \]

Statistical analysis was performed using analysis of variance for multiple comparisons followed by Tukey’s test for individual comparisons, from which a level of 0.05 was considered to be significant.

Results

Blood Glucose

The intraperitoneal injection of glucose (2.5 g/kg) 30 minutes prior to the occlusion of the common carotid arteries significantly increased plasma glucose concentrations from a control value of 9.30 ± 0.47 mM to a value of 26.14 ± 1.41 mM.

Energy Metabolism

Cortical glucose and the high-energy phosphates (ATP and phosphocreatine) were depleted by the end
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**ADENOSINE TRIPHOSPHATE**

**NORMOGYCEMIA**

![Graph showing adenosine triphosphate concentrations](image)

**P-CREATINE**

**NORMOGYCEMIA**

![Graph showing p-creatine concentrations](image)

**HYPERGELYCEMIA**

![Graph showing adenosine triphosphate concentrations](image)

**HYPERGELYCEMIA**

![Graph showing p-creatine concentrations](image)

**FIG. 2.** Graphs showing time course of tissue adenosine triphosphate concentrations in the cerebral cortex in gerbils during reflow activity for normoglycemic (upper) and hyperglycemic (lower) gerbils following global ischemia. Preischemic control values are indicated by the dotted lines. The solid lines and filled circles indicate changes seen after 5 minutes of ischemia; the dashed lines and filled squares indicate changes following 10 minutes of ischemia. Statistical details are as given in Fig. 1.

**FIG. 3.** Graphs showing time course of tissue phosphocreatine concentrations in the cerebral cortex during reflow activity for normoglycemic (upper) and hyperglycemic (lower) gerbils following global ischemia. Preischemic control values are represented by the dotted lines. The solid lines and filled circles indicate changes seen after 5 minutes of ischemia; the dashed lines and filled squares indicate changes following 10 minutes of ischemia. Statistical details are as given in Fig. 1.

5 minutes of ischemia in both normo- and hyperglycemic animals, reflecting a general energy failure in the four groups of gerbils. Glucose values in the hyperglycemic cortex of the control group were 2.2-fold greater than in the normoglycemic cortex. Cortical glucose in all groups was depleted at the end of the ischemia. Upon initiating reflow activity, tissue glucose levels remained significantly lower than those of the control group for up to 2 minutes. At the end of the reflow period, cortical glucose concentrations in normoglycemic animals had only recovered to 29% and 17% of control values for 5 and 10 minutes of ischemia, respectively (Fig. 1 upper). In contrast, cortical glucose levels in hyperglycemic brains had recovered to 37% and 17% of control levels by 2 minutes of reflow activity following 5 and 10 minutes of ischemia, respectively (Fig. 1 lower).

Even though the glucose concentrations remained depressed, ATP and phosphocreatine levels recovered toward those of control levels in both groups during the first 30 seconds of reflow activity following 5 minutes of ischemia (Figs. 2 and 3). The hyperglycemic brains showed a continual rise in high-energy phosphates throughout the first 2 minutes of recirculation, but the normoglycemic animals exhibited a secondary fall in high-energy phosphates between 30 and 120 seconds. By 2 minutes of reflow activity, the ATP concentrations were 88% of control levels in hyperglycemic animals but only 38% of control levels in the normoglycemic animals. Phosphocreatine concentrations at 2 minutes of reflow activity were 75% of control levels in the hyperglycemic animals, but only 28% of control levels in normoglycemic animals. Thus, the high-energy phosphate concentrations in the cortex of hyperglycemic brains were almost completely restored, while phosphate concentrations from normoglycemic
brains were less than 40% of control levels at 2 minutes of reflow activity following 5 minutes of global ischemia.

Prolonging the ischemic insult to 10 minutes did not adversely affect recovery of ATP and phosphocreatine values in brains from normoglycemic gerbils at 2 minutes of reflow activity, which reached 47% and 31% recovery, respectively (Figs. 2 and 3 lower panels). The near-total recovery of high-energy phosphates in hyperglycemic animals following 5 minutes of bilateral ischemia was compromised, however, by extending the ischemia to 10 minutes; as a result, the recovery became comparable to that observed in normoglycemic gerbils.

Lactate and Intracellular pH

Ischemia produced significant elevations in tissue lactate concentrations in all groups. Lactate levels in normoglycemic brains were 4.8- and 5.5-fold higher than the control value at 5 and 10 minutes of ischemia, respectively (Fig. 4 upper). In contrast, the lactate accumulation in hyperglycemic animals was 8.2- and 9.3-fold greater than those of the control group at 5 and 10 minutes of ischemia, respectively (Fig. 4 lower).

The pH_i values for normoglycemic brains were significantly decreased, from a control value of 7.05 ± 0.03 to 6.71 ± 0.04 and 6.68 ± 0.11, after 5 and 10 minutes of ischemia, respectively (Fig. 5 upper). Although it was restored at 2 minutes of reflow activity in the 5-minute ischemic group, the pH_i was only 6.89 ± 0.10 in the 10-minute ischemic group at the end of the reflow period, a value significantly lower than both the corresponding 5-minute ischemic group and the control value.

The intracellular acidosis in the cortex of hyperglycemic gerbils was greater than that in normoglycemic gerbils (Fig. 5 lower). There was also a significant difference in recovery of pH_i depending on the duration of ischemia. The pH_i decreased to 6.57 ± 0.10 at 5 minutes of ischemia and to 6.52 ± 0.24 at 10 minutes of ischemia. During the reflow period in the 5-minute hyperglycemic group, the pH_i initially increased, but exhibited a secondary decrease at 1 minute of reflow activity. While the pH_i was restored to that of control in the 5-minute ischemic group at 2 minutes of reflow, the pH_i in the 10-minute ischemic group was only 6.80 ± 0.11, which was significantly less than the control value.

Discussion

Restoration of energy metabolism and proton homeostasis is a necessary first step to the recovery of function following ischemia. The gerbil model of global ischemia was used because it allows the rapid in vivo fixation of brain tissue that is necessary to examine early periods of recirculation. The results from this study indicate that the onset of pH_i and metabolic recovery occurs rapidly upon the initiation of reflow activity and that the marked redistribution of both glycolytic and tricarboxylic acid cycle intermediates to an equilibrium state during ischemia is rapidly reversed with the reintroduction of glucose and oxygen. The regeneration of newly synthesized ATP is apparently used to correct ischemia-induced ion perturbations. Only after this correction is achieved can the cortical concentrations of the high-energy phosphates be fully re-established.

Although there is a clear relationship between the accumulation of lactate and the degree of acidosis during ischemia, the stoichiometric relationships may differ depending on the species of animal used and according to the type and duration of insult. During reflow activity, there is a significant recovery of pH_i in the absence of any detectable decrease of the elevated lactate levels, which indicates a dissociation between lactate concentration and pH_i. However, the time necessary to restore pH_i does appear to vary with respect to the animal species, the type of insult, and the technique used to determine pH_i. Duration of the insult is another important factor in the restoration of pH_i during the reflow period. While hyperglycemic brains...
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used in this study exhibited a greater acidosis and accumulation of lactate, the pH, in both normo- and hyperglycemic brains at 2 minutes of reflow activity was restored to that of control values in the 5-minute ischemic group but not in the 10-minute ischemic group. Thus, the duration of ischemia is the critical factor in the time needed to restore pH to a control value despite a greater degree of lactacidosis in hyperglycemic rats.

Researchers who have varied the preischemic glucose concentrations have found that the extent of acidosis during ischemia does not affect the recovery of brain energy metabolism or mitochondrial function during recirculation. However, our results indicate that the rate of energy restoration is facilitated in hyperglycemic brains after 5 minutes of global ischemia: high-energy phosphate levels recovered to 80% of control values at 2 minutes of reperfusion, while normoglycemic brains attained less than 40% of the control values. Recovery of high-energy phosphates after 10 minutes of ischemia was similar in both normo- and hyperglycemic brains, again reaching approximately 40% of control values at 2 minutes of reperfusion. Therefore, the additional 5 minutes of ischemia did not exacerbate the metabolic recovery of the normoglycemic brains; however, during this period the rate of recovery in hyperglycemic brains was reduced to a rate comparable to that of normoglycemic brains.

Other researchers have examined the recovery of pH with respect to recovery of high-energy phosphates during reflow activity and have reported that the recovery of ATP and phosphocreatine essentially mirrored that of pH in achieving control values within approximately 50 minutes of reflow activity. The gerbil model is different in at least two respects. First, the recovery in the 5-minute ischemic groups was more rapid than that seen in previous studies, reaching a resting pH within 2 minutes. Second, the recovery of pH preceded restoration of high-energy phosphates in hyperglycemic brains following 5 minutes of ischemia, although in the remaining groups there was simultaneous recovery. Thus, it seems that restoration of pH is an early step in recovery following global ischemia and can precede the restoration of high-energy phosphates in some situations.

One explanation for the differential recovery of pH and high-energy phosphates in normo- and hyperglycemic brains observed in this study may be that differences in the ionic workload had accumulated at the end of the ischemic insult. It was originally shown by Hansen that anoxic depolarization, characterized by a large efflux of potassium, is delayed by approximately 2 minutes in hyperglycemic rats subjected to cardiac arrest compared to normoglycemic rats. In the gerbil model, anoxic depolarization occurs in normoglycemic brains after 0.9 minutes, while anoxic depolarization is delayed until 3.1 minutes in hyperglycemic brains. This delay in hyperglycemic brains is thought to occur because additional energy provided by anaerobic glycolysis can sustain the ionic gradients for a longer period of time. It is possible that the normoglycemic gerbils in this study were exposed to a more prolonged energetic stress from redistribution of ionic gradients, because anoxic depolarization occurred earlier in them than in hyperglycemic animals. The normoglycemic brains subjected to 5 minutes of ischemia underwent 4.1 minutes of ischemia under conditions of anoxic depolarization compared to only 1.9 minutes in hyperglycemic brains. When the duration of ischemia was extended to 10 minutes, normoglycemic brains underwent 9.1 minutes of ischemia under conditions of anoxic depolarization compared to 6.9 minutes in hyperglycemic brains. Thus, it would appear that metabolic and pH recovery in short-term ischemia is determined much more by time of depolarization than duration of ischemia; however, when there are longer periods of ischemia, the discrepancy between the two time periods becomes negligible and therefore is not a factor in metabolic recovery.

The hyperglycemic delay in the onset of anoxic depolarization by means of increased anaerobic glycolysis may, however, come at a cost: namely, a greater accumulation of lactate. After 5 minutes of ischemia,
hyperglycemic brains may have a better short-term recovery, but edema, one of the long-term effects of additional lactate, and proton accumulation may outweigh these short-term benefits. When ischemia is extended to 10 minutes, any metabolic advantage provided by hyperglycemia is lost, and there is no difference in the recovery of pH, or high-energy phosphates between these groups. These results indicate that any additional damage suffered by hyperglycemic brains must occur after reflow activity has been initiated because hyperglycemia does not compromise the short-term restoration of energy metabolism. Furthermore, additional damage imposed by the glucose load does not appear to be due to a metabolic lesion in the restoration of energy metabolism.

Conclusions

Our study indicated that a reversal of intracellular acidosis occurred quite rapidly upon the initiation of reflow activity following ischemia and preceded the complete restoration of cortical energy stores. Although hyperglycemia improved recovery after 5 minutes of ischemia, such an effect was not evident after 10 minutes of ischemia. Thus, metabolic restoration was not markedly affected by increasing either blood glucose or the duration of ischemia, two factors that are known to increase damage following ischemia. Because the deleterious effects of ischemic duration and hyperglycemia may not be immediately evident and there is no clear evidence of improved metabolic function with ischemic epochs greater than 5 minutes, the avoidance of hyperglycemic conditions during even brief periods of ischemia is probably warranted. Finally, the rapid recovery of pH, after periods of ischemia that are insufficient to retard energy restoration suggests that measures designed to reverse ischemic acidosis may be unnecessary.

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