The Effect of Anoxia on the Amino Acid and Carbohydrate Metabolism of the Isolated Dog Brain

Kon Huh, David D. Gilboe and David H. Minskyer

Departments of Neurosurgery and Physiology, University Hospitals, Madison, Wisconsin, U.S.A.

(Received for Publication: November 29, 1967)

ABSTRACT

The isolated dog brain was perfused for a period of 4 minutes with blood normal in all constituents, but pathologically low in oxygen in order to follow the metabolic response of the brain to anoxia.

During anoxic perfusion, the brain appears to subsist on the free amino acids in the brain and on glucose taken up from the perfusion blood. Oxygen uptake is relatively constant increasing temporarily immediately after anoxia. The lactic acid formed within the brain during anoxia is not released in any quantity; instead it appears to be metabolized in the brain following the period of anoxia causing a lower than normal uptake of glucose. Brain ATP and GTP levels decrease significantly, but not markedly indicating that the brain’s energy requirements are being partially met during anoxic perfusion.

INTRODUCTION

The brain is assumed to require a continuous supply of oxygen to efficiently meet the enormous needs for energy to continue normal cerebral function. Since there is little reason to believe that the brain has any capacity to store substantial amounts of oxygen or high energy compounds, specific chemical reactions which partially meet the energy requirements of the brain anaerobically must take place within the brain in response to anoxia. Identification of the metabolic reactions associated with anoxia has been difficult since most methods which produce anoxia result in some impairment of cerebral circulation; the resulting ischemia presents an entirely new spectrum of problems associated with the reduced availability of nutrients and the impeded removal of metabolic waste products.

The environment of the surgically isolated brain preparation used for this study can be controlled to produce selected conditions such as anoxia without altering other variables. This report concerns results obtained from a preliminary investigation of the feasibility of producing cerebral anoxia for the study of brain metabolism. The efficiency of anaerobic processes in meeting the brain’s energy requirements during anoxia is judged on the basis of changes in the brain’s high energy phosphate content.

METHODS

The dog brains were isolated under sodium pentobarbital anesthesia following a procedure described previously (Gilboe et al., 1966) that involved the removal of the mandible, snout, and all extracranial soft tissues leaving only the brain case intact. At the level of the second cervical vertebra, a laminectomy was performed and the spinal cord and dura were ligated and transected. The internal carotid arteries, and the anastomotic branch of the internal maxillary segment of the external carotid arteries supplied blood to the isolated brain preparation (Figure 1). Venous blood was returned to the oxygen-
ator from the vertebral sinus. The micro disc oxygenator systems (Figure 2) were primed with 600 ml of fresh, compatible blood collected from a donor that had been anticoagulated with heparin. Arterial blood was propelled by a variable speed roller pump. A similar pump aided venous return.

**Fig. 1.** The carotid arteries and brain case in the dog after brain isolation.

**Fig. 2.** A diagrammatic representation of the 1 pump systems used in the study. A. Disc oxygenator B. Roller pump, C. Dacron wool filter, D. Heat exchanger, E. Bubble trap, F. Manifold, G. Blood pressure transducer, H. Head holder, I. Isolated brain, J. Venous return pump. The second system contains anoxic blood.

Viability was judged by the presence and quality of brain electrical activity (EEG) (Figure 3). The experiment was continued only if the brain showed relatively normal electrical activity after isolation. Pump arterial pressure and EEG were monitored continuously following isolation. Brain temperature, measured with a flexible thermistor inserted between the olfactory bulbs, was transcribed onto the polygraph record.

Following isolation, 2 sets of simultaneously drawn arterial and venous blood samples were taken at 30 and 15 minutes prior to anoxia to obtain control values for glucose and oxygen consumption and urea, ammonia and lactic acid formation. About 30 minutes after isolation, the brain was perfused for 4 minutes with blood which had been equilibrated with a nitrogen-carbon dioxide mixture to reduce the oxygen tension to near zero while
maintaining the carbon dioxide tension near 40 mm Hg. Simultaneous arterial and venous blood samples were drawn every 2 minutes during anoxia and at 2, 4, 15, and 30 minutes following anoxia. Frozen biopsy samples were removed for analysis of brain adenosine triphosphate (ATP) and creatine phosphate (GrP) in one group during anoxia, in a second group 10 minutes after anoxia (when the EEG returned) and in a third group 60 minutes following anoxia.

Blood glucose was assayed enzymatically by a modification of the method of Meller and Dahlquist (1966). Lactic acid was measured by the method of Olson (1962). The pO2 was determined by use of a polarographic electrode. Urea formation was measured by the method of Rosenthal (1957). Blood ammonia was determined colorimetrically by a modification of the ion exchange method of Forman (1962). Brain ATP was determined by a modification of the method of Ingel (1962) and the GrP by the method of Stone (unpublished). The brains were removed and weighed at the termination of each study.

RESULTS

The control oxygen uptake in these studies is about 70% of the normally accepted figure due to the depressing effects of sodium pentobarbital anesthesia on cerebral metabolism and oxygen uptake. There does seem to be an oxygen debt due to anoxia, but this appears to be made up during the first 2 minutes following anoxia since oxygen uptake is subnormal at 4 minutes and normal for the remainder of the 30 minute period (Figure 4).

Release of lactic acid by the brain does increase during the first 2 minutes of anoxia, but the rate of lactic acid release by the brain is lower than the control values for the remainder of the study. Posner and Plum (1967) have shown that the amount of lactic acid released

Fig. 4. The uptake of oxygen and glucose and the evolution of lactic acid by the isolated dog brain before, during and after anoxia.

into the blood by the brain is not necessarily a good indication of the quantity of lactic acid in the cerebral tissue and these data would bear out this observation. The brain must metabolize the excess lactic acid formed during this short period of anoxia rather than release it into the blood.

The uptake of glucose by the isolated brain seems to increase slightly during anoxia and decrease slightly during the first 4 minutes following anoxia, but neither set of values differ significantly from the control values. The decrease in glucose uptake at 15 and 30 minutes following the period of anoxia is significant and probably demonstrates preferential catabolism of the lactic acid accumulated in the brain during the period of anoxia.

An unusual feature of anoxic perfusion is the increased liberation of nitrogenous waste products by the brain (Figure 5). Urea is normally taken up or given off in small amounts by the brain during perfusion, but the brain releases the nitrogen equivalent of about 1.8 mg of amino
EFFECT OF ANOXIA ON METABOLISM OF THE DOG BRAIN

Fig. 5. The evolution of urea and ammonia by the isolated dog brain before, during and after anoxia.

acid/min/100 grams of brain during the second 2 minutes of anoxic perfusion and continues to give off urea at about 50% of that rate for the remainder of the study. The brain changes from the evolution of small quantities of ammonia prior to anoxia to the evolution of sizeable quantities of ammonia during and following anoxia, a factor believed to indicate that some proteolysis is taking place.

<table>
<thead>
<tr>
<th></th>
<th>Control Non-isolated brains</th>
<th>Isolated brains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 2.5 min. anoxia</td>
<td>10 min. post anoxia</td>
</tr>
<tr>
<td>AMP</td>
<td>0.04±0.05</td>
<td>0.50±0.13</td>
</tr>
<tr>
<td>ADP</td>
<td>0.43±0.06</td>
<td>0.58±0.09</td>
</tr>
<tr>
<td>ATP</td>
<td>2.02±0.12</td>
<td>1.59±0.21*</td>
</tr>
<tr>
<td>GrP</td>
<td>2.70±0.29</td>
<td>3.36±0.31*</td>
</tr>
</tbody>
</table>

All values in pM/gram of brain tissue
* Significant at 5% level compared to controls
# Significant at 1% level compared to controls

The adenine and creatine phosphate content of the brain was used to evaluate the state of energy metabolism before, during and after anoxia. There is no significant difference between the control values and either the 10 or 60 minute recovery values for brain high energy phosphate content. The 10 minute sample probably has slightly less ATP and GrP than normal, but it would require the analysis of many more specimens to demonstrate this abnormality. During anoxia there is a slight reduction in brain ATP which is significant at the 5% level and a more profound reduction in brain GrP which is significant at the 1% level.

DISCUSSION

The brain continues to take up glucose at about the normal rate during anoxic perfusion, but not enough glucose is taken up to provide the normal level of energy by the less efficient anaerobic metabolic pathway. The increased release of nitrogenous products of metabolism indicates that a larger than normal part of the brain's energy is derived from the breakdown of amino acids within the brain. Since there are more free amino acids than glucose in the brain, they would provide a logical source of energy. The amino acid breakdown accounted for by release of urea and ammonia during anoxia would not provide anywhere near the normal level of energy for the brain, however, there is no reason to believe that ammonia and urea leave the brain more rapidly than lactic acid. One may have to determine the urea and ammonia released by the brain for an extended period to quantitate amino acid catabolism during the period of anoxia. Obviously the role of the lipids in anoxia has been ignored in this study.

The brain does not release large amounts of lactic acid during or after anoxia despite the fact that lactate must be accumulating at 10 to 20 times the normal rate. Since the brain often has a substantially higher lactic acid level than the
blood, it probably consumes energy to retain this gradient and any great outpouring of lactic acid may signal loss of cellular integrity and irreversible brain damage.

The accumulation of lactic appears to be the most dangerous aspect of cerebral anoxia, since aerobic glycolysis in the brain is said to be severely curtailed below pH 6.6. The brain apparently protects itself oxygen is restored by deriving the major portion of its energy requirement from accumulated lactic acid rather than from glucose.

While factors such as species variation and the presence of a barbiturate anesthetic may effect high energy phosphate levels observed in this study, it does appear that the ATP levels do not drop as precipitously during pure anoxia as the results of other investigations might indicate (Dahl and Balfour, 1964). Most other studies have been complicated either poorly controlled anoxia or inadequate sampling techniques both of which tend to reduce ATP levels somewhat.

The reductions in brain ATP and Grp point to a slowing of brain energy metabolism during anoxia. This impression is reinforced by the chance observation that brain temperature is reduced slightly in the course of anoxic perfusion. The uptake of oxygen by the isolated brains in these studies was sufficient to form approximately 7 μM of high energy phosphate per gram of brain per minute if glucose was the substrate. The isolated brains lost an average of 1.77 μM of high energy phosphate per gram of brain during the mean 2.5 minute period of anoxia or about 0.7 μM gram of brain per minute. Unless the energy requirements of the brain are severely curtailed, anaerobic metabolism of the substrates available from the blood and within the brain may be capable of providing up to 90% of the energy necessary for sustaining the brain during anoxia. The relatively rapid recovery to normal ATP and Grp levels together with the normal EEG activity indicate that 4 minutes of uncomplicated anoxia is well tolerated by the brain.

There does not appear to be any direct relationship between the amount of ATP in the brain and the brain’s electrical activity. Whatever the reactions involved in causing the rhythmic changes in brain potential, they are dependent on the availability of oxygen, since 10 to 15 seconds of anoxic perfusion that depletes the oxygen reserve usually results in an isoelectric EEG while the ATP levels are only slightly diminished.

Acknowledgments: 1. Supported in part by Grants NB05-961 from the National Institute of Neurological Diseases and Blindness and R-195-66 from the United Cerebral Palsy Research and Education.

2. The authors thank Mrs. Martha Davis, Mr. Wilbert Heiman, Mr. Alton Mitnour, Miss Elisabeth Osborn and Mrs. Joanne Overman for their assistance during these studies.

BIBLIOGRAPHY


