Cerebral Energy Metabolism After Subarachnoid Hemorrhage

BY JACK M. FEIN, M.D.*

Abstract:
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The purpose of this study was to determine the primary effects of subarachnoid hemorrhage (SAH) on cerebral oxidative metabolism and energy balance. Rhesus monkeys were prepared so that cerebral metabolic consumption rates of oxygen, glucose and the lactate/pyruvate ratios of CSF were estimated after isobarically and hyperbarically induced SAH. A regional analysis was performed on brain sections after similarly induced SAH in the rat, for levels of tissue, glucose, lactate, pyruvate, ATP, ADP, AMP, PCr and the partition of hexokinase between soluble and mitochondrial bound forms. The presence of intracranial hypertension was associated with an immediate ischemia, and increased cerebral glucose extraction was initially noted. Specific tissue substrate concentrations in rat brain indicated that fresh intracisternal hemorrhage is associated with decreased glycosis at a time when high energy phosphate levels are normal. It is concluded that intracranial hypertension hastens the onset of ischemia after SAH. SAH alone produces a decrease in cerebral energy requirements which secondarily depresses the rates of consumption of energy yielding substrates. The depression in metabolic rates may be mediated through a brain stem mechanism.

Additional Key Words subarachnoid hemorrhage regional brain metabolism cerebral oxidative metabolism

Introduction

Recent studies of oxidative metabolism have clarified the relationship between nutritional supply and cerebral energy requirements after ischemic and anoxic insults. Disturbances of cerebral energy metabolism also may be responsible for the clinical sequelae of subarachnoid hemorrhage (SAH), yet, at present there is little available information in this regard. Cerebral blood flow is normally under the influence of cerebral metabolic requirements, but after SAH, spasm may render the microcirculation insensitive to alterations of cerebral energy needs.

The purpose of this study was to determine the effects of SAH on brain energy metabolism. A series of experiments was performed using the rhesus monkey, during which the average cerebral metabolic consumption of glucose and oxygen were measured in relationship to other parameters of developing ischemia. It became apparent that the presence of intracranial hypertension significantly modified the sequence of hemodynamic events. Thereafter, a regional metabolic analysis was performed in the rat, with normal intracranial pressure (ICP), to characterize the disturbances of regional energy metabolism induced by the presence of arterial blood in the subarachnoid space.

Methods

Adult rhesus monkeys weighing 3.5 to 4.5 kg were intubated, paralyzed with d-tubocurarine 0.25 mg per kilogram, and maintained on a 65% to 75% nitrous oxide/25% to 35% oxygen mixture. Arterial Pco2 was maintained between 34 and 40 mm Hg, arterial pH between 7.370 and 7.410 units, and arterial Po2 between 100 and 130 mm Hg. Aortic blood pressure was monitored by a PE 90

Neurological Sciences Division, Armed Forces Radiobiology Research Institute, National Naval Medical Center, Bethesda, Maryland 20014.

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Present address: Department of Neurological Surgery, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.

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catheter passed transfemorally and connected to a P-23-Db Statham transducer. Cisternal pressure was monitored and controlled by a double lumen scalp vein needle, one arm of which was connected to a P23V Statham transducer. Plasma glucose levels were maintained between 120 and 152 mg % by 5% dextrose and saline solution administered by intravenous infusion at a rate of 2 to 3 cc per kilogram per hour. Hydrogen gas then was administered by inhalation until saturation of the brain was achieved. Its exponential washout was then detected polarographically by a single platinum-platinum electrode placed in the distal end of the sagittal sinus. Average total cerebral blood flow was obtained by resolving the biexponential decay into its initial weights and half times. A-V glucose, A-V lactate/pyruvate ratios were determined. Lactate concentrations in CSF and serum were estimated spectrofluorometrically according to Bergmeyer. CSF pyruvate was determined according to the method of Lowry et al.

After control studies, the animals were divided into three groups:

1. Six animals underwent isobaric injection (ICP 5 to 7 mm Hg) of 0.50 cc of fresh isologous arterial blood into the cisterna magna (isobaric cisternal).

2. Six animals underwent bilateral trephination and unilateral isobaric injection of 0.50 cc of isologous arterial blood into the subarachnoid space over the right or left cerebral convexity (isobaric convexity).

3. Seven animals underwent the hyperbaric (ICP 20 to 24 mm Hg) injection of 0.50 cc of isologous arterial blood into the cisterna magna (hyperbaric cisternal). Serial blood flow and metabolic consumption studies were then performed up to 12 hours after induced SAH in all three groups.

A regional analysis of energy metabolism then was carried out using Sprague-Dawley rats weighing 200 to 300 gm. After brief ether induction, a tracheostomy was performed and arterial blood gases were controlled with closed ventilation using an anesthetic mixture of nitrous oxide/oxygen and curare. Arterial pH was maintained between 7.377 and 7.415, arterial Po2 varied between 100 and 140 mm Hg, and arterial Pco2 was maintained between 34 and 42 mm Hg. Aortic blood pressure and cisternal pressure were monitored as previously described. Plasma glucose levels were maintained between 125 and 144 mg % by a 5% dextrose and saline solution administered intravenously at a rate of 2 to 3 cc per kilogram per hour. Fresh homologous cardiac blood (0.25 cc), taken from a donor rat, was slowly injected into the cisterna magna. In 47 rats, cisternal pressure was maintained between 3 and 6 mm Hg after SAH by withdrawing 0.5 to 1.8 cc of bloody CSF. At selected intervals, both before (n = 5) and after (n = 19) induced SAH, substrate metabolism was interrupted by decapitation into liquid nitrogen. Representative chips of cerebrum, cerebellum, brain stem and basal ganglia were weighed and extracted in 3 M HC104 and neutralized with 2 M KHCO3.

The supernatant was assayed for levels of tissue glucose, lactate, pyruvate, phosphocreatine (PCr), adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP), according to methods described by Lowry et al. The energy charge potential (ECP) which describes the ratio of high energy phosphates to the total adenine nucleotide pool was calculated according to Atkinson as:

\[ \text{ECP} = \frac{\text{ATP} + 0.5 \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}} \]

Seventeen rats, in whom SAH also was induced, were killed by decapitation. Representative specimens of cerebrum, cerebellum, basal ganglia and brain stem were removed over ice and homogenized in 10 volumes of 0.25 M sucrose. Six rats were similarly prepared but SAH was not induced. The distribution of soluble, bound and latent hexokinase in brain homogenates was determined in control and SAH animals by the spectrophotometric determination of Knutt et al.

### Results

#### CEREBRAL METABOLIC CONSUMPTION OF SUBSTRATES

Mean values for three groups of monkeys did not differ significantly. Mean cerebral blood flow (40.6 cc/100 gm per minute), cerebral metabolic consumption rates for oxygen (3.7 cc/100 gm per minute), and glucose (5.9 cc/100 gm per minute), and lactate/pyruvate ratios (11.2) were obtained by pooling the means of all three groups. The changes in these values in subsequent measurements are expressed as percentage change from control measurements.

1. In the isobaric cisternal group (fig. 1), the metabolic consumption rates (CMR) of oxygen and
glucose decreased over the first hour after hemorrhage. The depression in cerebral blood flow was delayed for approximately two and one-half hours after SAH. The CSF lactate/pyruvate ratios remained unchanged until the fourth hour, although the concentration of both substrates promptly increased after SAH. Eventually, ischemic changes indicated by a depressed cerebral blood flow rate (fig. 2), and progressively increasing CSF lactate/pyruvate ratios were noted (fig. 3).

2. In the isobaric convexity group, CMR_{O_2} remained normal until ten hours (fig. 4), and CMR_{glucose} was transiently elevated. CBF remained at normal levels for six hours after SAH. However, evidence of ischemia was present earlier with a mild but persistent elevation of CSF lactate immediately after SAH (fig. 3).

3. In the hyperbaric cisternal group, the mean perfusion pressure was approximately 20 mm Hg lower than in the previous two groups. Loss of autoregulation was evident in the early decrease of CBF (fig. 5). The CMR_{O_2} was maintained near con-
Cerebral blood flow and metabolism in the isobaric convexity group. There was no detectable increase in average CBF after focal deposition of blood until ten hours after SAH. An increase in glucose extraction after four hours may reflect the presence of a mild degree of ischemia.

SAH TIME (HOURS)

FIGURE 4

The hexokinase distribution in normal brain was in favor of the bound form in cerebrum (63.6%), cerebellum (58.2%), and basal ganglia (58%). In the brain stem the relative amount in the bound form was consistently between 40% and 50% with a mean value of 43.5%. After SAH significant solubilization of hexokinase activity was noted in the cerebrum and cerebellum (P < 0.01, P < 0.01) lasting for three to four hours, after which an increased binding to mitochondria was present. By five hours 100% of the crude enzyme was bound to mitochondria in both regions. A slight solubilization of hexokinase activity was present in the basal ganglia for the first hours after SAH, following which binding returned to control values. An increased binding of hexokinase was noted in the first sample taken after SAH from the brain stem. Following this initial rise, the degree of binding was similar to control values for the remainder of the study (fig. 6C).

Mean lactate levels for individual regions in control animals were between 1.7 and 2.1 mM/kg/ww and pyruvate levels ranged between 8.2 and 11.7 mM/kg/ww. There were no significant changes in the lactate/pyruvate ratio of any of the regions examined for up to three hours after SAH (fig. 6B). The absolute lactate levels increased slightly in samples from brain stem. However, these were accompanied by corresponding rises in pyruvate levels. At four hours after SAH, tissue lactate increased 43% in cerebrum, 56%
Cerebral blood flow and metabolism in the hyperbaric cisternal group. Intracranial pressure was allowed to rise after injection of blood and the decreased cerebral perfusion pressure was sufficient to cause a 40% to 60% decrease in inflow between one and six hours after hemorrhage and barely perceptible flow values thereafter. After a transient increase in anaerobic metabolism, both oxygen and glucose were depressed. Only one study was performed at 12 hours.

in cerebellum and 58% in basal ganglia. These concentrations remained elevated through the period of observation, while pyruvate levels remained near control values. The overall lactate/pyruvate ratio increased (P < 0.001) from control values to 53 in cerebrum, 65 in cerebellum and 107 in basal ganglia. The rise in brain stem lactate/pyruvate ratio was of questionable significance (P < 0.1).

In control animals phosphocreatine concentration in cerebrum was 15 mM/kg/ww, cerebellum 1.63 mM/kg/ww (P < 0.1), brain stem 2.1 mM/kg/ww (P < 0.01) and basal ganglia 1.3 mM/kg/ww (P < 0.01). After SAH an initial decline of PCr to 40% of control values was noted in brain stem. This level was maintained until the fourth hour of SAH at which time a further drop was noted. After this period PCr was no longer detectable in brain stem. By contrast, PCr concentrations in cerebrum and cerebellum were unchanged through the second hour after SAH following which these levels progressively decreased (fig. 6D). There were no appreciable changes in phosphocreatine concentration throughout the period of observation within the basal ganglia.

Energy charge potential was calculated from the individual concentrations of adenine nucleotides and expressed in arbitrary units. Absolute levels of nucleotides varied in regional studies in the steady state condition, but the ratios of ATP, ADP and AMP remained approximately 110, 10, and 1. The energy charge potential for cerebrum was 0.951; for cerebellum, 0.933; for basal ganglia, 0.946; and for brain stem 0.961. There was no marked change in any of these values until four hours after SAH. At that time, the energy charge potential of cerebral sections progressively declined until levels of 0.690 were
Regional metabolic changes after isobaric cisternal SAH. Areas of the brain are identified as follows: cerebrum = open circle; cerebellum = closed circle; brain stem = open square; basal ganglia = closed square. A. Tissue glucose levels are expressed as percentage change from control values. The rise in mean values through the fourth hour are not significant (P > 0.1). Between the fourth and sixth hours glucose dropped to barely detectable levels in cerebrum. There was no appreciable change in the other regions (P > 0.1). B. Lactate/pyruvate ratios were between 5.2 and 11.7 in control animals. The balance between aerobic and anaerobic glycolysis in the preischemic period is noted. C. The percentage of crude hexokinase activity bound to mitochondria decreased in cerebrum, cerebellum and brain stem during the preischemic period. The onset of complete binding at four hours is consistent with an increased rate of glycolysis. D. Phosphocreatine levels remained at control levels until four to six hours. These levels became imperceptible in brain stem after six hours, in cerebellum after eight hours, and in cerebrum after ten hours. E. Energy charge potential which reflects the relative levels of high energy phosphates was maintained until PCr levels were significantly depressed. The maintenance of both energy sources in basal ganglia is noted.
reached at eight hours. The energy charge potential of cerebellum decreased more slowly and remained above 0.770 at the termination of the study. There was a transient decline in energy charge potential of brain stem between six and eight hours and only a minimal decline in energy charge potential of the basal ganglia at six hours after SAH.

Discussion
When intracranial pressure is increased by infusing saline into the subarachnoid space, average hemispheric blood flow remains unchanged as long as cerebral perfusion pressure remains above 35 to 40 mm Hg. Despite biochemical evidence of a shift to anaerobic metabolism during moderate intracranial hypertension, Siesjo has shown that energy balance is well maintained at these levels of perfusion pressure. After hyperbaric cisternal SAH, however, a 20 mm Hg decrease in cerebral perfusion pressure was sufficient to provide a dramatic change in cerebral blood flow. The altered redox state of the brain was reflected in an early rise of the CSF lactate/pyruvate ratio. This chemical evidence of ischemia was probably related to changes in the biomechanics of blood flow rather than to any primary alteration of brain metabolism by subarachnoid blood.

To study the latter relationship, it became necessary to assure a constant perfusion pressure, which in the model used here is mainly dependent on the control of intracranial hypertension. When that variable was adequately controlled, hemodynamic and metabolic effects of SAH were primarily related to the site of hemorrhage. On postmortem examination a predilection of the convexity hemorrhage for the ipsilateral side was noted, whereas intracisternal hemorrhage produced more diffuse staining of the subarachnoid space. Convexity hemorrhage alone did not produce a significant decrease of CBF although a slight increase in CSF lactate/pyruvate ratios was found.

Cisternal SAH caused an immediate fall in cerebral metabolic consumption rates of oxygen and glucose, largely due to a decrease in extraction rates of these substrates. The decreased extraction rates, however, may be related to a transient decrease in energy requirements or to pathological alterations of the metabolic machinery commonly seen after cerebral infarction.

The regional metabolic survey indicated that early after isobaric cisternal SAH in the rat there is a primary depression of oxidative metabolism unrelated to ischemia. Glucose supply was adequate in relation to its utilization, as evidenced by the normal tissue glucose concentration, and corresponds to the early period during which, in the monkey, substrate extraction is depressed. PCR levels as well as energy charge potential were near normal values and therefore rule out significant ischemic pathology. The braking system in this early or preischemic period is probably intimately related to the relatively small redistribution of hexokinase activity. These moderate changes in hexokinase activity noted after SAH may have a profound influence on gluolytic activity in the brain. Kinetic evidence derived from studies of Fromm and Copley indicates that the soluble and mitochondrial bound enzymes have different Ki values for glucose-phosphate with the Ki of the soluble form approximately five times lower than that of the mitochondrial form. It appears likely that a small redistribution of brain hexokinase can indeed contribute to the control of carbohydrate metabolism at the level of hexose phosphorylation, as had been proposed earlier by Wilson. While these observations confirmed the enzymatic and chemical rearrangement consistent with low energy turnover state, the mechanism by which blood in the subarachnoid space slows energy consumption and production remains unclear.

A direct toxic effect on neuronal metabolism is probably ruled out by the restriction of blood to the subarachnoid space. The selective depression of metabolism produced by cisternal injection suggests that a brain stem mechanism for the control of energy metabolism may have been involved.

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