Cerebral Uptake of Glucose and Oxygen in the Cat Brain After Prolonged Ischemia

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SUMMARY In cats, total cerebral ischemia of one hour's duration was produced by arterial clamping and lowering of the blood pressure. Following ischemia the brains were recirculated with blood for various periods between 30 minutes and six hours. CBF and uptake of O₂ and glucose were determined and correlated with the electrophysiological recovery of the brain.

In four of 20 cats recovery was absent because of respiratory or circulatory insufficiency. In 16 cats signs of progressive electrophysiological function returned. These animals exhibited postischemic hyperemia; electrical excitability of the brains recovered within 15 minutes, and the adenylate energy charge returned to more than 95% of the preischemic value within 30 minutes. O₂ uptake was significantly reduced for about one hour, but returned to or above normal as soon as spontaneous ECoG activity began to reappear. Glucose uptake was initially in the normal range until the cerebral glucose stores were replenished, but then followed a time course similar to that of O₂.

It is concluded that aerobic glycolysis may return after one hour's ischemia if the brain is adequately resupplied with glucose and O₂.

Introduction

WHEN THE BLOOD CIRCULATION and oxygen delivery to the body are arrested, the energy demands of the tissues must be covered by anaerobic metabolism. In the brain this is accomplished by utilizing the available stores of glucose and glycogen which are rapidly metabolized to lactate. However, the energy reserves are so small, and the anaerobic energy yield of glucose so low, that energy metabolism and hence all energy-dependent physiological and metabolic processes break down within a few minutes.

It has been recognized that suppression of energy metabolism is rapidly reversible after complete ischemia of at least one hour, although physiological functions and complex metabolic processes, such as spontaneous EEG activity or protein biosynthesis, are inhibited for considerably longer. The activity of energy metabolism is frequently expressed in terms of the adenylate energy charge, i.e., the relative amount of phosphorylated compounds in the adenylate pool. Following complete ischemia of 15 minutes, the energy charge may return to 99%, and after one hour of ischemia to 93%, even when severe functional suppression is still apparent.

A high energy charge, however, may overestimate the efficiency of energy metabolism as long as the energy demands of the tissue are reduced. The knowledge of the metabolic rate of substrates involved in energy metabolism is of interest, therefore, in determining the actual activity of energy metabolism. In the present paper cerebral uptake of oxygen and glucose was measured at different intervals following total ischemia of one hour in the cat brain, and the results obtained were correlated with electrophysiological recovery and the restoration of energy-rich compounds in the brain tissue.

Methods

Twenty-six adult cats of both sexes, weighing between 2 and 3.5 kg, were used. The animals were anesthetized by a single intraperitoneal injection of pentobarbital sodium (Nembutal®, 30 mg per kilogram), immobilized with gallamine triethiodide (Flaxedil®, 10 mg per kilogram), and ventilated artificially with room air. The respiration was adjusted to yield a Paco₂ of 28 to 30 mm Hg and a Pao₂ of more than 100 mm Hg. Blood pressure, temperature, tidal O₂ and CO₂, blood gases and serum electrolytes were monitored and kept within physiological limits.

The animals were divided into the following groups: three cats were sham-operated controls, three cats were submitted to one hour's complete cerebral ischemia without blood recirculation, and 20 cats were recirculated after one hour's complete ischemia for various predetermined periods from 30 minutes to six hours.

Ischemia was produced by intrathoracal clamping of the innominate and right subclavian arteries, i.e., by interrupting the blood supply to both carotid and both vertebral arteries. A collateral flow to the brain was prevented by ligating the internal mammary arteries and lowering the systolic blood pressure to below 80 mm Hg by the infusion of a ganglion-blocking agent (camphor sulphonate, Arfonad®). Completeness of ischemia was ascertained in the following way. Xenon was injected into the innominate artery immediately prior to ischemia and, using extracranial scintillation detectors, radioactivity in the head was recorded. The clamping was performed when the indicator reached the brain, and interruption of blood flow was considered to be complete only when cerebral radioactivity remained constant throughout the 60 minutes of ischemia.

During recirculation after ischemia the systolic blood pressure was raised to more than 180 mm Hg by the infusion of a sympathomimetic drug (norfenefrin, Novadral®), and the derangements of acid-base balance and electrolytes were corrected by the controlled infusion of buffers and electrolytes. Postischemic coagulopathy was treated with heparin, and hyperglycemia with insulin. Electrophysiological function of the brain was monitored by recording the electrocorticogram (ECoG) and the pyramidal response (PR) to the electrical stimulation of the sensorimotor cortex. The latter is a sensitive method for the assessment of electrical and synaptic excitability of cortical neurons.

For the determination of cerebral oxygen and glucose net uptake blood samples were withdrawn from the femoral...
artery and the sagittal sinus. In the latter case a small catheter with a tapered tip was introduced through a small incision in the dorsal wall of the sinus and directed toward the sinus confluence. Arterial and venous blood was sampled at an interval of two minutes in order to compensate for the mean transit time of the blood through the brain. Glucose content of the blood was determined by enzymatic analysis, and O₂ content with a direct-reading oximeter (Lex-O₂-Con M, Lexington). Cerebral blood flow (CBF) was measured by a modification of a venous sampling technique following 133xenon bolus injection into the innominate artery. Blood samples were withdrawn from the sagittal sinus at short intervals, and clearance curves were obtained by plotting radioactivity of 133xenon contained in each blood sample against the interval after injection. CBF was calculated by compartmental analysis of the initial ten minutes of desaturation and expressed as the weighed means of fast and slow flows. Hemoglobin was determined during each blood flow measurement, and the corresponding blood-tissue partition coefficients were taken from a table by Høedt-Rasmussen. Cerebral net uptake of O₂ and glucose was determined from the products of CBF and cerebral arteriovenous difference of O₂ and glucose, respectively. Availability of glucose and O₂ was calculated from the products of CBF and the respective arterial concentrations.

At the end of the experiment and with the animal under continuous ventilation, one hemisphere was exposed and freeze-clamped in liquid nitrogen, as described before. Glucose, glycogen, lactate, pyruvate, adenine monophosphate, diphosphate and triphosphate, and creatine phosphate were determined in the acid-soluble fraction by specific enzymatic analyses (for details 11).

**Results**

The functional state of the brains during and after one hour's ischemia was monitored by neurophysiological recording. Electrophysiological recovery was defined as the successive return of electrical excitability of cortical neurons within 15 minutes, synaptic excitability within 45 minutes, and spontaneous electrocortical activity within three hours after ischemia (fig. 1). Recovery was considered to be absent when these criteria were not fulfilled. The results obtained in animals with or without recovery will be described separately below.

**Animals With Electrophysiological Recovery After One Hour's Ischemia**

This group consisted of 16 animals. Prior to ischemia CBF was 69.7 ± 2.4 ml/100 gm per minute (mean ± SE). Upon recirculation after one hour of ischemia, CBF transitarily increased by more than 40% and then returned to or below normal after one hour.

Cerebral uptake of O₂ before ischemia was 5.3 ± 0.26 ml/100 gm per minute. During the initial 30 minutes of recirculation, O₂ uptake was less than 50% of the preischemic value (fig. 2). This was a result of reduced oxygen utilization of the brain and not of reduced oxygen availability which, in fact, was increased above normal during this time. As soon as hyperemia ceased, oxygen uptake began to increase and returned to, or even above, control value after two to three hours.

Changes in glucose net uptake after ischemia were triphasic (fig. 2). The control value before ischemia was 6.21 ± 1.44 mg/100 gm per minute. With the onset of recirculation, uptake was normal, but this was followed by a significant suppression after 30 minutes. Thereafter, glucose uptake again increased and reached a maximum of 16.60 ± 5.08 mg/100 gm per minute after three hours before it returned to the normal level after five hours of recirculation. In contrast, changes in glucose availability were monophasic (fig. 2). Following ischemia the glucose content of the arterial blood increased from 127 to more than 200 mg % and remained elevated throughout the remaining period of recirculation. Glucose availability, as a consequence, was...
maximal shortly after ischemia during the phase of post-ischemic hyperemia, i.e., at a time when glucose net uptake was severely suppressed.

The results of the biochemical analysis of the brains are summarized in figure 3. After one hour of ischemia, the reserves in energy-rich compounds were almost exhausted, the energy charge of the adenylate pool had fallen from 0.90 to less than 0.1, and the lactate/pyruvate ratio had increased from 14.4 to more than 700. When the brains were recirculated, glucose and pyruvate transitorily increased to supranormal levels, whereas lactate gradually decreased. Adenosine triphosphate and phosphocreatine returned to approximately two-thirds of the control value within 30 minutes, but very slightly improved at longer recirculation times. The adenylate energy charge, however, reached values as high as 0.87 after 30 minutes, which indicates that the energy supply and energy needs of the tissue were almost balanced. This is also suggested by the decrease in the lactate/pyruvate ratio which reflects the improvement in the cytosolic redox state of the brain.

The recovery of electrophysiological function correlated well with the metabolism. The restoration of the adenylate energy charge was accompanied by the return of electrical excitability, and the normalization of cerebral uptake of O$_2$ and glucose by the reappearance of spontaneous electrocortical activity. However, despite the high O$_2$ consumption, neurological function did not return and a direct relationship between the latter and the tissue concentrations of energy-rich compounds was not found.

**Animals Without Recovery**

Four animals showed no signs of progressive functional recovery. In two of these, postischemic hyperemia was absent and cerebral O$_2$ uptake remained below the pre-ischemic value. Glucose net uptake, on the other hand, came close to the normal, which suggests that some anaerobic glycolysis was maintained during recirculation. In the third case, postischemic hyperemia was present and electrophysiological as well as metabolic recovery proceeded in a normal way for two hours. Thereafter, blood flow decreased and the already recovered pyramidal response gradually disappeared together with a secondary suppression of O$_2$ and glucose net uptake. In the fourth case, the arterial Po$_2$ was below 50 mm Hg owing to severe respiratory insufficiency. Initially the pyramidal response returned, but the biochemical analysis revealed considerable lactacidosis (27.8 mmol per kilogram), a low adenylate energy charge (0.69), and a relatively low glucose level (3.62 mmol per kilogram). These findings are in agreement with the earlier conclusion that successful cerebral recirculation and reoxygenization are main factors contributing to the recovery of cerebral metabolism following ischemia.$^14$

**Discussion**

In the intact brain, energy for the maintenance of metabolic and physiological function is derived almost exclusively by aerobic metabolism of glucose absorbed by the brain from circulating blood. The present experiment was designed to investigate whether aerobic glycolysis is restored after prolonged cerebral ischemia, and to what extent electrophysiological recovery depends upon it. Measurements were performed by sampling venous blood from the sagittal sinus close to the sinus confluence. At this site, blood from the whole brain should be collected but the relatively high control values for blood flow and glucose uptake indicate that sampling concerned preferentially the cerebral cortex.

A precise evaluation of the metabolic rate of glucose or O$_2$ from the products of flow and arteriovenous concentration differences is possible only when flow and metabolism are at a steady state during a period which is at least as long as the longest transient time of blood flow through the tissues.$^{16}$ In the present experimental situation blood transient times were much faster than the postischemic changes in blood flow or uptake of glucose and oxygen; thus a near steady state existed during the time of measurement. However, a considerable error may be introduced when the tissue concentrations of the investigated substrates are not constant. This is not important in the case of oxygen because the oxygen stores of the brain are low, but the changes in glucose were such that they could not be neglected. During the initial 30 minutes of recirculation the glucose content of the brain increased from zero to about 10 mmol per kilogram which would correspond to an average glucose net uptake of 6 mg/100 gm per minute when the increase is linear, or to an even higher value when this concentration is reached after a shorter time. Since the measured glucose uptake during this period was 8 mg/100 gm per minute, most, if not all, of the incorporated glucose must have been used for the replenishment of glucose stores of the brain rather than for metabolism. This interpretation is supported by the fact that
after 30 minutes of recirculation, i.e., when tissue glucose had reached its peak, there was almost no glucose uptake. At longer recirculation times this relationship was reversed: the cerebral glucose concentration gradually decreased, and in consequence glucose net uptake was now slightly less than the actual glucose consumption.

With these considerations in mind, three metabolic stages can be distinguished in the course of recovery after complete ischemia of 60 minutes: an early period of recirculation during which oxygen and glucose consumption rates are suppressed; a second period during which glycolysis is increased; and a third period during which metabolism stabilizes near the preischemic level.

The first period was related to reactive postsischemic hyperemia and lasted up to one hour. Oxygen utilization was reduced by about 60%, and glucose consumption presumably even more in spite of the increased glucose and oxygen availability. During this time spontaneous EEG activity was absent which may explain the low metabolic rate. However, the restoration of the adenylate energy charge and the improvement in the redox state of the brain indicate that factors other than energy failure must be responsible for the functional and metabolic inhibition. The reasons for this inhibition are poorly understood, but postsischemic acidosis, shifts in the ionic balance, and changes in protein and amino acid metabolism may play a role.

The postsischemic suppression of glycolysis was followed by an increase of glucose and O2 consumption above normal after two to three hours of recirculation. This increase may be due to partial uncoupling of oxidative glycolysis but there is also evidence that certain metabolic pathways are activated after ischemia.

The restoration of aerobic glycolysis is surprising with regard to the length of ischemia, and methodological peculiarities of the present experiment should be considered. A collateral flow in the brain during ischemia can be excluded because clearance of 133 xenon was absent, and earlier experiments have confirmed that with the present method ischemia is complete. Protection of the brain by temperature drop is also unlikely because brain temperature decreases only by 0.2°C per minute, and the energy reserves are completely exhausted before the brain becomes hypothermic. There may be some protection by using barbiturate rates for anesthesia but the effect must be small because the drug was given in a single dose about four hours before the induction of ischemia.

On the other hand, our findings are supported by earlier reports of partial recovery after prolonged anoxia or ischemia. Drewes and coworkers found a return of O2 consumption to more than 70% after 30 minutes of anoxia in the isolated brain although cerebral glucose uptake was significantly inhibited. Lang et al., using isolated heads, demonstrated considerable restitution of cerebral metabolic rate of oxygen after 30 minutes of complete ischemia, and Subotka and colleagues had a normalization of the O2 saturation of cortical venous blood even after 60 minutes. Schutz and coworkers, with an in vitro model of isolated mitochondria, reported the reversibility of mitochondrial function after deprivation of O2 and substrates for 30 minutes. Thus, the combined evidence of these authors and the present investigation warrants the conclusion that suppression of aerobic glycolysis after prolonged anoxia is largely reversible when the brain is adequately resupplied with glucose and oxygen.

Four to five hours after recirculation the ECoG had improved further and the metabolic rate of O2 and glucose was in the normal range, but the neurological state of the animals was still severely compromised. This can be attributed to several factors. One is inhomogeneous perfusion of the brain, which would lead to an overestimation of the metabolic activity because arteriovenous differences are calculated from those areas of the brain which are well perfused, whereas the neurological performance depends upon the function of the whole brain. Another factor is the slow restitution of complex biochemical processes. Recent investigations have revealed that the sedimentation profiles of the polyribosomes do not normalize until 24 hours after ischemia, and that total adenylate recovers very slowly despite an increased purine de novo synthesis. Furthermore, postsischemic disturbances in the amino-acid metabolism cause a decrease in those substrates which are excitatory and an increase in others which are known to inhibit neuronal activity.

At present it is not known whether these changes eventually are reversible or not. However, the restoration of oxidative glycolysis shows that the necessary metabolic requirements for recovery are present even after one hour's complete ischemia.

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References

DIPLOPIA AND INVOLUNTARY EYE CLOSURE/Messert et al.

Diplopia and Involuntary Eye Closure in Spontaneous Cerebellar Hemorrhage

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SUMMARY Spontaneous cerebellar hemorrhage is of difficult clinical diagnosis. The causes can be varied, but the hemorrhage is most often associated with hypertensive cardiovascular disease. The neurological symptomatology is complex and often misleading. The diagnosis is mainly dependent on familiarity of the eye signs seen in this disease. Among these, the spontaneous unilateral eye closure is presented as an additional striking manifestation. The displacement of the brain stem by the hematoma is frequently associated with a seventh nerve palsy on the side of the hemorrhage. The patient, in an effort to obviate the diplopia caused by the gaze dissociations and extracocular motor palsy, has only the option to close the eye on the noninvolved side of the face, and thus the eye remaining open is on the side of the cerebellar hematoma. This paper presents reports of two patients with these symptoms.

THE CLINICAL DIAGNOSIS of spontaneous cerebellar hemorrhage is often a difficult one and few antemortem diagnoses are made. Reports by Fisher et al.1 and McKissock et al.2 have done much to describe the clinical aspects of the disease.

Spontaneous cerebellar hemorrhage in the majority of cases is associated with hypertensive vascular disease, and less frequently related to a number of other causes such as arteriogenous malformation, bleeding diathesis, infections of the central nervous system and trauma. The clinical spectrum of signs and symptoms can vary considerably from acute onset with coma and death within a few hours, to subacute and chronic courses simulating brain stem vascular infarctions or posterior fossa tumors.

The subacute cases are those presenting the greatest clinical challenge. The outstanding neurological manifestations are centered in the cranial nerves with diplopia, nystagmus, EOM abnormalities, paresis of conjugate gaze to one side, or even forced conjugate deviation to the other. A decreased corneal reflex on one side is frequently associated with a peripheral facial palsy on the same side.

The headache when unilateral, the facial palsy, the corneal reflex deficit, and the direction of gaze palsy all point to the side of the posterior fossa involvement and are homolateral to the hematoma.

The difficulty in diagnosis seems to reside in the rather unusual and paradoxical absence of expected manifestations. There is often no blood in the spinal fluid obtained by lumbar puncture. Significant neck rigidity is rare. There are few reports of observed papilledema. The pupils remain reactive, cerebellar ataxia, if present at all, is unimpressive and often absent, and often there are no paresis and no sensory change in the extremities. Thus, at this stage of the illness, the eye signs are by far the most characteristic features. The purpose of this report is to focus on the interesting phenomenon of the closure of one eye found early in the course.

This closure of one eye was noted by Fisher et al.1 and termed "involuntary closure of one eye." He thought the left eye was most usually involved and that it reflected brain stem damage. Lichtenstein,2 quoting Fisher, described "apparent ptosis of one eyelid" and stated that this was associated with an internal ophthalmoplegia. Photophobia and/or reflex blepharospasm are interpretations of the eye closure in other case reports.

It is our interpretation that the closure of one eye is only the reflex avoidance of diplopia in these patients who have a marked and complex ophthalmoplegia of acute onset; the side of closure is simply determined by the presence of the associated facial palsy (Case 1). However, when the facial palsy is minimal or even absent, the eye closure can occur randomly on either side (Case 2).
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