Focal Transient Cerebral Ischemia in the Squirrel Monkey

EFFECT ON BRAIN ADENOSINE TRIPHOSPHATE AND LACTATE LEVELS WITH ELECTROCORTICOGRAPHIC AND PATHOLOGIC CORRELATION

By Thoralf M. Sundt, Jr., and John D. Michenfelder

ABSTRACT

Cerebral adenosine triphosphate and lactate concentrations were measured in the squirrel monkey (Saimiri sciureus) before, during, and at selected times after various periods of middle cerebral artery occlusion. The gradual decrease in ATP to 55, 35, and 20% of normal and the increase in lactate to 7, 8, and 10 times normal after 2, 3, and 4 hours of occlusion, respectively, were reversible with restoration of flow. Electrocorticograms recorded in acute preparations indicated potential for recovery. The correlation of lactate levels with previous determinations of blood flow in this preparation supports the theory that loss of autoregulation and "luxury perfusion" in cerebral ischemia results from localized metabolic acidosis due to accumulation of lactic acid. In a separate group of monkeys, the ischemic lesions were found to be largely reversible after 2 or 3 hours and irreversible after 4 hours of middle cerebral artery occlusion; the cause of death in the monkeys subjected to 4 hours of occlusion was edema, which progressed even after restoration of flow.

KEY WORDS

ischemic tolerance cerebral autoregulation cerebral edema luxury perfusion metabolic acidosis

Lassen (1) originally hypothesized the concept of "luxury perfusion" in focal cerebral ischemia and suggested that this, along with a failure in autoregulation, resulted from a "focal metabolic acidosis" related to the intracellular accumulation of lactic acid. In a previous report (2), both the failure of autoregulation and the presence of luxury perfusion were documented in our laboratory preparation of focal cerebral ischemia. However, the status of the tissue's metabolism was not reported.

Although an increase in lactate accompanied by a decrease in adenosine triphosphate (ATP) has been demonstrated in various preparations of generalized, complete cerebral ischemia (3-5), a pattern of reversible accumulation has not been documented in a reliable model of focal, incomplete, transient cerebral ischemia. It is apparent from previous investigations that the tolerance of neural tissue to ischemia parallels the relative decrease in blood flow and is measured in minutes after total circulatory arrest (4) and in hours after occlusion of a single major vessel (6). Many hemodynamic characteristics of total circulatory arrest, such as the no-reflow phenomenon (7), are not present early in focal ischemia, in which there is a reactive hyperemia after restoration of flow (2). Therefore, many biochemical determinations based on total, generalized cerebral ischemia probably cannot be extrapolated to focal, incomplete ischemia.

In the present work to investigate brain metabolism before, during, and after middle
cerebral artery (MCA) occlusion, we compared cerebral ATP and lactate levels after restoration of flow to ischemic hemispheres (11 monkeys) with the levels determined during the period of ischemia (18 monkeys). The rates of ATP depletion and lactate accumulation previously determined for up to a 3-hour period of ischemia in 14 monkeys (8) were recalculated and extended to a 4-hour period by the addition of 3 more monkeys. To facilitate statistical analysis, an additional monkey with 2 hours of MCA occlusion was added. The normal hemisphere was studied in all of these preparations and both hemispheres were studied in 4 additional normal monkeys, providing nonischemic mean control values derived from 37 determinations. Electrocorticograms during ischemia and after restoration of flow, not previously reported in this preparation, were included for correlation with the chemical determinations.

To complement the metabolic studies, which explored only a facet of the dynamic evolving stroke, pathologic evaluation in a group of chronic preparations was included.

**Methods**

**Acute Preparation.**—Squirrel monkeys (Saimiri sciureus) weighing 600–1,200 g were anesthetized with sodium pentobarbital (15 mg/kg, ip). The monkeys were then fixed in a headrest, placed in the prone position, and covered with a heating blanket. The cutting current of an electrosurgical unit was used to reflect scalp and muscle flaps bilaterally. With the aid of an operating microscope, the right MCA was occluded with a miniaturized Mayfield clip through a retroorbital approach; this procedure did not involve retraction or manipulation of the brain itself (9). Next bilateral frontoparietal craniectomies were completed without violation of the dura, using a micropneumatic air drill and an operating microscope. The monkeys were then repositioned, and a catheter was placed in the femoral artery for blood pressure and blood gas measurements. Representative electrocorticograms were recorded from both hemispheres during ischemia in all monkeys. In those monkeys destined for restoration of flow, the occluding clip was removed after the desired period of ischemia.

At selected intervals after restoration of flow, the dura overlying both exposed hemispheres was excised, and bilateral cerebral biopsy specimens were taken simultaneously. In those monkeys used to enlarge the ischemic control group, flow was not restored, and biopsies were taken at an occlusion time of 2 or 4 hours.

**Chronic Preparation.**—Three groups of six monkeys each were prepared after occlusion times of 2, 3, or 4 hours. In these monkeys the surgical procedure consisted of a retroorbital approach with aspiration of the global contents and dissection along the sphenoid wing in the retroorbital space. The dura overlying the optic nerve was incised under the operating microscope, and the arachnoid surrounding the MCA was divided. A miniaturized Mayfield clip was applied for the appropriate time, after which it was removed, and the wound was closed in anatomic approximation. All these monkeys awoke from anesthesia, and their clinical course was recorded until they died or were killed. After death, the brains were removed, fixed in formalin, and photographed; then block hemispheric sections were stained with hematoxylin and eosin and luxol fast blue and examined for histologic changes. Infarct volumes were computed by the average and area method previously described (9).

**Vital Sign Measurements.**—Arterial blood pressures were measured by strain-gauge transducers in the femoral artery catheter. Brain temperatures were monitored with a temporal epidural thermistor, and the monkeys were kept at normothermia by use of small heating blankets. Arterial blood samples were taken for measurements of arterial Pao2, Pco2, pH, and microhematocrit. Immediately following the biopsy, the animals were killed.

**Electrocorticography.**—Electrocorticograms were recorded in all acute animals from electrodes brought into contact with the exposed dura through moist cotton wicks. The temporalis muscle was used for the ground. Tracings were recorded on a Consolidated Electrodynamic Corporation data graph on light-sensitive paper. An Analog Devices 603J differential low-wave, high-gain amplifier with a frequency response specialized for the normal frequency of electrocorticography was used as the intermediate unit (frequency response range, 0.5 to 120 Hz).

Recordings were obtained from both hemispheres in all monkeys at 30-minute intervals after occlusion of the MCA and were continued through the period of restoration of flow in the animals selected for temporary occlusion.

**Biopsy Technique.**—Biopsy specimens were collected simultaneously, immediately after excision of the overlying dura, from both exposed hemispheres at the predetermined time. The visual appearance of the brain was recorded, including the amount of apparent edema. The technique of biopsy was that described by Kramer et al. (10); within 1 second a 100- to
400-mg brain sample was removed and deposited in liquid nitrogen. Although the brain sample usually broke into only two units after it was aspirated and placed in the liquid nitrogen, sometimes a sample broke into many smaller units on contact with the liquid. This phenomenon has never been related to variation in control values, from which we conclude that the sample is uniformly and rapidly frozen regardless of the size and that no artifact is introduced as a result of a gas-phase insulation phenomenon.

Biopsies in the group in which flow was not restored were arranged at approximately 60-minute intervals, and samples were collected up to the time limit of 4 hours of occlusion. Biopsies in the group in which flow was restored were spaced at varying time intervals (20 to 50 minutes), and only a single biopsy of each hemisphere was obtained from an individual preparation. In all instances, the sites of biopsy were identical and corresponded to the central area of infarct previously demonstrated to occur ultimately in this species after permanent occlusion of the MCA (6). In most instances the biopsy was of sufficient depth to expose the lateral ventricles.

**Measurement of Brain ATP and Lactate.**—Each core of brain tissue was handled in a manner intended to minimize the possibility of thawing and artifactual change. In rapid sequence, the frozen brain tissue was removed from the liquid nitrogen, weighed, and, after the addition of 2 ml of cold (0-4°C) perchloric acid (8%), ground for 1 minute with a high-speed tissue homogenizer. During the grinding process, the tissue container was immersed in a dry ice-alcohol slush (−70°C). Thereafter, the homogenate, maintained at a temperature of 0-4°C, was centrifuged, neutralized with potassium hydroxide, buffered, and adjusted to a volume of 10 ml. The ATP concentration was determined by the firefly luminescence method (11-13). The lactate concentrations were determined by standard enzymatic techniques (14).

The brain specimens obtained contained variable small amounts of blood (2-5%), which is a potential source of error. Therefore, erythrocytes from a donor squirrel monkey were tagged with 51Cr and injected into each experimental animal prior to biopsy. The radioactivity of the brain specimens and of a sample of blood from each animal was subsequently measured. These values, along with measurements of blood ATP concentrations, permitted correction for this variable.

**Measurement of Brain Weight for Quantification of Cerebral Edema.**—Immediately after the monkeys were killed, the control and ischemic hemispheres were removed and weighed for wet weight. The hemispheres were then dried in a warm-air oven and reweighed at 24-hour intervals until the weights were constant. The difference between the change of weight in the two hemispheres gave an indication of the relative degree of edema.

**Statistical Analysis.**—Regression equations were calculated by the method of least squares. Significant differences between mean values were tested by Student’s t-test for unpaired data (P < 0.05 considered significant).

**Results**

**Systemic Stability of Acute Preparation.**—Blood loss during the entire procedure was less than 5 ml; it was never necessary to transfuse the animals. Based on the respiratory rate and the degree of spontaneous movements, none of the animals was in an excessively deep plane of anesthesia, and it was not necessary to supplement the original dose of the drug. The monkeys breathed room air spontaneously throughout the period of the experiment, and their respiratory rate averaged 30/min.

Systemic parameters measured are summarized in Table 1 for the 11 monkeys in which the clip was removed and flow restored prior to biopsy and for the 4 monkeys used to extend the regression lines of ATP and lactate to 4 hours. Except for a somewhat higher arterial PO2 and lower pH there was no significant difference between these parameters in the current group of monkeys and in the original group from which the primary 3-hour regression lines were calculated (8). The higher mean arterial PO2 in the monkeys of this study (90 mm Hg vs. 77 mm Hg) is presumably accounted for by the greater

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (±SEM)</th>
<th>Significance</th>
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<tbody>
<tr>
<td>Brain temperature (degrees C)</td>
<td>36.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>92 ± 4</td>
<td></td>
</tr>
<tr>
<td>Arterial PO2 (mm Hg)</td>
<td>90 ± 3</td>
<td></td>
</tr>
<tr>
<td>Arterial PCO2 (mm Hg)</td>
<td>39 ± 3</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>BB+ (mEq/liter)</td>
<td>49 ± 1</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

**BB+ = buffer base.**
elapsed time from administration of pentobarbital until biopsy (clip time + restored flow time) and hence a lighter level of anesthesia. This is not considered an important difference since hemoglobin saturation at either arterial Po$_2$ exceeds 95%. The lower pH (7.41 vs. 7.47), which relates in part to an insignificant difference in arterial Pco$_2$, is unexplained and is believed to be of no consequence. Otherwise, the uniformity in these measurements ensured that the brain samplings were based on highly stable preparations. No animal deteriorated during the period of investigation or appeared to be terminal prior to the sampling.

Cerebral ATP and Lactate Concentrations.—The mean cerebral ATP and lactate values in the normal hemispheres of the monkeys in this study were virtually identical to those previously reported and were $2.00 \pm 0.04$ μmoles/g (SE) and $2.38 \pm 0.27$ μmoles/g, respectively. The slopes of the previous 3-hour regression lines and of the new 4-hour regression lines (Fig. 1) were essentially the same. Individual cerebral ATP and lactate concentrations determined in the 11 monkeys following restoration of MCA flow are shown in Table 2 and compared to individual values previously determined in monkeys without restored flow. These individual values (animals with restored flow only) are also plotted in Figure 1. Without exception, the ATP concentrations in all monkeys with restored flow were above the calculated 4-hour regression line, and in 9 of 11 monkeys the lactate concentrations were below the regression line. The differences between these combined individual values and the combined individual values from which the regression equations had been calculated at 2, 3, and 4 hours were statistically significant. In addition, there was an apparent relationship among the duration of MCA occlusion, the duration of restored flow, and the concentrations of ATP and lactate that suggests a progressive return toward control levels. This relationship is indicated in Figure 1 by the suggested recovery slopes after 30 minutes, 2 hours, 3 hours, and 4 hours of MCA occlusion.

Cerebral Edema.—In most animals, visual inspection of the brain just prior to biopsy
suggested that the hemisphere subjected to MCA occlusion was edematosus, as compared to the control side. This seemed particularly apparent in animals subjected to ischemic periods of 3 hours or more. This impression was supported by the wet and dry weight determinations in that the hemispheres exposed to 3 or more hours of MCA occlusion always contained a greater percent of water than did the control hemispheres, but the differences, although statistically significant, were small (0.5–2.0%). These measurements may be misleading since the edema was presumably focal at this early stage rather than hemispheric, and the area of maximal edema was probably removed at the time of biopsy.

Electrocorticograms.—The electrocorticogram recorded from the nonischemic hemisphere under these experimental conditions consistently showed two basic patterns of electrical activity (Fig. 2). The first pattern consisted of single or serial, high-voltage (300–600 µV), 2- to 5-Hz waves. The second pattern consisted of rhythmic, lower-voltage (80–200 µV), 11- to 14-Hz waves. This second pattern could occur independently of or be superimposed on the first pattern. Additional, more irregular, activity below the 2-Hz range was occasionally seen in the nonischemic left hemisphere. However, this activity was not consistently present.

The electrical activity in the ischemic hemisphere showed a variable degree of change when compared to the nonischemic hemisphere. When the changes were minor, there was only a decrease in amplitude of both of the patterns mentioned above (Fig. 2, animal 4). In the more severe changes, there was total absence of the two types of activity with replacement by lower-voltage, more irregular components.

After restoration of flow to the ischemic hemisphere, there was a gradual improvement in the electrocorticogram. This improvement was progressive with time. There was slow recovery of both of the two basic patterns of electrical activity.

Chronic Preparation.—The results are summarized in Table 3. In general, the clinical course paralleled the autopsy findings. The level of consciousness indicated the relative amount of edema, and the degree of paresis indicated the relative amount of internal capsule involvement by the lesion. In those animals dying from edema or surviving but with
infarcts, histologic changes were always most marked in the region of the insula, the center point of biopsy in acute preparations.

In all the animals with 4 hours of MCA occlusion, death ensued prior to a clearly delineated infarct, usually 16-24 hours after the operation. The major changes were swelling in both the cortex and subcortical white matter in the insula, frontal operculum, and superior temporal gyrus (Fig. 3). Myelin stained poorly in the external capsule but still stained clearly in the internal capsule. The putamen invariably was increased in size, at times approaching a 50% increase in transverse diameter. The globus pallidus usually was spared at this point in the temporal profile of edema. Neurons stained poorly and, although the laminated cytoarchitecture of the cortex was still identifiable, there was widespread vacuolation and pallor of the neuropil. In some animals (those surviving longer), the edema spread in the white matter to involve the deeper longitudinal association tracts and corona radiata.

Small infarcts found in the monkeys with 2 and 3 hours of MCA occlusion involved chiefly the insula, external capsule, frontal operculum, and superior temporal gyrus. In larger infarcts the putamen, head of the caudate, and internal capsule were included. In one monkey only the head of the caudate, putamen, and isolated cortical areas of the insula were involved and the internal capsule was spared (this animal was not hemiplegic when killed). Two of the six infarcts had significant areas of perivascular hemorrhage and vascular engorgement, but in general the infarcts were pale and typical of 5- to 7-day-old infarcts (15). Ischemic necrosis was clearly delineated and polymorphonuclear cells were present in the parenchyma. At the periphery of the infarct, phagocytic cells were present and there was occasional hyperplasia of the capillaries.

**TABLE 3**

Clinical Course and Pathologic Findings in Chronic Preparations

<table>
<thead>
<tr>
<th>Period of MCA occlusion (hours)</th>
<th>No. animals</th>
<th>Clinical course</th>
<th>Autopsy result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6</td>
<td>1, died 6 hours postoperative</td>
<td>Massive edema</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2, mild hemiparesis</td>
<td>Small infarcts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3, no deficit</td>
<td>No infarcts</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>2, hemiplegia</td>
<td>Moderate infarcts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2, hemiparesis</td>
<td>Small infarcts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2, no deficit</td>
<td>No infarcts</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>All died 16-24 hours postoperative</td>
<td>Severe edema</td>
</tr>
</tbody>
</table>

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METABOLISM IN ISCHEMIA

FIGURE 3

Gross sections from three chronic preparations showing, from left to right, effect of 2, 3, and 4 hours of MCA occlusion. No infarct is apparent at 2 hours of ischemia. After 3 hours of ischemia an infarct involves insula, frontal operculum, and superior aspect of temporal lobe; external capsule, basal ganglia, and internal capsule are spared. This animal had a transient hemiparesis but was normal by the time it was killed. After 4 hours of MCA occlusion, massive edema, with a clearly defined shift of midline structures, caused early death; this early death occurred prior to tissue necrosis or delineation of an infarct.

Discussion

The close correlations between the biochemical determinations and electrocorticograms in the acute preparations and between the neurologic evaluations and the postmortem histologic studies in the chronic preparations reinforce the findings of previous investigations (2, 6, 8, 16). It is obvious that there is a difference between physiologic paralysis and cell death. The tolerance of neural tissue to ischemia is related to the severity and duration of the decrease in blood flow to which it is subjected.

Although biologic variation makes it necessary that conclusions be drawn from analysis of the animals as a group and interpretations not be based on observations or findings in single animals, it is important to note that in each preparation there was close correlation between the electrocorticogram and the relative ATP and lactate levels. It was possible, as indicated in Figure 2, to predict from the relative amount of electrocorticogram activity just prior to biopsy the approximate ATP and lactate levels. Those monkeys with a very inactive electrocortical tracing just prior to biopsy had relatively low ATP and high lactate values; in contrast, monkeys with a more nearly normal tracing had relatively high ATP and low lactate values.

Figure 4 illustrates the effect of MCA occlusion for 2 hours, shown by average parameters constructed from the group as a whole. The cerebral cortical blood flow, determined from previous investigations (2) (using $^{85}$Kr and calculated by the kinetic analysis of Zierler [17] with the Geiger tube placed over the area of biopsy in the present
group), decreased after occlusion from levels of approximately 1.6 ml/g min\(^{-1}\) to 20-50% of the preocclusion flow. ATP and lactate curves, derived from Figure 1, show a calculated regression during this period of ischemia that lags considerably behind the changes in blood flow, ATP measuring 1.07 \(\mu\)moles/g (ATP = 1.79 - 0.006 \(\times\) minutes) and lactate measuring 13.6 \(\mu\)moles/g (lactate = 7.6 + 0.05 \(\times\) minutes) after 2 hours of ischemia. Sequential electrocorticograms, representative of tracings in these monkeys, show that the cerebral physiologic function is acutely sensitive to this degree of ischemia, becoming grossly abnormal after occlusion. However, with restoration of flow the electrocorticogram gradually returns toward normal. Although flow remains relatively constant during the early period of ischemia (if systemic blood pressure is stable), ATP continues to decrease and lactate continues to increase. With release of the occluding clip, there is prompt reactive hyperemia or luxury perfusion, and, after restoration of flow, there is a gradual increase in ATP and a decrease in lactate.

The high levels of lactate during the period of ischemia and early after the restoration of flow coincide with a loss of autoregulation and a reactive hyperemia, respectively, in this model. These lactate values are much higher than values reported for models of generalized cerebral ischemia, in which the degree of ischemia is more severe but the duration of lactate accumulation is much shorter (3-5). Differences between the two types of models and clinical situations—cardiac arrest or shock vs. stroke—make comparisons difficult. However, even at lactate levels considerably below those of our study, Siesjö and Zwetnow (5) found an eventual failure of autoregulation in their model of hypovolemic hypotension.

The high brain lactate levels which we observed are not believed to be related to increased blood lactate levels since, in animals not included in this study, we have observed no increase in arterial blood lactate over a 2-hour period after MCA occlusion done under conditions similar to those of this study (Michenfelder and Sundt, unpublished data).

Brain pyruvate levels were determined in the animals in this study but have not been reported since their inclusion, along with the

FIGURE 4

Effect of 2 hours of temporary MCA occlusion, illustrated by parameters from the group as a whole. Refer to text for description of curves and data analysis.
calculated ratio of lactate to pyruvate, adds nothing to the interpretation of the data. Pyruvate concentrations were within the normal range in all samples; hence, the ratio of lactate to pyruvate was normal in the nonischemic hemisphere (mean = 11) and was increased in the ischemic hemisphere to the same degree as were the lactate levels.

The metabolic changes in the ischemic cell are complex, and lactate is only one facet of the problem. Although it is probable that the brain can metabolize part of the lactic acid (18), excessive accumulation could ultimately impair oxidative phosphorylation by creating an intracellular acidosis (1), by acting as a rate-limiting factor, or by changing cellular osmolality. It is possible that cellular lactic acidosis is one of the critical factors related to cell death and that the recovery of tissue ATP is dependent on correction of the acidosis. Lysosomal enzyme release and autolysis follow, in hours (19, 20), these early metabolic changes and therefore are not likely to be related to early cellular swelling or physiologic paralysis.

The cause of cerebral edema in ischemia and after restoration of flow must await future investigation. Perhaps the edema during the period of ischemia and the edema after restoration of flow are from different mechanisms, the former being related to acidosis or abnormalities in the energy-dependent cell membrane and the latter being related to a reactive hyperemia. Should some means be achieved for controlling the edema, the period of reversibility for this model and for clinical stroke might well be extended. Furthermore, as Leaf (21) has recently indicated, the edema of ischemia may be of considerable importance in the heart and the kidney as well as in the brain. Although there are established differences in metabolism in these tissues (22, 23) there may be more similarities than differences in their individual edemas of ischemic origin.

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