Cerebral ATP and Lactate Levels in the Squirrel Monkey Following Occlusion of the Middle Cerebral Artery

BY JOHN D. MICHENFELDER, M.D., AND THORALF M. SUNDT, JR., M.D.

Abstract: Cerebral ATP and Lactate Levels in the Squirrel Monkey Following Occlusion of the Middle Cerebral Artery

Previous studies have shown that occlusion of the middle cerebral artery (MCA) of the squirrel monkey results in a consistent large infarct; that in the initial two hours after occlusion of the MCA, blood flow in the central area of ischemia continues at a reduced rate (20% to 50% of normal); and that restoration of normal flow within three hours results in a survival rate of 65% without infarction. In this study, cerebral adenosine triphosphate (ATP), lactate, and pyruvate concentrations were measured at various time intervals after occlusion of the MCA. ATP decreased slowly during a three-hour period to 30% of normal, and lactate, after an initial rapid accumulation, increased slowly to about eight times normal. This compares to the effects of circulatory arrest which, in the dog, results in a reduction of the ATP level to 25% of normal within four minutes and a reciprocal increase in the lactate level. Because the effects of total cerebral anoxia are potentially reversible prior to four minutes, and, therefore, at an ATP concentration above 25% of normal, the slow rate of ATP depletion observed in the ischemic monkey brain supports the view that a significant period exists after occlusion of a major intracranial vessel wherein the ischemic effects are potentially reversible. Using the methods of this study, future investigations should permit a meaningful evaluation of the relative merits of those measures recommended for the treatment of acute cerebral ischemia.

ADDITIONAL KEY WORDS cerebral metabolism cerebral ischemia cerebral infarct stroke

Introduction

In man, total circulatory arrest at normothermia results in irreversible anoxic brain damage within five minutes. Based on this observation, it is generally assumed that occlusion of a major intracranial vessel will likewise result in rapid irreversible anoxic damage of that portion of the brain normally supplied by the occluded vessel. Observations made in the experimental animal have not supported this assumption. In the squirrel monkey, permanent occlusion of the middle cerebral artery (MCA) consistently produces a large infarction of the involved hemisphere. However, in the immediate two-hour period after occlusion, cerebral blood flow (CBF) in the area of the eventual infarction does not cease but instead is reduced to 20% to 50% of normal. Furthermore, if flow in the MCA is reestablished within three hours after occlusion, most monkeys will survive without infarction. The tolerance for occlusion might even exceed this three-hour period if it were...
not for the development of cerebral edema which by mass action jeopardizes collateral circulation. The metabolic events that occur in the involved hemisphere after occlusion of the MCA have not previously been examined in this model.

In this study, cerebral adenosine triphosphate (ATP), lactate, and pyruvate concentrations in the ischemic hemisphere of the squirrel monkey were measured at various time intervals after occlusion of the MCA. As opposed to the rapid depletion of cerebral ATP that occurs during total circulatory arrest, only a very slow depletion in ATP was observed over a three-hour period after occlusion of the MCA. Similarly, cerebral lactate accumulated at a relatively reduced rate. These observations provide further support for the view that a significant period exists after occlusion of a major intracranial vessel wherein the ischemic changes are potentially reversible.

Methods

Eighteen unpremedicated squirrel monkeys (Saimiri sciureus) weighing 600 gm to 1,200 gm were anesthetized with intraperitoneally administered sodium pentobarbital (15 mg/kg). Each monkey was then placed in the prone position and covered with a heating blanket; the head was fixed in a Waltz headrest. In 14 monkeys the right MCA was occluded under the operating microscope with a miniature Mayfield clip through a retro-orbital extradural approach that avoids retraction and manipulation of the brain itself. The time of occlusion was noted. Bilateral frontal parietal craniectomies were then completed. The animal was repositioned and a femoral arterial catheter was placed for blood pressure and blood gas measurements.

At various time intervals after occlusion of the right MCA (28 to 189 minutes), the dura overlying both exposed hemispheres was excised, and simultaneous biopsy specimens of the right (MCA occluded) and left (control) cerebral hemispheres were taken. The technique of biopsy was that described by Kramer et al. which, within one second, removes a brain sample of 100 mg to 400 mg and deposits it into liquid nitrogen. Time intervals between occlusion of the MCA and biopsy were approximately 30 minutes in five monkeys, one hour in three monkeys, two hours in three monkeys, and three hours in three monkeys. In four monkeys, the surgical preparation was identical to that described except the right MCA was not exposed and occluded. In these animals, simultaneous bilateral cerebral biopsy specimens were taken as soon as surgery was completed. In all instances, the site of biopsy was identical, and corresponded to the central area of infarct previously demonstrated to eventually occur in this species after permanent occlusion of the MCA. In most instances, the biopsy specimen was of sufficient depth to expose the lateral ventricles. Prior to biopsy, arterial blood pressure was measured intermittently by strain gauge, brain temperature was monitored with a temporal epidural thermistor, and arterial blood samples were taken for measurement of $P_aO_2$, $P_aCO_2$, pH (IL electrodes), and hematocrit.

After biopsy, the monkeys were killed. Blood loss during the entire procedure was usually less than 5 ml. Blood loss in excess of 5 ml was replaced with heparinized blood taken from a donor squirrel monkey. With one exception, mean arterial blood pressure (MABP) remained higher than 70 mm Hg. Brain temperature was maintained between 35.5°C and 38.5°C. In none of the monkeys was the depth of anesthesia judged to be excessive and, in most instances, some spontaneous movement was noted prior to biopsy. The monkeys breathed room air spontaneously throughout the experiment.

Thebrain specimens contained various amounts of blood. This is a potential source of error in the measurement of cerebral ATP concentrations because blood contains significantly less ATP than does brain. To eliminate this source of error, red cells from a donor squirrel monkey were tagged with $^{51}$Cr, and an aliquot was injected into each experimental monkey prior to biopsy. The radioactivity of the brain specimens and of a sample of blood from each monkey was subsequently measured. This, along with measurement of the blood ATP concentration, permitted correction for this variable.

Each core of brain tissue was handled in a manner intended to minimize the possibility of thawing and artifactual change in the ATP and lactate concentrations. In rapid sequence, the frozen brain tissue was removed from the liquid nitrogen, weighed, and, after the addition of 2 ml of cold (0°C to 4°C) perchloric acid (8%), ground for one 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°C to 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. The lactate and pyruvate concentrations were determined by standard enzymatic methods.
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TABLE 1
Brain Temperature, Arterial Pressure, and Blood Values Prior to Biopsy (18 Monkeys)

<table>
<thead>
<tr>
<th>Brain temperature, °C</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>92</td>
<td>5</td>
</tr>
<tr>
<td>PaCO₂, mm Hg</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>7.47</td>
<td>0.02</td>
</tr>
<tr>
<td>BB⁺, mEq/l</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>41</td>
<td>2</td>
</tr>
</tbody>
</table>

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
The mean values for arterial pressure, brain temperature, blood gases, pH, and hematocrit in the 18 monkeys immediately prior to biopsy were tabulated (table 1). There were no significant differences between values observed for the 14 monkeys with occluded right MCA and those for the four monkeys without occlusion. Hence all values were pooled. The mean control cerebral ATP, lactate, and L/P values were tabulated separately for these two groups of monkeys (table 2). There were no significant differences between values obtained from the control cerebral hemispheres (left) of monkeys with occluded right MCA and those obtained from both hemispheres of monkeys without occluded MCA. Accordingly, these values were pooled.

The individual cerebral ATP values obtained from the right hemisphere of the 14 monkeys with occluded right MCA were plotted in relation to time after occlusion (fig. 1). In these monkeys the ATP concentrations of the right cerebral hemisphere were always less than the mean control value and, at the four time intervals investigated after occlusion, the mean values were progressively diminished. The latter suggests a linear depletion of cerebral ATP, although in three monkeys (nos. 4, 9, and 11), cerebral ATP was considerably less than that suggested by the calculated linear regression equation. The lowest of these was in the one monkey (no. 9) that became hypotensive (MABP of 40 to 60 mm Hg) after occlusion of the MCA. With this one exception, none of the ATP values were less than 25% of control. There was no evidence to suggest that a new steady level of ATP was reached after occlusion of the MCA.

Individual cerebral lactate concentrations obtained from the right hemisphere of monkeys with occluded right MCA were plotted (fig. 2). All of these values were considerably greater than the mean control lactate concentration (none less than three times). The mean values at the four time intervals investigated were progressively larger and suggest a linear rate of lactate accumulation between 30 and 190 minutes after occlusion. However, at some time prior to the 30-minute interval, a considerably greater rate of lactate accumulation occurred because extrapolation of the calculated regression line to zero time yielded a lactate value almost four times the mean control value. The cerebral L/P increased progressively with time of occlusion such that after three hours the L/P was 16 times the mean control value (fig. 2).

The gross appearance of the brains prior to biopsy varied according to the duration of MCA occlusion. In all monkeys, focal cortical pallor of the right hemisphere was noted and some darkening of the cortical venous blood was apparent. Gross edema of the right hemisphere was only noted in the monkeys

<table>
<thead>
<tr>
<th>Control Values for Cerebral Adenosine Triphosphate (ATP), Lactate, and Lactate to Pyruvate (L/P) Ratio</th>
</tr>
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<tbody>
<tr>
<td>Control hemisphere (14 monkeys)</td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>ATP, μmol/gm</td>
</tr>
<tr>
<td>Lactate, μmol/gm</td>
</tr>
<tr>
<td>L/P</td>
</tr>
</tbody>
</table>
Cerebral ATP concentrations after occlusion of the right middle cerebral artery (MCA). Individual animal values (identified by number) and mean values calculated at four time intervals after occlusion are plotted. ATP levels gradually reduced over a three-hour period to approximately 25% of the mean control value.

Cerebral lactate concentrations and L/P values after occlusion of the right MCA. Individual animal lactate values (identified by number) and mean lactate values calculated at four time intervals after occlusion are plotted. In the initial 30 minutes after occlusion, lactate levels increased rapidly to about four times the mean control value. Thereafter, lactate levels increased slowly to about eight times control after three hours. The mean L/P ratio increased progressively to about 16 times control at three hours after occlusion.
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studied after 120 minutes of occlusion. This was evident in two of the three monkeys in whom biopsy specimens were taken at the two-hour period (nos. 6 and 10) and was more pronounced in all three monkeys in whom biopsy specimens were taken at the three-hour period. The left hemisphere appeared normal in all monkeys.

Discussion

In order to discuss the results of this study, it is necessary to briefly review the pertinent findings of previous investigations done in these laboratories concerned with the effects of MCA occlusion on morbidity, mortality, and CBF and the effects of total circulatory arrest on cerebral ATP and lactate concentrations.

After development of the surgical technique used to clip the MCA of the squirrel monkey, it was shown in 20 chronic preparations that removal of the clip after three hours of occlusion (either in untreated monkeys or in monkeys supported with concentrated serum albumin) resulted in a survival rate of 65%, with no morbidity of the survivors. Histological studies with light microscopy of these brains (seven days or more after temporary occlusion of the MCA) showed primarily occasional areas of focal cellular destruction or damage.

For periods of MCA occlusion extending over three hours there was an extremely high mortality rate that was directly related to the development of massive cerebral edema. In separate studies, regional CBF (krypton-85) was measured intermittently before and for two hours after occlusion of the MCA. During occlusion, CBF in core areas of ischemia decreased to 20% to 50% of CBF values before occlusion and became pressure-dependent. There was no evidence of gradual failure of collateral circulation during the two-hour period of observation and before the development of cerebral edema.

The effects of total cerebral anoxia (produced by decapitation) on cerebral ATP and lactate concentrations were studied in dogs in the presence of different anesthetics and during hypothermia. At normothermia, regardless of the anesthetic, the cerebral ATP level decreased rapidly in a linear fashion to 25% of control within four minutes and was accompanied by a reciprocal increase in cerebral lactate level. Hypothermia (30°C) reduced the rates such that the ATP level did not reach 25% of control until seven or eight minutes after decapitation. Because the effects of total cerebral anoxia are potentially reversible within four minutes at normothermia and within seven or eight minutes at 30°C, it was postulated that a cerebral ATP concentration higher than 25% of normal was indicative of a potentially reversible anoxic circumstance.

In this study, a correlation between the previously observed anatomical and hemodynamic effects of temporary occlusion of the MCA and the cerebral ATP concentrations after occlusion is apparent. The calculated linear regression line for ATP depletion, when extrapolated, intercepts the postulated critical ATP concentration of 25% of normal at three hours and ten minutes after occlusion. Furthermore, in only five of the 14 monkeys were the ATP levels below this regression line. If these five values were eliminated, the new regression line would reach 25% of normal ATP approximately four hours after occlusion. That 65% of the values approximated such a rate of ATP depletion and that, in the chronic studies, 65% of the monkeys tolerated three hours of occlusion without morbidity is likely more than coincidental.

These combined observations plus the demonstration of a measurable CBF in the region of maximal ischemia after occlusion of the MCA provide further evidence to support the view that a significant period exists after occlusion of a major cerebral vessel during which the ischemic effects are potentially reversible. If these observations can be extrapolated to man, the concept that the brain will tolerate only four to five minutes of occlusion of a major vessel should be discarded. Clearly, in the squirrel monkey, the initial anatomical, hemodynamic, and metabolic events after occlusion of a major vessel are distinctly different from those after total circulatory arrest.

Observations in this study, and in previous studies, make it possible to speculate about the relative contribution of the various factors that combined to determine the rates of cerebral ATP depletion and lactate accumulation after occlusion. The depletion in ATP could be accounted for either by a gradual depletion of ATP in all of the cells in the ischemic brain or by a gradual reduction in the number of viable cells. The latter interpretation

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implies a decreasing population of cells with normal ATP and an increasing population of cells with little or no ATP. If this were so, then the effects of ischemia should be rapidly irreversible both functionally and anatomically. No such evidence was found in our chronic monkey studies. A gradual reduction of ATP in all of the cells implies complete reversibility until energy reserves reach a critical level.

The energy requirements of the ischemic brain for the maintenance of viability (and hence reversibility) are not known. For normal monkey brain, it can be assumed that the rate of oxygen consumption in the cerebral hemispheres is between 3 and 5 ml O$_2$/100 gm/min$^{12}$ or 1.3 to 2.2 $\mu$mol O$_2$/gm/min. On the assumption that the P:O ratio is 3.0, the normal aerobic rate of ATP production (and approximately, therefore, of ATP utilization) would be 8 to 13 $\mu$mol ATP/gm/min. Such a rate of ATP utilization may be viewed as the resultant of two major determinants, that is, the energy required for the maintenance of cellular integrity and the energy required for the maintenance of cerebral function. After MCA occlusion, function of the ischemic brain is presumably reduced and the energy requirements would decrease accordingly toward a minimal level necessary for the maintenance of cellular integrity. In the dog, anoxia results in immediate cessation of cerebral function (as evidenced by the EEG) and the estimated rate of ATP utilization in the initial four minutes was reduced to 3.0 to 3.5 $\mu$mol ATP/gm/min. $^4$ In the cat, after occlusion of the MCA, the electrocorticogram of the ischemic hemisphere becomes distinctly less active, as evidenced by a lower amplitude and slower frequency. $^{13}$ These changes progress to virtually no cortical activity when CBF is reduced to below 30% of normal. Thus, it is reasonable to conclude that the rate of ATP utilization in the ischemic hemisphere of the monkey was reduced to between 3.0 and 8.0 $\mu$mol ATP/gm/min.

The available sources for the production of ATP in the brain are limited primarily to aerobic glycolysis, anaerobic glycolysis, and the phosphorylation of adenosine diphosphate (ADP) by creatine phosphate. Creatine phosphate may be considered a source of stored high-energy phosphate. In the event of hypoxia sufficient to decrease aerobic ATP production, this source is immediately utilized to maintain ATP concentrations and is not replenished until ATP production returns toward normal. During anoxia, creatine phosphate is rapidly depleted. $^{14}$ It is probable that creatine phosphate is likewise rapidly depleted after occlusion of the MCA. Normal concentrations of cerebral creatine phosphate (in those species studied) are between 2.5 and 3.5 $\mu$mol/gm. $^{15}$ This will phosphorylate ADP to form ATP on an equimolar basis. Even at the suggested reduced rate of ATP utilization, such a source for the prolonged maintenance of ATP concentrations is of minor importance. Anaerobic glycolysis as compared to aerobic glycolysis is an inefficient means of producing ATP. The aerobic breakdown of 1 mole of glucose to H$_2$O and CO$_2$ produces 38 moles of ATP, whereas anaerobically, 1 mole of glucose yields only 2 moles of ATP (and 2 moles of lactate). The rate of lactate accumulation observed in the monkeys cannot be equated to the rate of lactate production because loss into the CSF (primarily) and the blood (unproved) must be occurring simultaneously. However, even on the assumption that the rate of lactate accumulation was twice the rate of lactate accumulation, the ATP produced by this means would be only 0.1 $\mu$mol/gm/min. Thus, even from these crude estimates, the major source of ATP production after occlusion of the MCA must continue to be by aerobic glycolysis.

At some time in the initial 30 minutes after occlusion of the MCA, the rate of lactate accumulation was considerably greater than that calculated after 30 minutes. This rate change could be explained by an increase in the loss of lactate to the CSF and the blood or by a decrease in lactate production secondary to either a decrease in available glucose or the accumulation of a rate-limiting factor (that is, lactate or one or more of the intermediate products of anaerobic glycolysis). It is also possible that, as suggested by Lassen, $^{16}$ the accumulated lactate and the resulting intracellular acidosis caused cellular swelling. This in turn might be expected to reduce the metabolic activity of the cell and thus the rate of anaerobic glycolysis. The observations of this study do not permit speculation as to which of these factors was dominant. Whatever the true rate of lactate production was, it is interesting to note that an increase in the rate of lactate production of less than 0.01 $\mu$mol/gm/min
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would have theoretically been sufficient to maintain a normal ATP (assuming an unchanged rate of ATP-utilization).

There were several potentials for error inherent in the methods used for this study. The site from which the biopsy specimens were taken may not have been the area of maximal ischemia; this would introduce a variability unrelated to the time of biopsy. However, the squirrel monkey was selected for this study because of the consistent, large infarct that is produced by permanent occlusion of the MCA. This is not true for other species such as the cat and dog in whom smaller and more variable infarcts occur. The site selected for the biopsy specimen in the squirrel monkey was the central region of eventual infarction. Because of the onset of edema two to three hours after MCA occlusion, the measured weights of the brain specimens taken at that time were necessarily falsely high in relation to the brain specimens taken prior to two hours. This error would consistently produce falsely low values for ATP and lactate and, therefore, would not significantly alter the interpretation of the data. In taking the brain biopsy specimens, the lateral ventricles were frequently entered. This produced a brain specimen with a variable amount of frozen CSF attached. Fortunately, while frozen, the CSF could be easily separated from the brain specimen. Assuming some residual CSF on these brain specimens, the error introduced would again cause falsely high weights and low ATP and lactate concentrations.

Our control values for cerebral ATP and lactate concentrations and L/P were similar to those reported for other species. The range of reported mean cerebral ATP concentrations in other species (rat, mouse, dog, and cat) is 2.0 to 2.7 μmol/gm. In the dog studies done in these laboratories using methods identical to those of the present study, the mean control cerebral ATP was 2.3 μmol/gm. To our knowledge, cerebral ATP in the monkey has not previously been reported. Cerebral lactate levels reported for other species range from 0.8 to 2.7 μmol/gm and cerebral L/P values from 11 to 18.

The methods used in this study may provide a valid means for evaluating the efficacy of the various suggested therapeutic measures for minimizing the ischemic effects of occlusion of a major intracranial vessel. In the past, such suggested measures have been primarily directed toward efforts to increase flow in the ischemic brain and have included hypocarbia, hypercarbia, hemodilution, hypertension, and surgical restitution of blood flow. In the future, measures directed toward preservation of the existing flow and support of the marginal cellular metabolic functions may show greater promise. The demonstration that such measures can significantly alter the concentrations of ATP after occlusion from that described in this communication would provide evidence either for or against these measures and should permit a meaningful evaluation of their relative merits.

References

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