Cerebral Oxygen Availability and Blood Flow During Middle Cerebral Artery Occlusion: Effects of Pentobarbital

PAUL J. FEUSTEL, PH.D., MALIN C. INGVAR, M.D.,
AND JOHN W. SEVERINGHAUS, M.D.

SUMMARY To determine whether barbiturate administration can improve oxygenation, oxygen availability (aO2) and local cortical blood flow (ICBF) were measured in cats before and during middle cerebral artery occlusion (MCAO) using 10 platinum electrodes distributed over the cortex. Halothane/N2O anesthesia was used during the surgical preparation and N2O with a relaxant thereafter. After 15 to 30 min of MCAO, 50 mg/kg of pentobarbital was infused slowly. Measured from electrodes in severely ischemic cortex, aO2 increased if the blood pressure was maintained with dopamine. Control animals in which no pentobarbital was given showed no change in aO2 over the same period of time. In areas of cortex not affected by middle cerebral artery occlusion the aO2 did not change from control values despite a decrease in blood flow from 72.7 ± 49.8 to 48.9 ± 26.7 ml/min/100 g. Thus, pentobarbital appears to decrease ICBF and metabolism proportionally in well perfused cortex so that aO2 remains constant, while improving the flow to metabolism ratio in poorly perfused cortex so that aO2 rises.

BARBITURATES have been shown to reduce infarct size following middle cerebral artery occlusion in some species of animals.1-4 The mechanism of protection is unknown. This study was designed to determine the relative O2 tension and blood flow responses of normal and ischemic cat cerebral cortex to large doses of barbiturates. Changes in cortical O2 may help explain how barbiturate treatment results in smaller infarct sizes following middle cerebral artery occlusion (MCAO).

Branston et al.8 have reported that barbiturates increased flow in micro-areas of baboon cortex where flow during MCAO was less than 20 ml/min/100 g. Their results are consistent with an inverse steal hypothesis for barbiturates. This hypothesis states that vasoconstriction of non-ischemic cortex results in an elevated microvascular pressure which can then lead to increased flow through anastomotic channels to ischemic tissue. A second hypothesis is that barbiturate protection is secondary to the resulting decreased metabolic rate and oxygen requirement. However, all agents which decrease metabolic rate do not have a protective effect.4,5 The important variable for tissue oxygenation is the ratio of flow to metabolism. This determines the amount of oxygen available for metabolism and also the gradient for diffusion to the mitochondria. Thus, increases in flow combined with decreases in metabolism may amount to a more substantial improvement in tissue oxygenation than flow measurements alone would indicate. Therefore, it is desirable that either flow and metabolism both be measured at the same location or that a single parameter, which reflects the flow to metabolism ratio, be measured. Clark et al.7 have defined oxygen availability (aO2) as the amount of oxygen reduced at a platinum cathode in tissue. It is an estimate of the oxygen delivery minus the locally metabolized oxygen. Therefore, aO2 was measured in micro-areas of cat cortex before middle cerebral artery occlusion (MCAO), during MCAO, during MCAO with pentobarbital administration and during MCAO with pentobarbital and restoration of blood pressure. Local cerebral blood flow was also measured before and after these interventions. When blood pressure was maintained, pentobarbital was seen to improve tissue oxygenation in areas of focal ischemia.

Methods

Electrodes were cut from 25 micron diameter Teflon-coated platinum (Pt) wire (Medwire Inc.). They were approximately 4 centimeters in length. One end was held briefly over an alcohol lamp to remove the Teflon and allow connection to the amplifiers. The other end was cut at a 45° angle to facilitate placement into the cortex through the pia mater. The Pt electrodes were maintained at virtual ground while the reference electrode was held at +0.5 v (E(Pt-Ag) = —0.5 v) for the oxygen reduction reaction and at —0.3 v (E(Pt-Ag) = 0.3 v) for the hydrogen (H2) oxidation reaction. The reference electrode was silver with a silver chloride coating. The potential of an additional driver electrode was placed subcutaneously on the back. Nitrous oxide does not interfere with O2 current.

Cats of either sex were anesthetized with halothane, intubated, paralyzed with gallamine triethiodide (1.0 mg/kg) and artificially ventilated. Inspired gas consisted of 70% N2O and 30% O2 with halothane added to achieve an end-tidal fraction of 0.8%.
Anesthetic and respiratory gases were monitored with a Perkin-Elmer 1100 mass spectrometer. An added peak detection circuit sampled the maximum CO₂ as end-tidal CO₂ for monitoring and recording. A femoral arterial cannula was used for sampling arterial blood and monitoring blood pressure. A femoral venous line was used for administration of bicarbonate to correct any metabolic acidosis. Temperature was monitored with a rectal probe and controlled with a heating pad. The head was fixed and a 20 mm by 3 mm opening was made on the right side of the skull parallel and approximately 10 mm lateral to the midline along the suprasylvian and ectosylvian gyri from the rostral suprasylvian sulcus to the caudal suprasylvian sulcus in the occipital cortex. A smaller 3 mm by 3 mm hole was made on the left, corresponding in position to the anterior portion of the contralateral opening. The dura was left intact until the orbit was prepared.

The transorbital approach to the middle cerebral artery was used. The right eye was removed. Using an operating microscope (Ziess OPMI1), the optic foramen was enlarged laterally so that when the dura was opened the middle cerebral artery was accessible. The dura was then opened and the electrodes were inserted into the cortex a distance of 1 to 2 mm. Pial vessels were avoided. Three electrodes were placed into the left cortex. Seven electrodes were placed on the right side over an area extending from the suprasylvian gyrus to the occipital cortex. Following initial preparation, halothane anesthesia was withdrawn and analgesia maintained with 75% N₂O. Gallamine triethiodide (2.5 mg/kg) was used as a relaxant. Monitoring of end-tidal CO₂ and periodic blood gas determinations (Paco₂, PO₂, and pH) prevented CO₂ changes and systemic acidosis throughout the experiment.

Three electrodes, end-tidal PCO₂, and arterial blood pressure were recorded on a Gilson polygraph. The output from all 10 electrodes together with the blood pressure and end-tidal PCO₂ signals were digitized and stored by the PDP 11/34 computer. The digitized signals were returned to an ADM display terminal for monitoring. O₂ current and H₂ current were sampled at two and one hertz respectively.

Local cerebral blood flow was measured by the H₂ clearance technique. After polarizing to E(Pt-Ag) = −0.5 v and waiting 20 min for stabilization, H₂ current was determined by 30 sec of N₂O delivery. After control determinations of flow and PO₂, the middle cerebral artery (MCA) was exposed by opening the dura in the enlarged optic foramen. The MCA was dissected from the surface of the brain as far medially as possible and clamped. Mean PO₂ measurements were made during the 15 to 30 min after MCAO so that electrodes could later be compared on the basis of this initial decrease. After 15 to 30 min, pentobarbital (60 mg/kg Diabutal, i.v.) or no anesthesia was added to the N₂O. In the pentobarbital treated group PO₂ was again measured during the resultant hypotension. The blood pressure was restored to the pre-pentobarbital post-MCAO level by appropriate intravenous dopamine infusions and PO₂ determinations made 60 to 90 min after MCAO. All PO₂ measurements are the means of a 5 min period of sampling at one hertz.

The PO₂ “waves” are characteristic oxygen tension fluctuations observed initially by Clark et al. which are believed to represent arteriolar vasomotion. Our criteria for PO₂ waves were fluctuations of PO₂ with a frequency of 6 to 15 cycles per min, an amplitude of at least 10% of the mean PO₂, and a sequence of at least 10 waves with the same period. If the fluctuations showed beats, then the last criterion was extended over several beats.

Zero PO₂ current was determined by 30 sec of N₂O breathing. During anoxia, electrode current fell rapidly to a plateau, which was assumed to be zero PO₂, and was subtracted from all other PO₂ determinations for that electrode. The electrodes were then polarized for blood flow measurements and multiple determinations made after a 20 min stabilization period.

Because of the wide variability in PO₂ due to differences in electrode characteristics (the available surface area for O₂ reduction in particular) and the heterogeneity of the cortex PO₂, all PO₂ measurements are expressed as a ratio to the pre-MCAO control measurement. All values are means ± the standard deviation. Data for PO₂ and PE were not normally distributed. Statistical comparisons of PO₂ values were done with the non-parametric Wilcoxon paired
sample test for data not normally distributed. Values for ICBF were compared with the paired Student's t-test.

**Results**

Current for aO₂ averaged 27.0 ± 23.8 nanoamps (mean ± SD, n = 54) in normal cerebral cortex. Assuming an oxygen consumption for cortical grey matter of 7 ml O₂/100 g/min, \( \text{aO}_2 \) would utilize as much O₂ as a sphere of cortical tissue with a radius of 68 microns.

MCAO resulted in a bimodal distribution of aO₂ reductions (fig. 1). Based on this histogram the electrodes were arbitrarily divided into 2 groups for analysis. Electrodes in which aO₂ was reduced by more than 50% were placed into Group I. Group I included 33 electrodes, 23 of which were in 6 cats subsequently treated with pentobarbital and 10 in the 3 non-treated cats. Group II, which included an electrode with a 3-fold increase in aO₂, consisted of 28 electrodes, 18 in animals subsequently treated with pentobarbital and 10 in untreated animals. The more severely ischemic areas were located in the more anterior areas. There was substantial variability in the extent of the ischemia among animals. In some animals the MCA was seen to supply the brain covered by a large fraction of electrodes on the occluded hemisphere but in others only a small fraction of electrodes was affected. Group I ranged from 25% to 86% of the electrodes on the occluded side.

In the 10 Group I electrodes in 3 untreated cats, MCAO resulted in a decrease in aO₂ to 0.30 ± 0.12 of control (fig. 2). After one hour aO₂ was unchanged at 0.24 ± 0.16 of control (\( p > 0.20 \)). There was a small decrease in blood pressure associated with MCAO in the control animals but not in the experimental animals. Except for the hypotensive period immediately following pentobarbital treatment, dopamine infusion held blood pressure at the level measured immediately following MCAO. The Group II electrodes in the same animals showed no change in aO₂ with MCAO or following one hour of MCAO. Thus, when no intervention was made, there was little or no improvement of aO₂ from the measurement made at 15 minutes to that made at one hour following MCAO. When halothane (0.8% end tidal) was added to the N₂O after the one-hour measurement and blood pressure supported by dopamine infusion, the aO₂ was unchanged at 0.32 ± 0.31. Halothane was then withdrawn and following a stabilization period, local cerebral blood flow was found to be significantly below control ICBF in Group I electrodes (\( p < 0.01 \)) but was unaltered in Group II electrodes.

In those animals treated with pentobarbital, MCAO resulted in aO₂ reductions to 0.16 ± 0.09 of control in Group I and in no change to 1.13 ± 0.53 of control in Group II (fig. 3). With pentobarbital treatment the blood pressure fell from 112 ± 24 to 66 ± 27 mm Hg. No change in mean aO₂ in either group of electrodes was seen (aO₂ was 0.28 ± 0.43 in Group I and 1.03 ± 0.73 in Group II). One animal became so severely hypotensive that it was necessary to begin dopamine infusion before the entire dose of pentobarbital was given. Following restoration of blood pressure by dopamine infusion (mean rate was 0.64 ± 0.42 mg/kg/min) the aO₂ in the Group I electrodes rose to 0.61 ± 0.74 (\( p < 0.01 \), Wilcoxon paired sample test compared with pre-pentobarbital values) and was unchanged at 1.20 ± 0.52 in the Group II electrodes. Thus, in Group II electrodes the aO₂ remained unchanged both during the hypotensive period and with restitution of the blood pressure by dopamine infusion. The preservation of aO₂ in Group II electrodes occurred despite a reduction in the normotensive blood flow from 72.7 ± 49.8 to 48.7 ± 26.9 ml/min/100 g. Results are the same if only un-occluded hemisphere electrodes are compared. Flow in the Group I electrodes was found to be 61.8 ± 34.8 ml/min/100 g before MCAO and 27.3 ± 21.9 ml/min/100 g after MCAO, pentobarbital and

![Figure 1. Frequency histogram of electrodes. The abscissa is the ratio of the post MCAO aO₂ to the pre MCAO aO₂ and thus represents the severity of the occlusion at each electrode. The electrodes were arbitrarily divided into two groups. Those on the left are electrodes in areas where oxygen had been decreased to less than 50% of the control value (Group I). The remaining electrodes are in Group II.](http://stroke.ahajournals.org)
dopamine administration. All areas in which aO₂ was increased to values in excess of pre-MCAO values were hyperemic.

Waves of aO₂ were present in 8 of the 41 electrodes before MCAO. After MCAO, pentobarbital and restoration of blood pressure to the pre-pentobarbital level, the aO₂ waves appeared in 6 of 18 electrodes in Group II and in 6 of 23 electrodes in Group I. Barbiturates restored aO₂ waves in ischemic cortex where mean aO₂ had been reduced by as much as 85%.

Discussion

Although Black et al. showed decreased infarct size in cats treated with barbiturates, Michenfelder and Milde were unable to show a therapeutic benefit. The
latter authors attributed the lack of measurable protection to the variability of collateral circulation in cats. Our results also indicate a large range of responses among animals in similar areas of cortex. By comparing electrodes, rather than animals, the present model is not vulnerable to this variability. However, it introduced the additional complication of variability with time. The control experiments indicate that in most areas further establishment of collateral circulation does not occur in the absence of interventions between fifteen minutes and one hour following occlusion. Local cerebral blood flow in ischemic cortex has been shown to decrease with time in halothane anesthetized cats.16

During the hypotensive period immediately following pentobarbital administration, aO2 in several Group II areas was substantially reduced suggesting that pentobarbital induced hypotension may increase the area of cortex subject to severe aO2 reductions. There was restoration of aO2 in most of these areas when blood pressure was returned to pre-pentobarbital levels. In Group I areas, and probably in border zone areas, aO2 was very sensitive to blood pressure changes. With restoration of blood pressure in Group I areas, mean aO2 was seen to double, whereas in Group II mean aO2 was increased only 20% (not significant).

In areas not severely affected by MCAO (Group II), including those electrodes on the unoccluded side, pentobarbital with blood pressure maintenance did not alter aO2 despite a drop in ICBF. Thus, flow and metabolism were decreased proportionally so that the aO2 was unchanged. This is consistent with the known reduction of metabolism by barbiturates19, 30 and the well established relationship between metabolism and flow.31

In severely ischemic cortex (Group I) pentobarbital caused an increase in aO2 if pressure was restored to pre-pentobarbital levels by dopamine infusion. Several areas showed a marked hyperemia. It is not clear why some micro-areas showed more substantial increases in aO2 than did other areas, although there is a tendency for electrodes over the less severely ischemic areas within Group I to show a greater increase in aO2. The increase in aO2 reflects an increase in the metabolic requirements of these areas.

Although without measurements of ICBF in the post-MCAO pre-pentobarbital period the present studies offer no direct evidence for the inverse steal effect, Branston et al.6 have shown flow increases which may be due to an inverse steal effect. For this mechanism to be effective, the increased microvascular pressure which arises from a constriction of distal vascular resistance must not be offset by a decrease in systemic arterial blood pressure. Thus, in order to test an inverse steal effect it is necessary to restore or maintain arterial pressures. Blood pressure maintenance was a part of therapy in studies demonstrating reduced infarct size with barbiturates.34 At constant perfusion pressure, increases in flow, if based on an inverse steal effect, would be directly proportional to the fraction of total vascular resistance distal to the proposed anastomotic connections. Assuming that as much as 30% of the total cerebrovascular resistance is proximal to the anastomotic site, which places the anastomosis arteries smaller than 50 microns,28 then a doubling of proximal resistance in normal tissue would produce the drop in cerebral blood flow observed in Group II electrodes and would result in an increase in collateral perfusion pressure from 70 to 85 mm Hg. Vessels in ischemic cortex, assuming they are fixed and dilated, would see an increase in flow of 21%. Unless aO2 increases very steeply at low aO2 for small changes in flow, this increase in flow may not be sufficient to explain the observed increase in aO2.

The increase in aO2 in Group I electrodes is not necessarily due solely to an increase in flow. Additional mechanisms might include the barbiturate induced decrease in the oxygen consumption of ischemic or near ischemic tissue such that aO2 rises, or recruitment of capillaries by elevated collateral vessel perfusion pressure resulting in a better distribution of flow independent of an increase in absolute flow. The present studies offer some support for these 2 mechanisms. Metabolism may have decreased. This must have occurred in the non-ischemic cortex in order for the aO2 to remain the same with a lower flow. One might extrapolate that a similar effect is occurring in near ischemic cortex, although without knowing the flow post-MCAO pre-pentobarbital no definite statement can be made. The restitution of aO2 "waves" in some Group I areas indicates that the potential for vasomotion has been restored in a previously dilated area. The second possible mechanism is that aO2 improves because oxygen extraction is improved. A more efficient distribution of capillary flow may occur. The restoration of vasomotion in some areas by pentobarbital may indicate that very local arteriolar pressures have been elevated to levels above the critical pressure for opening and that some tissue metabolic demands are met by existing flow. Areas of cortex which had been deprived of flow by low resistance channels would be better served by perfusion than by diffusion from a maximally diluted but far removed capillary. Pentobarbital may have restored this dilated vessel to the more physiological intermittent flow typical of capillaries in vascular beds operating at less than maximal flow requirements. Such vasomotion would result in a more efficient delivery of oxygen to all parts of tissue. The more efficient capillary distribution will result in more oxygen extraction from an equivalent amount of blood flow.

Thus, in the absence of hypotension, barbiturates decrease flow and metabolism proportionally in non-ischemic cortex so that aO2 remains unchanged while increasing the flow to metabolism ratio in ischemic cortex. Such an increase in aO2 may be due to one or several mechanisms including 1) increased flow by the inverse steal mechanism, 2) decreased metabolic requirements or 3) a more efficient homogeneous flow.
distribution at the microcirculatory level as vasomotion is restored. Barbiturates have the potential to restore vasomotion in ischemic cortex where mean $aO_2$ had been reduced by as much as 85%, indicating that the nutritional needs of that area were being met despite the reduction in mean $aO_2$ and ICBF.

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P J Feustel, M C Ingvar and J W Severinghaus

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