Incomplete Transient Ischemia: A Non-Destructive Evaluation of In Vivo Cerebral Metabolism and Hemodynamics in Rat Brain

N. Wiernsperger, Ph.D., A. L. Sylvia, Ph.D., and F. F. Jöbsis, Ph.D.

SUMMARY Differential near infrared spectrophotometry was used to monitor sequential in vivo alterations in cerebral hemoglobin saturation, blood volume, and cytochrome c oxidase reduction/oxidation responses during and after a period of incomplete transient ischemia (acute, reversible common carotid artery occlusion). In this study the rat brain was monitored non-invasively in the intact skull by transillumination. The data show that an increase in cerebral deoxygenation of hemoglobin, which occurs simultaneously with a decrease in blood volume subsequent to carotid ligation, acts as a compensatory mechanism to assist in maintaining aerobic energy metabolism. The observations also demonstrate that in this species the effects of bilateral carotid occlusion on the cerebrovascular parameters are not necessarily irreparable. The intramitochondrial metabolic alterations, as evaluated by cytochrome c oxidase redox transitions, are reversible as long as the systemic arterial blood pressure does not fall below a value of approximately 40 mm Hg. These data suggest the possibility of being able to use a critical reduction level of cytochrome c oxidase as an early indication of ischemia-induced cerebral metabolic dysfunction prior to major changes in high energy stores.

Stroke, Vol 12, No 6, 1981

THERE IS ample evidence to support a close coupling relationship of brain functional activity, oxidative energy metabolism, and cerebral blood flow. Normally, cerebral blood flow, nutrient substrate delivery and oxygen sufficiency, are carefully regulated in direct proportion to regional tissue metabolic demands. However, in pathological states such as stroke-related oligemia, this regulatory capacity becomes impaired because the equilibrium between tissue metabolic supply and demand has been disturbed. Clinical assessment of progressive cerebral ischemic damage, therefore, demands a precise knowledge of sequential changes in the interrelationships between tissue energy metabolism and brain hemodynamics.

The major route of cellular energy production, in the form of adenosine triphosphate (ATP), occurs via the process of oxidative phosphorylation. In terms of tissue oxygen consumption, O2 utilization is directly proportional to the rate of electron transfer from cytochrome c oxidase (Cyt. a,a3) to molecular oxygen. Since the Cyt. a,a3 reduction/oxidation (redox) state is affected by variations in the level of tissue oxygenation within the physiological range in situ, changes in the in vivo redox state of this enzyme may be used as an indicator of intracellular oxygen sufficiency and bioenergetics.

In the present study, we have explored the consequences of incomplete transient ischemia (acute, reversible common carotid artery occlusion) on the tissue oxidative metabolic state and hemodynamic alterations in rat brain. A newly developed technique of differential near infrared spectrophotometry is used whereby it is possible to monitor nondestructively and simultaneously sequential in vivo alterations in blood volume, hemoglobin saturation states, and cytochrome c oxidase redox responses. The metabolic significance of these observations during and after a period of oligemia are addressed.

Methods

Adult male Sprague Dawley rats were anesthetized with urethane (780 mg/kg, i.p.). The common carotid arteries were carefully exposed and separated from the vagal nerves on both sides of the neck. Cotton ligatures were loosely placed around the arteries. One femoral artery was cannulated for anaerobic blood sampling, i.e. Pco2, Po2, pH (Radiometer Analyzer, Copenhagen); and the other was cannulated for continuous blood pressure monitor (Statham transducer). A femoral vein was also cannulated for i.v. administration of solutions when necessary. The animals were tracheotomized and immobilized using tubocurarine chloride (1.2 mg/kg, i.v.). Upon cessation of spontaneous breathing, the animal was attached to a rodent respirator and ventilated with a gas mixture containing 30% O2/70% N2. The tidal volume and the respiratory rate were adjusted to obtain Paco2 values between 35–40 mm Hg and PaO2 values above 100 mm Hg. The body temperature was maintained close to 37°C.

A depilatory cream was used to remove the hair on both sides of the head. The rats were placed on an elevated surgical table so as to leave the carotid artery ligatures freely exposed and accessible for inducing occlusion. The head of the animal was positioned between an optical fiber bundle on one side, and a photomultiplier detector on the other; thus permitting transillumination directly through the head in the region immediately posterior to the eyes. In view of the extremely low concentration of cytochrome ox-
idase in skin and bone tissue, the signals from this enzyme arise almost exclusively from cerebral tissue. The optical probes were pressed with sufficient force against the sides of the skull so that changes in blood flow in the tissues external to the skull are eliminated. Therefore, the blood signals also reflect, for all practical purposes, the cerebrovascular changes only. Black cloth was then draped over the preparation in order to exclude extraneous light.

A differential spectrophotometric technique employing 3 wavelengths in the near infrared provided continuous and simultaneous measurements of changes in the cytochrome c oxidase redox state \([845 - 0.5 (784 + 883) \text{ nm}]\), cerebral blood volume \([784 + 883 \text{ nm}]\), and hemoglobin saturation \([883 - 784 \text{ nm}]\). The 3 signals were displayed simultaneously and independently on a strip chart recorder. The data are presented as changes in optical density units from the initial steady state baseline. Taking the intensity of the light signals in the physiological baseline condition as the original light signal intensity \((I_0)\), then according to the Beer-Lambert Law, the intensity resulting from surgical manipulations \((I)\) was recorded electronically according to the equation \(\Delta \text{O.D.} = \log_{10} (I_0/I)\).

After calibration, the preparation was monitored for a 10 min period of control baseline signal stabilization. Then small weights sufficient to occlude the carotid artery without vessel damage were attached to the exposed ligatures. The left carotid was occluded first followed 5 minutes later by occlusion of the right carotid artery. After a period of 20 min bilateral occlusion, both arteries were opened to reestablish perfusion; starting with the left carotid and 5 min later the right carotid. Cerebral recovery from transient partial ischemia was subsequently monitored for 15 min.

**Results**

Representative traces of cerebral hemodynamic and cytochrome c oxidase \((\text{Cyt. } a,a_t)\) metabolic responses to incomplete transient ischemia (ITI) are shown in figure 1. In addition to monitoring the phasic responses during ITI, all experiments were terminated using 100% \(\text{N}_2\) so that the maximal reduction level of \(\text{Cyt. } a,a_t\) could then be compared with that obtained after unilateral and bilateral common carotid ligation. The results show only a partial reduction associated with unilateral ligation and that bilateral occlusion did not produce \(\text{Cyt. } a,a_t\) reduction levels of the magnitude ultimately attained upon tissue death. The remaining oxidation is ascribed to non-carotid perfusion of the brain which is relatively vigorous in the rat.

The composite pattern of cerebral hemodynamic and metabolic responses to ITI is depicted in figure 2. The opened data points are the mean values \(\pm \text{ SEM}\) for 9 animals. Unilateral carotid artery occlusion produced an immediate decrease in cerebral blood volume simultaneous with a decrease in hemoglobin oxygenation and a concomitant increase in the level of reduced \(\text{Cyt. } a,a_t\). The mean arterial blood pressure (preligation values 115 \(\pm 3.4 \text{ mm Hg SEM}\)) transiently decreased coincident with ligation, frequently being followed by a brief period of hypertension, thereafter normalizing to the preligation values. During the 5 min interval following unilateral occlusion, both blood volume and hemoglobin saturation remained fairly constant while \(\text{Cyt. } a,a_t\) showed a tendency toward reoxidation. Subsequent bilateral occlusion was associated with a more significant drop in blood volume, decrease in hemoglobin saturation, and a marked shift of cerebral \(\text{Cyt. } a,a_t\) towards greater reduction. During the remaining period of brain underperfusion (20

**FIGURE 1.** Continuous and simultaneous changes in hemoglobin saturation, cerebral blood volume, and the cytochrome c oxidase redox state during and after a period of incomplete transient ischemia in rat brain. The traces are calibrated in optical density units from steady state baseline levels. Also shown are the hemodynamic and metabolic responses to 100% nitrogen termination.
min), cerebral blood volume and hemoglobin oxygenation progressively decreased while Cyt. a.a tended toward a slight reoxidation.

When cerebral blood flow was reinstated by unilateral carotid artery opening, the vascular and metabolic signals abruptly shifted toward partial normalization. However, after one min the blood volume again tended to fall and the hemoglobin signal indicated a further slight disoxygenation. However, the cytochrome redox state remained practically at a constant reduction level until removal of the second carotid clamp.

After establishing flow in both carotids (second ligation removal), we obtained an immediate hypermetabolic "overshoot" as indicated by a higher than steady state level of oxidized Cyt. a.a. This occurred coincident with significant increases in cerebral blood volume and hemoglobin oxygenation. During the remaining 15 minute period of recoverability, blood volume remained at a constant level below baseline while the blood oxygenation level continued to decrease simultaneous with the progressive hyperoxidation of Cyt. a.a (approximately 0.025 O.D. units above baseline values 15 min into recovery).

The closed data points in figure 2 represent mean values from 3 animals which were deliberately not included in the pooled data (n = 9) since after the monitored recovery period, the mean arterial blood pressure (MABP) progressively fell to levels well below 40 mm Hg. In all other cases, the MABP sustained a value exceeding 70 mm Hg. Although the pattern of cerebral blood volume and hemoglobin saturation was similar to that obtained in the pooled group of animals, the hypermetabolic "overshoot", as indicated by a higher than steady state level of cytochrome oxidation, was absent. These 3 animals died abruptly within 10 min after the last data points were recorded.

The statistically significant and most relevant data points from the pooled group of 9 animals are summarized in the table.

### Discussion

The cerebral circulation of the rat is characterized by an unusually vigorous contribution from collateral channels such as the posterior communicating arteries. Therefore, bilateral carotid occlusion produces a decrease in cerebral blood flow amounting to only 50% of the normal value. Despite this degree of reduction in cerebral perfusion, the energy state of the tissue, as evaluated by biochemical analysis, remains unaltered provided the animal is normotensive. Likewise, microelectrode measurements reveal that in spite of carotid ligation, adequate levels of tissue oxy-

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<th>TABLE</th>
<th>Cerebral Metabolic and Hemodynamic Responses to Unilateral and Bilateral Common Carotid Artery Ligation and Changes Associated with Flow Reinstatement</th>
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<tr>
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<td>1st clamp on</td>
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<tr>
<td>Cyt. a.a, Cu₃</td>
<td>-0.0187 (± 0.0034)</td>
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<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Blood volume</td>
<td>-0.0569 (± 0.0093)</td>
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<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
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<tr>
<td>Blood oxygenation</td>
<td>-0.0247 (± 0.0025)</td>
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<td>p &lt; 0.001</td>
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All values are means ± SEM for 9 animals. The degree of significant difference from the initial steady state baseline was calculated using Student's t-test.
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...presumably can be maintained in young but not in aged rats. This interpretation stems from the observation that the normal distribution of oxygen within the cerebral cortex has a maximum falling in the range of 15-25 Torr. Notwithstanding the apparent sufficiency of oxygen levels for normal function of Cyt. a,a when the enzyme is studied in vitro, present data show a considerable immediate as well as lasting effect of such oligemic episodes on the redox steady state of the enzyme.

If prior to bilateral occlusion the mean arterial blood pressure (MABP) is deliberately reduced, a threshold is reached beyond which chemical analysis...er...mortal...hemoglobin, i.e., the optical properties of the blood was observed towards a greater deoxygenation of hemoglobin, i.e., the optical properties of the blood...hemoglobin deoxygenation. Since direct cortical stimulation normally gives rise to an oxidation rather than a reduction of Cyt. a,a...4.89 to 3.33 mMol/kg wet wt. within a 5 min period...18 However, less is known regarding the intramitochondrial metabolic status of the tissue in vivo. For this reason, our protocol incorporates measurements of changes in the redox state of the enzyme cytochrome c oxidase which reacts directly with molecular oxygen in the obligatorily coupled process of energy production, i.e., oxidative phosphorylation.

In the present study, it is clear that carotid occlusion produces a significant drop in the cerebral blood volume. This would be expected to interfere with normal cellular oxidation. However, an associated shift was observed towards a greater deoxygenation of hemoglobin, i.e., the optical properties of the blood in the brain became more venous-like. This occurs immediately after occlusion and denotes increased oxygen extraction thereby contributing to better maintenance of aerobic energy metabolism. This compensatory action is indicated by the slow, progressive reoxidation of cerebral cytochrome c oxidase and the continuing deoxygenation of hemoglobin during the 20 min interval of bilateral occlusion. However, this increased extraction is apparently not sufficient to normalize the intramitochondrial redox state as evidenced by a sustained Cyt. a,a reduction amounting to 0.038 O.D. units below the original steady state baseline value. A similar observation has been made in exposed cat brain under analogous experimental conditions.

The more reduced level of Cyt. a,a throughout the period of carotid occlusion signals a decrease in cellular oxygen availability in agreement with direct tissue Po4 measurements made in conjunction with Cyt. a,a redox changes during transient cortical ischemia in cats. We interpret the persistence of the increased reduction level of Cyt. a,a during the 25 minute period of brain underperfusion to reflect an early lesion in cellular energy metabolism. Apparently this occurs even without the earliest detectable changes in brain high energy stores, creatine phosphate, as in animals having MABP values exceeding the 100 mm Hg threshold.

The relatively small hemodynamic and intramitochondrial metabolic recovery after restoration of flow in one carotid artery is somewhat unexpected in view of previous CBF measurements. Our results are, nevertheless, corroborated by the observations of LaManna et al. in open skull preparations which demonstrated compromised blood flow and tissue Po4 after flow reinstatement. Changes in intracranial pressure due to mild edema would lead to vascular compression and thus exacerbating the situation. The recorded, continuous but slow decrease in brain blood volume points to the same conclusion.

The immediate hyperoxidation of Cyt. a,a upon complete restoration of carotid flow indicates accelerated tissue oxygen extraction. This is supported by the fact that cerebral blood volume continues to increase simultaneously with a sustained higher level of hemoglobin deoxygenation. Since direct cortical stimulation normally gives rise to an oxidation rather than a reduction of Cyt. a,a...18 However, less is known regarding the intramitochondrial metabolic status of the tissue in vivo. For this reason, our protocol incorporates measurements of changes in the redox state of the enzyme cytochrome c oxidase which reacts directly with molecular oxygen in the obligatorily coupled process of energy production, i.e., oxidative phosphorylation.

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doi: 10.1161/01.STR.12.6.864

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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