Reduced Nicotinamide Adenine Dinucleotide Fluorescence and Cortical Blood Flow in Ischemic and Nonischemic Squirrel Monkey Cortex. 2. Effects of Alterations in Arterial Carbon Dioxide Tension, Blood Pressure, and Blood Volume

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Abstract:
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The controversy regarding the effects of hypercapnia or hypocapnia on blood flow in regions of focal ischemia can be resolved only by determining the associated changes in the oxidation-reduction state of the tissue and the concurrent focal metabolic acidosis. Similarly, it is necessary to know the effects of alterations in mean arterial perfusion pressure (MABP) and blood volume, although these are more generally recognized and there is more unanimity of opinion regarding their role. As emphasized recently by both Plum and Waltz, one must define the model studied, distinguish between anoxia and ischemia, and establish the relative severity of the ischemia.

The studies presented here were performed with the preparation and methods described in detail in Part 1 of this report. This technique allows direct observation of a specific intracellular biochemical event in areas of focal incomplete cerebral ischemia in the squirrel monkey (Saimiri sciureus).

Methods

ANIMAL PREPARATION AND NADH FLUORESCENCE RECORDING
The standard preparation is described in detail in Part 1 of this report. A catheter with a three-way stopcock was inserted into the femoral artery for continuous measurement of MABP; microsamples of blood were taken intermittently for measurement of blood gases. All animals were under barbiturate anesthesia for operation and were ventilated with a Harvard respirator after paralysis with curare. Blood oxygen tensions were maintained between 120 and 140 mm Hg. Alterations in Paco were achieved through changes in the proportion of CO2 in the inspired gas. Relative changes in cerebral blood flow (CBF) were determined with an infrared microscope focused on an area of brain adjacent to that used for NADH recordings by the method described in Part 1. Core body temperatures were maintained at 36° ± 1°C by a heating blanket; room temperature was uniform at 24°C.

EXPERIMENTAL PROTOCOL
Each experiment was performed according to the same protocol. NADH fluorescence was recorded during a period of spontaneous respiration, during which the focus of the assembly was adjusted. Curare then was given and controlled ventilation was started; the Paco, was varied from 60 to 20 to 40 mm Hg. Then the middle cerebral artery (MCA) was occluded and the Paco, again was varied from 40 to 60 to 20 to 40 mm Hg. The clip was then removed from the
MCA and, with Paco, held constant at 40 mm Hg, recordings were continued during the period of luxury perfusion. In the vertical illumination group the animal was killed without recording of NADH levels at death. In the circumferential illumination group, NADH levels were recorded at death (produced by occlusion of the endotracheal tube).

ANIMAL GROUPS
Ten animals were studied by vertical illumination with the pulse-and-hold system: five were normotensive and normovolemic, and five were rendered hypotensive and hypovolemic by removal of 20 ml of blood and replacement with an equal amount of saline. Ten other animals were studied by circumferential illumination with the pulse-and-hold system; again, five were normotensive and normovolemic and five were hypotensive and hypovolemic.

Results

VERTICAL ILLUMINATION GROUP
The levels of NADH fluorescence, relative CBF, and MABP at various levels of Paco, before, during, and after focal cerebral ischemia are summarized in figure 1 for both normotensive and hypotensive animals. There was a significantly higher level of NADH during the period of ischemia in the hypotensive-hypovolemic animals, indicating increased vulnerability to focal ischemia. Prior to MCA occlusion the normotensive group did not demonstrate a remarkable change in NADH with alterations in Paco, and CBF. Relative changes in CBF cannot be compared in the two groups because the technique used here is not a quantitative measurement of CBF but only indicates relative changes in the same animal.

During the period of occlusion, alterations in NADH with changes in Paco, were difficult to evaluate for two reasons: (1) alterations in Paco, had an associated change in MABP that was probably important during the period of occlusion; (2) despite the use of the pulse-and-hold system, photodecomposition was a problem to the extent that relative alterations in NADH were difficult to evaluate over an extended period.

CIRCUMFERENTIAL ILLUMINATION GROUP
The data are summarized in figure 2. Again, the NADH level was significantly higher during the period of ischemia in the hypotensive-hypovolemic group. Photodecomposition was conspicuously absent in this group. NADH fluorescence increased to greater levels during the period of ischemia in the animals that were hypotensive and hypovolemic, compared to the first group. Hypocapnia seemed to increase the level of NADH fluorescence in both the normotensive and the hypotensive animals, but this was also associated with a significant decrease in MABP, so interpretation of this change is difficult.

Discussion

PHOTODECOMPOSITION
The obvious gradual decrease in NADH levels in the studies with vertical illumination reflects an unacceptable amount of photodecomposition in spite of the use of a pulse-and-hold system. Fortunately, this was obviated in the group studied with circumferential illumination and a less-intense excitation energy.

NONISCHEMIC CORTEX
Cortical blood flow studies with krypton-85 in this model have demonstrated a variation in CBF from 0.8 ml/gm per minute at a Paco, of 20 mm Hg to 1.8 ml/gm per minute at a Paco, of 60 mm Hg. The uniform levels of NADH fluorescence found in this study indicate the amazing ability of normal brain to adjust to changes in flow and Paco, instantaneously and are consistent with metabolic studies of the effect of hypercapnia on the energy state of normal brain. Similarly, with the preservation of normal autoregulation, major fluctuations in MABP did not

NADH fluorescence recorded by vertical illumination technique had artifacts from photodecomposition (see text). Animals rendered hypotensive and hypovolemic were more vulnerable to focal ischemia.
NADH IN CEREBRAL CORTEX. 2.

NADH fluorescence recorded by circumferential illumination technique (minimal excitation energy) produced measurements free from artifacts related to photodecomposition. Hypovolemic and hypotensive animals were more vulnerable to focal ischemia. Alterations in Paco₂ in nonischemic cortex did not change levels of NADH fluorescence. During ischemia, increased Paco₂ did not decrease NADH levels. Decreasing the Paco₂ was not apparently beneficial but was associated with a predictable decrease of MABP. NADH levels were not as high during focal incomplete ischemia as during total anoxia.

produce alterations in NADH until the MABP had fallen to below 50 mm Hg; this is consistent with the critical perfusion pressure documented by Siesjö and Zwetnow. 21

ISCHEMIC CORTEX

To interpret the NADH levels in ischemic cortex, it is necessary to review results of CBF studies with krypton-85 in this model. Those studies demonstrated a decrease in CBF of 60% to 70% after MCA occlusion. During the period of ischemia there is a loss of autoregulation, CBF becomes both pressure-dependent and volume-dependent, and there is a failure of response to alterations of Paco₂ in nonischemic cortex did not change levels of NADH fluorescence. During ischemia, increased Paco₂ did not decrease NADH levels. Decreasing the Paco₂ was not apparently beneficial but was associated with a predictable decrease of MABP. NADH levels were not as high during focal incomplete ischemia as during total anoxia. However, it should be acknowledged that the production of hypocapnia was accompanied by a modest decrease in MABP.

The results of the present investigation are consistent with these CBF studies and indicate that, in areas of ischemia, cerebral metabolic function is adversely affected by a decrease in MABP or blood volume (the latter possibly exerting an influence through a decrease in cardiac output secondary to a decrease in venous filling pressure). Changes in Paco₂ did not produce changes that could be evaluated independently of fluctuations in MABP. Previous metabolic studies in this preparation have indicated that hypocapnia produces a significantly lower tissue ATP level in areas of ischemia but hypercapnia fails to produce a protective effect. In this regard, the secondary effects of hypercapnia on intracellular pH also must be considered, effects perhaps compensated for in normal brain but not tolerated by ischemic tissue.

We had expected NADH levels to increase gradually in the region of ischemia (although cortical flow remains constant at a new but lower value) because ATP levels have been found to decrease to approximately 50% of normal after two hours of MCA occlusion, along with an increase in lactate to nine times normal. This constant NADH level may reflect a new steady state with a uniform but inadequate rate of oxidative phosphorylation. However, it also is possible that both the CBF measurements and the NADH recordings fail to reflect the state of deeper tissue which is more ischemic and in which metabolism is failing more rapidly (this tissue is included in the measurements of brain ATP and lactate).

ISCHEMIC TOLERANCE

In general, most clinical studies using xenon-133 fail to reflect the true degree of regional ischemia because of "look through," Compton's scatter, and the temporal relationship of the flow study to the onset of ischemia. The major exception to this generalization is measurements obtained during carotid endarterectomy with the indicator arriving in the area predestined for ischemia prior to the onset of ischemia. These measurements have indicated that the critical CBF under halothane anesthesia at normocapnia required to sustain a normal electroencephalographical pattern, and therefore presumably adequate oxidative phosphorylation, is 18 ml/100 gm per minute. Experimental studies support this value and have either cited a similar critical level or reported a percentage decrease in flow that approximates this amount. Blair and Waltz plotted the regional changes in an area of infarction in this model against time after MCA occlusion and found gradations in flow from values approaching zero in the core area to regions of hyperemia on the margins. The no-reflow phenomenon and a tissue tolerance of four minutes of total ischemia may
apply to the core area of major infarctions but most certainly do not at the margins.

The prompt return of NADH fluorescence levels after restitution of flow gives further evidence for the reversibility of certain degrees of ischemia. This coincides with previous metabolic studies in this preparation indicating a return toward normal of the electrocorticogram and ATP and lactate levels with restoration of flow after two hours of MCA occlusion and a failure to produce an anatomical infarction from this duration of partial ischemia. It emphasizes the necessity of considering the degree of ischemia when determining the tolerance of neural tissue to ischemia.

ANOXIA VERSUS ISCHEMIA

Siesjö and associates emphasized the profound difference between anoxia and ischemia. In the former there is normal perfusion pressure, a tolerated local tissue acidosis producing a homogeneous increase in tissue perfusion, and sufficient flow to remove metabolic waste products. In the latter there is a failure in tissue perfusion pressure, a pattern of "patchy" perfusion (at a microscopic level not yet recorded by standard flow studies), and an accumulation of lactic acid and waste products of metabolism. These differences are important when evaluating the results of studies of the type reported here.

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