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

BY THORALF M. SUNDT, JR., M.D., AND ROBERT E. ANDERSON

Abstract: The fluorescence of reduced nicotinamide adenine dinucleotide (NADH) from cerebral cortex was measured before, during, and after middle cerebral artery (MCA) occlusion and then at death of the animal. In normal cortex, NADH remained constant throughout a wide range of variations in blood pressure and Paco₂. In ischemic cortex, NADH levels were higher in hypovolemic hypotensive animals than in normotensive normovolemic animals. Neither hypercapnia nor hypocapnia was effective in decreasing NADH in regions of ischemia, but the latter was associated with a degree of hypotension that interfered with interpretation of data. NADH returned to normal with restoration of flow, supporting the reversibility of this degree of ischemia. The high levels of NADH at death, compared to those during ischemia, are consistent with incomplete ischemia in this model of cerebral infarction.

Additional Key Words: steal, cerebral autoregulation, reverse steal

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 (MAP) 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 MAP, 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 Paco₂ 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 Paco₂ was varied from 60 to 20 to 40 mm Hg. Then the middle cerebral artery (MCA) was occluded and the Paco₂ 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.

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.19, 23 The major exception to this generalization is measurements obtained during carotid endarterectomy with the indicator arriving in the area predisposed for ischemia prior to the onset of the ischemia.23, 24 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.18-19, 25-27 Blair and Waltz28 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 phenomenon29 and a tissue tolerance of four minutes of total ischemia30 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 coin-
cides with previous metabolic studies in this prep-
paration indicating a return toward normal of the elec-
trocorticogram 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 accumula-
tion of lactate and waste products of metabolism.

These differences are important when evaluating the
results of studies of the type reported here.

References

1. Brawley BW, Stranand DE Jr, Kelly WA: The physiologic
response to therapy in experimental cerebral ischemia. Arch
Neurul 17:180-187 (Aug) 1967

2. Symon L: Experimental evidence for "intracerebral steal"
following CO₂ inhalation. Scand J Clin Lab Invest 22

3. Lassen NA, Palvögyi R: Cerebral steal during hypercapnia
and the inverse reaction during hypocapnia observed by the
133Xe-technique in man. Scand J Clin Lab Invest 22

changes in carbon dioxide pressure and arterial pressure on
blood flow in ischemic regions of the brain in dogs. Circula-
tion Research 24:557-565 (Apr) 1969

5. Poulsen OB, Lassen NA, Skinhøj E: Regional cerebral blood
flow in apoplexy without arterial occlusion. Neurology
(Minneapolis) 20:125-138 (Feb) 1970

hyperventilation on experimental cerebral infarction.
Neurology (Minneapolis) 21:479-485 (May) 1971

graphic blood flow changes in experimental cerebral ischemia;
Effects of arterial carbon dioxide studied by fluorescence
angiography and xenon 133 clearance. J Neurosurg 32:115-166
(Aug) 1970

8. Yamaguchi T, Regli F, Waltz AG: Effect of Paco on
hypoperfusion and ischemia in experimental cerebral infarction.
Stroke 2:139-147 (Mar-Apr) 1971

cerebral blood flow: Response to carbon dioxide inhalation
in cerebrovascular disease. Arch Neurol 27:403-412 (Nov
1972

10. Lassen NA: The luxury-perfusion syndrome and its possible
relation to acute metabolic acidosis localized within the

11. Michenfelder JD, Sundt TM Jr: The effect of Paco, on the
metabolism of ischemic brain in squirrel monkeys. Anesth.
Analg. 43:445-453 (May) 1973

12. Folbergrová J, MacMillan V, Siesjö BK: The effect of
moderate and marked hypercapnia upon the energy state
and upon the cytoplasmatic NADH/NAD⁺ ratio of the rat

13. Meyer JS, Denny-Brown D: The cerebral collateral circula-
tion. Factors influencing collateral blood flow. Neurology
(Minneapolis) 7:147-158 (July) 1957

14. Waltz AG: Effect of blood pressure on blood flow in ischemic
and in nonischemic cerebral cortex: The phenomena of
autoregulation and luxury perfusion. Neurology (Minneapolis)
18:613-621 (July) 1968

15. Plum F: The clinical problem: How much anoxia-ischemia
damages the brain? Arch Neurol 29:359-360 (Dec) 1973

16. Waltz AG: Pathophysiology of cerebral ischemia. In
McDowell FT, Brennan RW (eds): Cerebral Vascular
Diseases, Eighth Princeton Conference. New York, Grune
& Stratton, p 110-126, 1973

cerebral circulation (cortical blood flow) with an infrared
microscope. Stroke 1:100-103 (Mar-Apr) 1970

18. Sundt TM Jr, Waltz AG: Cerebral ischemia and reactive
hyperemia: Studies of cortical blood flow and microcircula-
tion before, during, and after temporary occlusion of middle
cerebral artery of squirrel monkeys. Circulation Research
28:426-433 (Apr) 1971

19. Hanson EJ Jr, Anderson RE, Sundt TM Jr: Comparison of
133Xe- and 133Xe cerebral blood flow measurements
before, during, and following focal, incomplete ischemia in
the squirrel monkey. Circulation Research (in press)

hypercapnia upon tissue levels of NADH, lactate,
pyruvate, phosphocreatine, and adenosine phosphates of rat

21. Siesjö BK, Zweitman MT: The effect of hypovolemic hyper-
perfusion upon extra- and intracellular acid-base parameters and
79:114-124 (May) 1970

22. Sundt TM Jr, Michenfelder JD: Focal transient cerebral
ischemia in the squirrel monkey: Effect on brain adenosine
triphosphate and lactate levels with electrotocorticographic
and pathologic correlation. Circulation Research 30:703-712
(June) 1972

blood flow measurements and electronecephalograms
during carotid endarterectomy. J Neurosurg 41:310-320 (Sept
1974

Neurol Scand 49 (Suppl 52):1-86, 1973

yhydrate metabolism of man during respiratory and

26. Eklof B, Siesjö BK: Cerebral blood flow and cerebral energy

cerebral blood flow and cerebral metabolism. Surg Forum
23:417-419, 1972

28. Blair RDG, Waltz AG: Regional cerebral blood flow during
NADH IN CEREBRAL CORTEX. 2.

acute ischemia: Correlation of autoradiographic measurements with observations of cortical microcirculation. Neurology (Minneapolis) 20:802-808 (Aug) 1970


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

THORALF M. SUNDT, JR. and ROBERT E. ANDERSON

Stroke. 1975;6:279-283
doi: 10.1161/01.STR.6.3.279

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1975 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/6/3/279

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at: http://stroke.ahajournals.org//subscriptions/