Pial Arterial Pressure in Cats Following Middle Cerebral Artery Occlusion — II. Relationship to Regional Disturbance of Energy Metabolism

W. PASCHEN, PH.D.,* T. SHIMA, M.D.,† AND K.-A. HOSSMANN, M.D., PH.D.*

SUMMARY Focal cerebral ischemia was produced in 16 cats by occluding the left middle cerebral artery (MCA) for 120 min. Cortical blood flow and pial artery pressure were determined prior to vascular occlusion and after 15, 60 and 120 min. At the end of the experiments (after 120 min MCA occlusion) heads were frozen in situ with liquid nitrogen. Cooled brains were cut into 0.5 cm thick slices. From these slices twenty-micron sections passing through the territory of the MCA were prepared in a cryostat and used in the pictorial presentation of glucose and ATP. NADH-fluorescence was recorded from the tissue slice, immersed in liquid nitrogen. In addition, tissue samples were taken from regions of interest and used for quantitative determination of biochemical substrates.

In all but two animals permanent MCA occlusion led to disturbances in the energy-producing metabolism, as indicated by reduction in glucose and ATP, and increase in lactate. The regions exhibiting bright NADH-fluorescence were much smaller than those in which ATP was absent. In 6 animals NADH-fluorescence was not increased but even decreased in areas with disturbed energy-producing metabolism. A close correlation was obtained after comparing cortical blood flow measured 15 min after MCA occlusion with the area of ATP-depletion at the end of the experiments. However, the size of ATP-depletion did not correlate with flow measured 60 or 120 min after MCA occlusion. It is concluded that in focal ischemia the size of an infarct depends on the reduction in flow occurring immediately after vascular occlusion and that reduction in the NAD/NADH pool may account for the disturbances of the energy-producing metabolism observed following temporary focal ischemia.

Material and Methods

Biochemical reagents were obtained either from Sigma, St. Louis, USA, (firefly lanterns) or Boehringer, Mannheim, FRG (all enzymes and coenzymes necessary for glucose-induced bioluminescence and quantitative measurements of biochemical substrates).

Animal Preparation

Sixteen adult cats of either sex, weighing 2.0–3.5 kg, were initially anesthetized with 3% halothane, immobilized with curarine (Pancuronium, Organo, FRG) and subsequently ventilated with a gas mixture containing 0.5% halothane, 70% N₂O and the remainder O₂. Arterial pCO₂ was maintained between 28 and 30 mm Hg, and arterial pO₂ above 100 mm Hg by adjustment of the respirator and addition of oxygen. The temperature was maintained at 37°C using a thermoccontrolled heating pad.

The MCA was exposed by the transorbital approach as described by O'Brien and Waltz. Following craniotomy over the parietal region of the ipsilateral hemispheric pial artery pressure and cortical blood flow were measured in 10 animals at different times as described in the first part of this investigation. The exposed regions of the cortex were covered with saline-soaked cotton-wool balls and rinsed with warm saline to prevent drying and cooling of the cortical surface.

Biochemical Studies

Following stabilization of physiological parameters and the recording of control values, the left MCA was occluded. After 120 min occlusion brains were frozen in situ with liquid nitrogen. Heads, cooled with liquid nitrogen, were cut with a band saw in 0.5 cm thick coronal sections, which were subsequently cleaned from bone and soft-tissue in a low temperature cabinet
ATP-induced bioluminescence reactions, using the bioluminescence techniques: 12–14 freeze-dried brain slices were covered by 60 μm thick slices of frozen enzyme/substrate solutions containing all necessary enzymes, coenzymes, and cofactors for glucose- and ATP-induced bioluminescence reactions, using the bioluminescence system of luminiferous bacteria and firefly lanterns. NADH-fluorescence was recorded from the cut surface of the tissue section as described by Welsh and Rieder.15

Small samples (about 20 mg tissue) were then taken from both regions of high and low ATP-bioluminescence, and phosphocreatine (PCr), adenosinetriphosphate (ATP), adenosinediphosphate (ADP), adenosinemonomophosphate (AMP), glucose, lactate, and pyruvate determined quantitatively. Metabolites were extracted using perchloric acid (660 mmol × 1⁻¹). After centrifugation the pellets were washed with perchloric acid. The combined supernatants were neutralized with KOH (7.1 mol × 1⁻¹) and the pellets redissolved in NaOH (1 mol × 1⁻¹) and assayed for protein16 using crystalline bovine serum albumin as standard. Substrates were measured using fluorometric enzymic techniques as described by Lowry and Passonneau.17

In order to correlate metabolic disturbances and changes in cortical blood flow or pial artery pressure, the size and localization of the ATP-depleted regions varied considerably: on average 44.1% ± 5.5% of the cross-section of the ipsilateral hemisphere. However, in the other two animals there was only a moderate reduction as indicated by ATP-depletion of brain tissue. In 14 out of 16 animals, energy-producing metabolism was found to be intact (PCr), adenosinetriphosphate (ATP), adenosinediphosphate (ADP), adenosinemonomophosphate (AMP), glucose, lactate, and pyruvate determined quantitatively. Metabolites were extracted using perchloric acid (660 mmol × 1⁻¹). After centrifugation the pellets were washed with perchloric acid. The combined supernatants were neutralized with KOH (7.1 mol × 1⁻¹) and the pellets redissolved in NaOH (1 mol × 1⁻¹) and assayed for protein16 using crystalline bovine serum albumin as standard. Substrates were measured using fluorometric enzymic techniques as described by Lowry and Passonneau.17

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Results

Physiological Parameters

The general physiological and hemodynamic changes observed in the present study have been described in detail elsewhere.4 The changes in pial artery pressure and cortical blood flow before and during MCA occlusion are summarized in Table 1. Prior to vascular occlusion the pial artery pressure was 50.4 ± 3.2 mm Hg (mean ± S.E.M.) and the cortical blood flow, expressed as cortical heat conductance, was 15.2 ± 1.0 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹. Occlusion of the MCA led to an abrupt decrease in heat conductance and pial artery pressure to about 10 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹ and 7.8 mm Hg, respectively. Heat conductance improved within the first few minutes after MCA occlusion and remained stable at about 11.8 × 10⁻⁴ cal cm⁻¹ sec⁻¹ °C⁻¹ between 15 and 120 min; however, the pial artery pressure increased substantially from 8.1 to 15.0 mm Hg during this period.

Regional Biochemical Findings

Occlusion of the left MCA leads to disturbances in the energy-producing metabolism in 14 out of 16 animals, as indicated by ATP-depletion of brain tissue. In the other two animals there was only a moderate reduction in ATP. The size and localization of the ATP-depleted regions varied considerably: on average 44.1% ± 3.6% (mean ± S.E.M.) of the ipsilateral hemisphere was affected (range 18.7% to 61.7%). In 8 animals ATP-depleted regions were sharply demarcated: there was no visible border-zone that exhibited only a partial decrease in ATP (fig. 1). Within the ATP-depleted tissue the content of glucose and high energy phosphates was low, leading to marked

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TABLE 1 Hemodynamic Parameters prior to and after Middle Cerebral Artery Occlusion in Cats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 10)</th>
<th>15 min (n = 10)</th>
<th>60 min (n = 10)</th>
<th>120 min (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial PCO₂ (mm Hg)</td>
<td>29.2 ± 0.5</td>
<td>29.2 ± 0.3</td>
<td>29.9 ± 0.3</td>
<td>29.6 ± 0.2</td>
</tr>
<tr>
<td>Systemic arterial pressure (mm Hg)</td>
<td>113 ± 3.2</td>
<td>113 ± 3.0</td>
<td>111 ± 2.9</td>
<td>113 ± 3.0</td>
</tr>
<tr>
<td>Cortical blood flow*</td>
<td>15.2 ± 0.3</td>
<td>11.8 ± 0.2</td>
<td>11.9 ± 0.3</td>
<td>11.9 ± 0.3</td>
</tr>
<tr>
<td>Pial artery pressure (mm Hg)</td>
<td>50.4 ± 3.2</td>
<td>8.1 ± 0.6</td>
<td>11.9 ± 1.0</td>
<td>15.0 ± 1.0</td>
</tr>
</tbody>
</table>

*Cortical blood flow was expressed as cortical heat conductance (λ). Values are means ± SEM.

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**Figure 1.** Regional distribution of glucose (A), ATP (B), and NADH-fluorescence (C) in a cat brain after 120 min left middle cerebral artery occlusion. Pictorial representation of glucose and ATP was achieved by means of bioluminescence techniques. Tissue samples were taken from regions of interest as indicated in (A) and extracted for quantitative determination of metabolites — phosphocreatine (PCr), adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), glucose (Glu), lactate (Lac), pyruvate (Pyr) — and calculation of energy charge (EC) and lactate/pyruvate ratio (L/P). Values are expressed in nmol/mg protein.
reduction in the energy charge (fig. 1, Region 1–3). In all animals the region exhibiting high NADH-fluorescence was much smaller than that in which ATP-bioluminescence was absent: In figure 1 the basal ganglia of the left hemisphere show bright homogeneous NADH-fluorescence, whereas no bright NADH-fluorescence was apparent in the cerebral cortex (fig. 1, Region 3) although the tissue was depleted from ATP. In 6 animals regions with moderately reduced ATP content were apparent in the vicinity of ATP-depleted areas (fig. 2, Region 1). Within this border-zone glucose and high-energy phosphates were reduced to about 50% of the control value (fig. 2). Again, in these animals the area of bright NADH-fluorescence was much smaller than that of low ATP content: In figure 2 the sulcus (fig. 2, Region 2) fluoresced strongly indicating high NADH content, whereas there was little NADH-fluorescence in the ectosylvian gyrus.

In 6 of these 14 animals which exhibited ATP-depletion tissue fluorescence was not increased but even decreased indicating low NADH content in areas with disturbed energy-producing metabolism. As illustrated in figure 3, the basal ganglia of the left hemisphere exhibited low tissue fluorescence although this region was depleted of ATP (fig. 3, Region 2).

Correlation of Blood Flow and Size of ATP-depleted Tissue

A close negative correlation (correlation coefficient $r = -0.83$) was obtained after comparing cortical blood flow measured 15 min after MCA occlusion with the area of ATP-depletion at the end of the experiments, i.e. after 2 hours (fig. 4A). However, the size of the ATP-depleted region did not correlate with the cortical blood flow measured 60 or 120 min after MCA occlusion (fig. 4B, C) (correlation coefficients $r = -0.15$ and $r = -0.33$, respectively).

Correlation of Pial Artery Pressure and Size of ATP-depleted Tissue

From table 4 it can be seen that pial arterial pressure improved substantially during 120 min MCA occlusion. However, no close correlation was found between the pial artery pressure measured 15, 60 or 120 min after MCA occlusion and the tissue areas exhibiting disturbances of the energy-producing metabolism (correlation coefficients $r = -0.55$, $r = -0.65$, $r = -0.52$, respectively) (fig. 5A–C).

Discussion

Occlusion of the MCA led to disturbances in the energy-producing metabolism in 14 out of 16 cats. Disturbances in the energy-producing metabolism were evaluated by planimetric measurement of the ATP-depleted area on the cross-sections. Since cross-sections were taken from similar planes of each brain the area of ATP-depletion reflected the volume of damaged brain tissue.

An important finding in the present series of experiments is the observation that the volume of ATP-depleted tissue correlated closely with cortical blood flow following 15 min vascular occlusion but not with blood flow after 60 or 120 min occlusion. These results indicate that in focal ischemia the size of an infarct depends on the reduction in flow occurring immediately after vascular occlusion, and that any later increase in blood flow, even within the first hour of ischemia, has no or only minor influence on the severity of the metabolic disturbances. This is in accordance with recent observations that 120 min reversible MCA occlusion in cats leads to irreversible damage of the energy-producing metabolism.18

The sequence of glycolytic changes following occlusion of a main supplying brain artery are well-

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### Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>PCr</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>EC</th>
<th>Glu</th>
<th>Lac</th>
<th>Pyr</th>
<th>L/C</th>
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<tr>
<td>1</td>
<td>8.8</td>
<td>2.2</td>
<td>1.5</td>
<td>1.1</td>
<td>1.5</td>
<td>19.3</td>
<td>15.3</td>
<td>0.61</td>
<td>193.7</td>
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<tr>
<td>2</td>
<td>1.5</td>
<td>5.6</td>
<td>3.6</td>
<td>2.2</td>
<td>3.0</td>
<td>7.5</td>
<td>31.0</td>
<td>0.49</td>
<td>710.9</td>
</tr>
<tr>
<td>3</td>
<td>3.0</td>
<td>20.1</td>
<td>2.2</td>
<td>0.6</td>
<td>0.6</td>
<td>9.0</td>
<td>30.0</td>
<td>1.02</td>
<td>62.5</td>
</tr>
<tr>
<td>4</td>
<td>36.2</td>
<td>21.9</td>
<td>5.1</td>
<td>1.1</td>
<td>0.8</td>
<td>35.3</td>
<td>24.1</td>
<td>0.70</td>
<td>30.5</td>
</tr>
<tr>
<td>5</td>
<td>35.9</td>
<td>20.9</td>
<td>4.0</td>
<td>1.5</td>
<td>0.8</td>
<td>37.3</td>
<td>51.4</td>
<td>0.68</td>
<td>75.6</td>
</tr>
</tbody>
</table>

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### Figure 2

Regional distribution of glucose (A), ATP (B), and NADH-fluorescence (C) in a cat brain after 120 min left middle cerebral artery occlusion. Other details as described in legends to figure 1. Note the low ATP content and low NADH-fluorescence in the basal ganglia of the left hemisphere.
ATP-depleted tissue. The size of the regions exhibiting ATP-depletion was measured planimetrically and expressed as per cent of the cross-section of the ipsilateral hemisphere. Cortical blood flow was determined 15 min (A), 60 min (B), and 120 min (C) after middle cerebral artery occlusion. Note the close correlation between size of ATP-depleted tissue and cortical blood flow determined 15 min after artery occlusion (A).

Low NADH-fluorescence may be caused by a change in the redox state or by a depleted NAD/NADH pool. In fact, reduction in the NAD/NADH pool has been shown recently to occur after reversible cerebral ischemia. These authors observed reduced NAD in brain regions with low ATP and in some areas tissue was almost depleted of NAD (reduction of about 90%). Since the NAD content estimated in tissue samples is an average value for the different cellular compartments (cytoplasm, mitochondria), the reduction in NAD observed by Welsh et al may be even more pronounced in the cytoplasm where glucose metabolism takes place. NAD is a cofactor for enzymes involved in glucose metabolism. Thus, glucose utilization may be influenced by reduced NAD if it becomes the limiting factor. If this assumption is valid, namely, that disturbances of the energy-producing metabolism are caused by a reduction in the NAD/NADH pool following focal cerebral ischemia, stimulation of NAD synthesis or inhibition of NAD degradation may prove beneficial in the therapeutic approach to improve the outcome after an ischemic episode. Experiments are under way to test this hypothesis.

**Acknowledgement**

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**References**

Regional Blood Flow in Canine Brain During Nicotine Infusion: Pentobarbital vs. Chloralose Anesthesia*

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SUMMARY We compared vasoactive effects of intravenous nicotine (36 µg/kg/min) in regional cerebral circulations under pentobarbital and chloralose anesthesia. Experiments were conducted in three groups of dogs: Group I, pentobarbital anesthesia with fixed ventilation; Group II, chloralose anesthesia with fixed ventilation; Group III, chloralose anesthesia with free breathing. Values for regional cerebral blood flow measured with 15 µm radioactivity microspheres were used to compute regional cerebral vascular resistance (rCBR). In Group I, nicotine had no effect on rCBR in cerebral cortex, and it increased significantly rCBR in cerebellum (+17%), pons (+13%), medulla (+23%), and spinal cord (+19%). Using chloralose instead of pentobarbital in dogs with fixed ventilation (Group II), caused a significant reduction in rCBR in the cerebral cortex during nicotine, although it did not alter significantly nicotine-induced changes in rCBR in other regions of the brain. Hypocapnic alkalosis during nicotine-induced hyperventilation (Group III) resulted in significant increases in rCBR in all regions of the brain; however, the increases in rCBR in non-cortical regions more than doubled those in the cerebral cortex. The present results indicate: 1) Nicotine-induced vasodilation in cerebral cortex was blunted by pentobarbital anesthesia. 2) Nicotine-induced vasodilation in cerebral cortex under chloralose anesthesia was sufficient to nullify in part the potent vasoconstrictor effect of hypocapnic alkalosis.

INTRAVENOUS NICOTINE activates vasomotor mechanisms which cause pronounced systemic cardiovascular responses, i.e., marked elevations in arterial blood pressure and total peripheral resistance. These mechanisms include the sympathoadrenal system and vasopressin released into the systemic circulation from the posterior pituitary gland. Activation of the sympathoadrenal system is via the arterial chemoreceptors, the sympathetic ganglia (including the adrenal medulla), and the central nervous system. We have demonstrated in pentobarbital-anesthetized, artificially-ventilated dogs that the vasomotor mechanisms activated by nicotine affect vascular resistance in cerebral cortex, but not in other regions of the brain. In the cerebral cortex, nicotine caused predominant beta-adrenergic receptor mediated vasodilation, although it also activated alpha-adrenergic (vasoconstrictor) receptors, and a non-adrenergic, non-cholinergic vasodilator mechanism.

Pentobarbital is a barbiturate which may have blunted nicotine-induced cerebral vasomotor responses in our earlier study because of its ability to 1) depress autonomic reflex pathways; 2) depress activity and metabolic rate in the central nervous system, and 3) reduce responsiveness of vascular smooth muscle. Therefore, in the present study nicotine-induced changes in regional cerebral blood flow and vascular resistance in artificially-ventilated dogs anesthetized with pentobarbital were compared to those in artificially-ventilated dogs anesthetized with chloralose. Chloralose is a non-barbiturate general anesthetic, which is relatively free of the depressant effects of Pentobarbital on cerebral vasomotor mechanisms. In addition, we assessed the strength of nicotine-induced cerebral vasodilation by testing its ability to override the cerebral vasoconstrictor effect of chemoreflex-mediated hypocapnic alkalosis in chloralose-anesthetized, free-breathing dogs.

Methods Experiments were performed in 21 mongrel dogs of both sexes ranging in weight from 16 to 24 kg. These dogs were randomly divided into three equal groups. Dogs of Group I were anesthetized with pentobarbital sodium, 30 mg/kg i.v., while dogs of Groups II and III were anesthetized with alpha chloralose, 100 mg/kg.
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