Effects of Hypoxia–Reoxygenation on Microvascular Endothelial Function in the Rat Hippocampal Slice

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Background: Cerebral ischemia and hypoxia may cause injury to both neuronal and vascular tissue. The direct effects of hypoxia on endothelial function in intraparenchymal cerebral arterioles are unknown. Using a modification of the rat brain slice preparation, allowing continuous imaging of these previously inaccessible vessels, microvessel dilation was evaluated before and after a brief hypoxic episode.

Methods: Rat brain slices were superfused with oxygenated artificial cerebrospinal fluid. Hippocampal arterioles were visualized using computerized videomicroscopy, and their diameters (range, 12–27 μm) were measured using image analysis. After preconstriction with prostaglandin F₂ₐ and controlled pH and carbon dioxide tension, graded concentrations of either acetylcholine (endothelium-dependent vasodilation) or sodium nitroprusside (endothelium-independent vasodilation) were given before and after a 10-min period of hypoxia.

Results: Sodium nitroprusside (100 μM) caused similar dilation before and after hypoxia (mean ± SEM: 9.6 ± 0.6% vs. 13.0 ± 0.9%). Acetylcholine (100 μM) caused significantly less dilation (P < 0.05) after hypoxia (mean ± SEM: 9.3 ± 1.8% vs. 3.6 ± 1.2%). The decreased acetylcholine-induced dilation after hypoxia was not reversed by pretreatment with L-arginine (1 mM), the precursor of nitric oxide (mean ± SEM: 8.8 ± 1.3% vs. 4.4 ± 0.7%).

Conclusions: Even brief periods of hypoxia may cause endothelial dysfunction in intraparenchymal cerebral arterioles. This does not seem to be related to a deficiency of the nitric oxide substrate, l-arginine. Endothelial dysfunction and impaired endothelium-dependent dilation of microvessels may decrease oxygen delivery and increase neuronal injury during cerebral hypoxia–reoxygenation. (Key words: Brain; cerebral blood flow; hippocampus; microcirculation; vasodilation.)

CEREBRAL ischemia and hypoxia are major causes of morbidity and mortality. These insults may damage both neuronal and vascular tissue. Early restoration of blood flow and oxygen delivery is considered desirable but may, paradoxically, increase the injury caused by the primary insult.¹ A critical event in the pathogenesis of cellular injury is endothelial dysfunction and impaired endothelium-dependent dilation.² This phenomenon has been described during hypoxia in isolated large cerebral arteries in vitro,³,⁴ and after hypoxia–reoxygenation⁵ and ischemia–reperfusion⁶–⁹ in pial arteries and arterioles in vivo. It has not been previously described in intraparenchymal cerebral arterioles. Although these arterioles are of similar size to the smaller pial arterioles examined in in vivo studies, they may differ from pial vessels with respect to their responsiveness,¹⁰ regulation,¹¹,¹² and physiologic function.¹³,¹⁴ A modification in the preparation of the rat brain slice has allowed high-resolution, continuous imaging of intracerebral arterioles using computerized videomicroscopy.¹⁰,¹⁵,¹⁶ These vessels remain embedded in the neuropil, where they are under the influence of the local neurohormonal activity, which may alter vascular tone in vivo. Using this model, we previ-
ously demonstrated a direct and potent hypoxia-induced microvascular dilation.\textsuperscript{17}

The aims of the current investigation were to evaluate the response of intracerebral microvessels to endothelial-dependent (acetylcholine [ACh]) and endothelium-independent (sodium nitroprusside [SNP]) vasodilators and to examine the effect of a brief, 10-min period of hypoxia on microvascular endothelial function as reflected by the response to these vasodilators.

Materials and Methods

All experimental procedures used in this investigation were reviewed and approved by the Animal Use and Care Committee of the Medical College of Wisconsin, with protocols completed in accordance with the Guiding Principles in the Care and Use of Laboratory Animals of the American Physiological Society and in accordance with National Institutes of Health guidelines. All animals used in this investigation were housed within the animal facilities of the Medical College of Wisconsin, accredited by the American Association for the Accreditation of Laboratory Care.

**General Preparation**

Adult male Sprague Dawley rats (weight: 150–300 g, with no significant weight differences between animals in each group) were used in these experiments. The animals were placed in a specially designed holding chamber and subsequently anesthetized by breathing halothane (Anaquest Inc., Madison, WI) in oxygen. After achieving sufficient anesthesia, a midline thoracotomy was performed, and 20 ml of 0.9% saline at room temperature was slowly infused intracardially into the left ventricle while simultaneously making a right atrial incision for blood drainage. The animals were then decapitated, and the brains were rapidly removed and rinsed with nutrient medium (artificial cerebrospinal fluid [aCSF]) of the following composition: 124 mM NaCl, 5 mM KCl, 2.4 mM CaCl\textsubscript{2}, 1.3 mM MgCl\textsubscript{2}, 10 mM glucose, 1.24 mM KH\textsubscript{2}PO\textsubscript{4}, and 26 mM NaHCO\textsubscript{3}. Nutrient medium was prepared daily and equilibrated with 95% O\textsubscript{2} and 5% CO\textsubscript{2} to achieve a p\textsubscript{H} of 7.4.

Brains were cut freehand into blocks containing the hippocampus. A vibratome mechanical tissue slicer (OTS-3000-03; FHC, Brunswick, ME) was used to immediately section the block into coronally oriented tissue slices (250–280 \mu m thick). Throughout the slicing procedure, tissues were continuously bathed in the oxygenated aCSF at room temperature. Subsequently, the slices were transferred to a Plexiglas holding chamber (M&G Plastic Specialists, West Allis, WI) and maintained at interface with oxygenated aCSF at the same temperature. Individual slices were then transferred for examination to a recording chamber mounted on an inverted halogen transillumination microscope (Nikon Diaphot 200, Yokohama, Japan).

The recording chamber, designed in our laboratory, consisted of a main recording/superfusion compartment with a volume of 2.8 ml and an elevated side chamber for vacuum suction that prevented significant fluid waves. Slices were submerged onto a nylon mesh, allowing continuous superfusion of aCSF on all sides of the brain slices. Flow through the recording chamber was at a rate of 2.0 ml/min, thus completely exchanging the chamber volume in less than 2 min. The chamber temperature was continuously monitored and maintained at 34°C using a thermoelectric Peltier device coupled to a sensor thermistor. The temperature at which brain slices have been successfully maintained ranges from room temperature to normal body temperature. Brain slices last longer and remain healthier at lower temperatures (30–35°C).\textsuperscript{18} Despite the apparently low temperatures used in this preparation, we and other investigators have previously shown that vessels under these conditions react appropriately to hypercarbia and hypocarbia.\textsuperscript{19} graded hypoxia,\textsuperscript{17} vasoconstrictors such as vasopressin and prostaglandin F\textsubscript{2}α (PGF\textsubscript{2}α),\textsuperscript{11,17} and vasodilators such as volatile anesthetics and SNP.\textsuperscript{10,16}

The slices were maintained in this chamber and continuously superfused with the oxygenated aCSF for approximately 1 h before the initiation of the experimental protocol. During this equilibration period, an intracerebral microvessel was located. The aCSF, which superfused the brain slices, was equilibrated with a mixture of oxygen and carbon dioxide sufficient to maintain the oxygen tension (P\textsubscript{O},) at approximately 500 mmHg and the pH and carbon dioxide tension (P\textsubscript{CO},) within normal limits. Gas analysis (Radiometer ABL\textsubscript{3}, Copenhagen, Denmark) of the superfused fluid was obtained during the equilibration period and repeated before each measurement of vessel diameter. The P\textsubscript{O}, was also continuously measured with an inline flow-through P\textsubscript{O}, electrode (DO-166FT; Lazar Research Laboratories, Los Angeles, CA), which was positioned immediately proximal to the inflow of aCSF into the recording chamber.

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Microvessel Analysis

A microvessel (range, 12–27 μm in diameter) was located within the hippocampal neuronal tissue, and the vessel was continuously monitored with videomicroscopy. An inverted halogen transillumination microscope equipped with a 40× objective (Olympus WPlanFL 160/0, Tokyo, Japan) and a 2.25× video projection lens (Nikon CCTV/Microscope Adapter, Yokohama, Japan) transmitted the image to a video camera (CCD 72; Dage MTI, Michigan City, IN) for display on a video monitor (Sony HR Trinitron, Tokyo, Japan). Vessel imaging was recorded on videotape using a VHS video recorder (Magnavox, Rebersburg, PA), and diameters were analyzed using a computerized imaging analysis system (Metamorph Imaging System; Universal Imaging Corp., West Chester, PA) with an IBM-compatible computer. All measurements of cerebral microvessel diameters were performed within 5 h of the tissue slice preparation.

Experimental Protocol

There were two main experimental groups, the ACh group and the SNP group. During baseline conditions (P_{O_2} 500 mmHg), PGF_{2α} infusion for preconstriction was started and continued throughout the entire protocol in both groups. After a stable control diameter was attained, vessel diameters were measured after each dose of either ACh or SNP. After again returning to the control diameter, hypoxia was instituted for 10 min (P_{O_2} < 115 mmHg) followed by a 30-min control period (P_{O_2} 500 mmHg). Vessel diameters were again measured before, during, and after ACh or SNP administration. All drugs were diluted in ACSF and infused directly into the recording chamber. ACh and SNP were each given at bath concentrations of 1 μM, 10 μM, and 100 μM in sequence, for 10 min at each dose. PGF_{2α} was given to produce a stable control diameter and to approximate physiologic myogenic tone. The bath concentration was varied (1–2 μM) to produce preconstriction of 10–15% from the baseline diameter before drug administration and to allow a return of the vessel to its preconstricted diameter after drug administration and after hypoxia. Hypoxia was induced by changing the gas mixture oxygenating the brain slices from 95% O_2/5% CO_2 to 95% N_2/5% CO_2 and was continued to the end of the experiment.

To determine if alterations in the posthypoxia dilator response were caused by a deterioration in endothelial responsiveness over time, the experimental protocol for the ACh group was repeated, but without the induction of hypoxia (time-control experiment for ACh). Vessels were preconstricted with PGF_{2α}, and incremental doses of ACh (1 μM, 10 μM, and 100 μM) were given on two occasions, with a time interval of 90 min between the first and second administrations. Bath P_{O_2} remained at 500 mmHg throughout this protocol.

Data Analysis

Microvessel diameters were derived as an average of 10–13 measurements taken every 6–10 μm along approximately 80 μm of vessel length. The percentage constriction of the cerebral arteriole from the baseline diameter was calculated using the following equation:

\[
\%\ Constriction = \left(\frac{D_{BL} - D_N}{D_{BL}}\right) \times 100
\]

where \(D_{BL}\) is the baseline diameter at the start of the experiment, and \(D_N\) is the new diameter. The percentage dilation of the cerebral arteriole was calculated as a percentage of the control diameter using the following equation:

\[
\%\ Dilation = \left(\frac{D_N - D_{CTRL}}{D_{CTRL}}\right) \times 100
\]

where \(D_N\) is the new diameter, and \(D_{CTRL}\) is the control diameter. Before hypoxia, dilation was calculated using the preconstricted diameter as the control diameter. After hypoxia, dilation was calculated using the diameter at the end of the posthypoxia control period as the control diameter.

Statistical Analysis

The percent constriction, percent dilation, and results of gas analysis within each group were analyzed by one-way repeated measures analysis of variance. The baseline arteriolar diameters, percent constriction, percent dilation, and the results of gas analysis in different groups, were analyzed by one-way analysis of variance.
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Table 1. Baseline, Prehypoxia, and Posthypoxia Arteriolar Diameters and Percentage Constriction from Baseline Diameter

<table>
<thead>
<tr>
<th></th>
<th>SNP Group</th>
<th>ACh Group</th>
<th>ACh + L-arg Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of slices</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Baseline diameter (μm)</td>
<td>17.6 ± 1.0</td>
<td>17.2 ± 0.9</td>
<td>16.4 ± 2.0</td>
</tr>
<tr>
<td>Prehypoxia control diameter (μm)</td>
<td>15.5 ± 1.0</td>
<td>15.3 ± 0.9</td>
<td>14.6 ± 1.8</td>
</tr>
<tr>
<td>Constriction from baseline (%)</td>
<td>11.7 ± 1.0</td>
<td>10.8 ± 1.6</td>
<td>11.0 ± 0.9</td>
</tr>
<tr>
<td>Posthypoxia control diameter (μm)</td>
<td>15.8 ± 1.0</td>
<td>15.3 ± 0.8</td>
<td>14.5 ± 1.7</td>
</tr>
<tr>
<td>Constriction from baseline (%)</td>
<td>10.0 ± 1.1</td>
<td>11.1 ± 1.4</td>
<td>11.6 ± 0.8</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. There were no significant differences between the groups or between pre- and post-hypoxia control diameters within any group. SNP = sodium nitroprusside; ACh = acetylcholine; L-arg = L-arginine.

In both cases, follow-up multiple comparisons were made with Duncan’s multiple range test. Differences were considered statistically significant when the $P$ value was < 0.05. All data are expressed as mean ± SEM.

Results

A total of 56 hippocampal slices were obtained from 27 animals. Brain slices were excluded from the analyses if microvessels could not be adequately visualized or if the luminal diameters of the microvessels were not clearly discernible throughout the experiment. The number of slices that completed the protocol, arteriolar diameters, and percentage constriction are shown in table 1. There were no differences between groups in baseline diameters, prehypoxia or posthypoxia control diameters, or percent constriction from baseline. Within each group, there was no significant difference between the prehypoxia and posthypoxia control diameters. The duration of the experiments was typically <$5$ h, which is less than the established viability of brain slices in this preparation (>10 h with excellent preservation of vascular reactivity as determined by vasoconstrictor responses to potassium chloride administration\(^{15}\)). The results of gas analyses are shown in table 2. There were no differences in pH, $P_{CO_2}$, or $P_{O_2}$ between groups at any time measured during the protocol. Although there was a significant decrease in $P_{O_2}$ during hypoxia as compared with the $P_{O_2}$ during baseline, there were no differences in pH or $P_{CO_2}$ within any group throughout the experimental protocol. Hypoxia-induced dilation was 10-14% from baseline, similar to that which we described previously.\(^{15}\)

In the SNP group, SNP caused increasing dilation be-

Table 2. Results of Gas Analysis

<table>
<thead>
<tr>
<th></th>
<th>SNP Group</th>
<th>ACh Group</th>
<th>ACh + L-arg Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline pH, CO₂ (mmHg)</td>
<td>7.40 ± 0.02</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>35.8 ± 0.6</td>
<td>36.3 ± 0.7</td>
<td>37.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>496 ± 46</td>
<td>431 ± 31</td>
<td>402 ± 35</td>
</tr>
<tr>
<td>SNP or ACh 100 μM pH, CO₂ (mmHg)</td>
<td>7.39 ± 0.01</td>
<td>7.38 ± 0.03</td>
<td>7.36 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>36.6 ± 0.3</td>
<td>39.6 ± 1.9</td>
<td>37.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>469 ± 49</td>
<td>495 ± 42</td>
<td>428 ± 44</td>
</tr>
<tr>
<td>Baseline pH, O₂ (mmHg)</td>
<td>7.37 ± 0.01</td>
<td>7.39 ± 0.02</td>
<td>7.38 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>37.3 ± 0.8</td>
<td>39.2 ± 0.7</td>
<td>38.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>114 ± 5</td>
<td>95 ± 8*</td>
<td>109 ± 5*</td>
</tr>
<tr>
<td>SNP or ACh 100 μM pH, CO₂ (mmHg)</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.03</td>
<td>7.39 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>36.8 ± 0.7</td>
<td>39.5 ± 1.6</td>
<td>36.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>460 ± 51</td>
<td>446 ± 17</td>
<td>435 ± 53</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. There were no differences between the groups, while there were significant decreases in $P_{O_2}$ levels during hypoxia in all groups.

SNP = sodium nitroprusside; ACh = acetylcholine; L-arg = L-arginine. $P_{CO_2}$ and $P_{O_2}$ = partial pressure of carbon dioxide and oxygen, respectively.

* $P < 0.05$ versus baseline values.
Fig. 1. Dilation of hippocampal arterioles in response to increasing doses of sodium nitroprusside (SNP; an endothelium-independent vasodilator) before and after a hypoxic period of 10 min. There was no significant difference between prehypoxia and posthypoxia dilation. Error bars represent SEM. *P < 0.05 vs. sodium nitroprusside.

fore and after hypoxia (fig. 1). Vessel diameters were significantly different from their control diameters at concentrations of 10 μM (7.4 ± 1.1% dilation) and 100 μM (9.6 ± 0.6% dilation) before hypoxia and at all three concentrations (5.9 ± 1.2%, 9.8 ± 1.2%, and 13.0 ± 0.9% dilation at 1 μM, 10 μM, and 100 μM, respectively) after hypoxia. Although posthypoxia dilation to SNP tended to be greater than prehypoxia dilation, this difference did not reach statistical significance at any concentration evaluated (fig. 1).

In the ACh group, superfusion with ACh dilated microvessels before hypoxia, with maximal dilation occurring during 100 μM ACh (fig. 2). Vessel diameters were significantly different from their control diameters at all three concentrations before hypoxia (5.4 ± 2.1%, 7.4 ± 2.3%, and 9.3 ± 1.8% dilation at 1 μM, 10 μM, and 100 μM, respectively). However, diameters were not different from control during any concentration of ACh after hypoxia (maximal dilation, 3.6 ± 1.2% during 100 μM). In contrast to the effects of SNP, ACh caused significantly less dilation after hypoxia as compared with prehypoxia dilation. The vasodilation caused by SNP and ACh were not different before hypoxia at any concentration, whereas the ACh-induced dilation was significantly less than that caused by SNP after hypoxia at all concentrations.

In the group that received ACh plus l-arginine, ACh 100 μM caused dilation of 8.8 ± 1.3% before hypoxia, which was similar to that observed in the ACh group. After hypoxia, in the presence of l-arginine, vasodilation was significantly attenuated (4.4 ± 0.7%) as compared with prehypoxia dilation (fig. 2). In other words, l-arginine infusion did not prevent the decreased ACh-induced dilation after hypoxia, as the dilation caused by ACh was similar in the presence and absence of l-arginine. In addition, vessel diameters were significantly different from their respective controls before, but not after hypoxia.

In the time-control experiment for ACh (n = 5), vessel diameters were significantly greater than their control diameters at all three concentrations during both the first and second administrations of ACh (fig. 3). There was no significant difference in dilation at each dose between these administrations (11.9 ± 1.6% and 12.3 ± 1.8% during the first and second administrations of 100 μM ACh).

Fig. 2. Dilation of hippocampal arterioles in response to increasing doses of acetylcholine (ACh; an endothelium-dependent dilator) before and after a hypoxic period of 10 min. The experiment was repeated at the highest dose of ACh (100 μM) in the presence of l-arginine. l-arginine was given during and after the period of hypoxia. There was no significant difference between the effect of ACh 100 μM in the presence or absence of l-arginine. Error bars represent SEM. *P < 0.05 vs. prehypoxia. $P < 0.05 vs. ACh 1 μM.
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The major finding of this investigation is that hypoxia specifically impaired microvascular dilation caused by ACh but not that caused by SNP. ACh-induced vasodilation requires the presence of endothelial cells and the release from these cells of NO or a NO-containing compound.21 NO activates vascular smooth muscle-soluble guanylate cyclase, which increases the synthesis of cyclic guanosine monophosphate and leads to vasodilation.21 In the brain, NO is produced by two isoforms of NO synthase (NOS), endothelial NOS and neuronal NOS.21 In this preparation, the dilator response of arterioles to ACh is blocked by the nonselective NOS inhibitor, N-nitro-L-arginine, but not by the specific neuronal NOS inhibitor, 7-nitroindazole.11 The presence of a vasodilator response to ACh is evidence of intact endothelial function after tissue preparation. In contrast, SNP-induced vasodilation does not require the presence of endothelium and is not decreased by NOS inhibitors, as SNP spontaneously generates NO.22

The decreased ACh-induced dilation after hypoxia suggests the presence of microvascular endothelial dysfunction and decreased endothelium-dependent dilation. The preserved dilation in the absence of hypoxia shows that this was not simply a time-related phenomenon. The unchanged response to SNP is evidence that hypoxia did not impair the guanylate cyclase pathway or overall smooth muscle function. Although increasing doses of SNP caused more dilation after hypoxia than before hypoxia, this difference was not statistically significant. This response may be related to a decreased basal level of cyclic guanosine monophosphate in vascular smooth muscle and a subsequent exaggerated response to activation of guanylate cyclase.23 Although basal release of NO contributes to the resting tone of microvessels in this preparation,11 our protocol tested agonist (ACh) stimulated rather than basal release of NO. Vessel diameters were similar before and after hypoxia as a result of the titration of the dose of PGF2α to ensure similar control diameters and allow a comparison of ACh-induced dilation before and after hypoxia.

Fig. 3. Time-control experiment for acetylcholine (ACh) showing ACh-induced dilation of hippocampal arterioles in the absence of hypoxia (bath P02, 500 mmHg). ACh was given at times corresponding to those in the ACh group. There was no significant difference in dilation between the first and second administrations of ACh at each dose. In both cases, dilation was similar to that in the ACh group before hypoxia. Error bars represent SEM. $p < 0.05$ vs. ACh 1 μM.

Discussion

The present findings agree with in vitro observations of isolated basilar arteries, where hypoxia caused a decrease in endothelium-dependent dilation.3,4 Hypoxia-reoxygenation5 and ischemia-reperfusion7,9 also decreased endothelium-dependent, rather than endothelium-independent, dilation of pial arteries and arterioles. Just as cerebral arterial responses may not predict those of cerebral arterioles,24,27 pial and intraparenchymal arterioles may differ in their sensitivity to vasodilators,10 their regulation by endogenous neurotransmitters,11,12 and their role in regulating cerebral blood flow.13,14 Although pial arterioles may act as pressure modulators, conducting and distributing blood from large arteries to the penetrating arterioles,13,14 intraparenchymal arterioles may play a principal role in regulating local cerebral blood flow to functional cerebral cortical units.14

The in vitro brain slice model allows examination of the microvascular effects of hypoxia independent of changes in other factors that may affect cerebral blood flow.28 Delivery of oxygen to the brain in vivo occurs via a network of capillaries.29 In the brain slice preparation, this must occur by diffusion from the surface of the slice, necessitating higher concentrations of oxygen in the surrounding fluid.30 The standard hypoxic insult chosen in most brain-slice studies is administration of 95% N2/5% CO2.30 Although this is 0% O2, the bath PO2 has been shown to be 89–111 mmHg after 30 min.29 However, 95% N2/5% CO2 is an effective hypoxic stimulus as reflected by reversible, electrophysiologic changes,30 including hippocampal synaptic silence within 2–3 min.31 In addition, using a microelectrode, brain slice tissue PO2 at a depth of 100 μm was between 0 and 20 mmHg, whereas bath PO2 remained approximately 90

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mmHg. We have previously shown that these vessels significantly dilate when the bath Po, is ≤ 200 mmHg.

The potential causes of the impaired ACh-induced dilation in this study include decreased synthesis and release or inactivation of NO. The synthesis of NO requires the presence of both L-arginine and molecular oxygen. L-arginine did not prevent the decrease in ACh-induced dilation after hypoxia. Thus, the cause of the blunted response does not seem to be a deficiency of this substrate for the production of NO. This is in contrast with the ability of L-arginine to restore ACh-induced pial arteriolar dilation after light-dye endothelial injury. However, it is in agreement with other studies that suggest that the availability of L-arginine is usually not rate-limiting for NOS activity in cerebral endothelium.

Oxygen-derived free radicals, produced during hypoxia-reoxygenation or ischemia-reperfusion, may directly injure endothelium and inactivate NO. We cannot exclude the possibility that oxygen-derived free radicals were generated during the administration of 95% O2 and in the presence of a bath Po, of 500 mmHg. However, we believe that free radical-mediated injury, if present, would play a minor role in view of the results of our time-control experiments with ACh, which demonstrate no attenuation of the dilator response over time.

It is unlikely that impaired ACh-induced dilation was caused by muscarinic receptor dysfunction because ischemia may cause impairment of endothelium-dependent dilation as a result of other agents such as calcium ionophore A-23187 and bradykinin. In addition, hypoxia-reoxygenation does not affect the dissociation constant of ACh to endothelial M2 muscarinic receptors.

It is unclear whether the endothelial dysfunction observed in this investigation arose during hypoxia, reoxygenation, or both. Cerebral ischemia without reperfusion in mice decreased endothelium-dependent dilation of pial vessels but caused no change in endothelium-independent dilation. It is likely that in the present study, damage to the endothelium occurred during both hypoxia and reoxygenation as reperfusion may greatly increase the degree of vascular injury that has been caused by ischemia. In isolated middle cerebral arteries examined after in vitro ischemia—reperfusion, arteries that had been reperfused for 24 h, but not 1-2 min, had a reduction in ACh-induced dilation and abnormal myogenic reactivity. Ischemia—reperfusion may cause multiple ultrastructural alterations in the endothelial cells of cerebral microvessels. Changes may be observed after as little as 10 min of ischemia. In the isolated aorta, 15 min of hypoxia and 30 min of reoxygenation significantly increased the number of dead endothelial cells. This increase was prevented by pretreatment with superoxide dismutase and catalase.

We cannot exclude the possibility that ACh caused the release of NO from nonendothelial sites, e.g., neurons, and that hypoxia may have affected this release. However, we consider this unlikely because inhibition of neuronal NOS with 7-nitroindazole has no effect on ACh-induced dilation in this preparation. In addition, ACh causes normal or increased dilation of pial arterioles in neuronal NO gene-deficient (knockout) mice. However, ACh also causes dilation in endothelial NOS knockout mice, and this response seems to be partially NOS- and cyclic guanosine monophosphate-dependent.

As in other in vitro vascular experiments, the vessels in our preparation have minimal myogenic tone; therefore, vasodilator responses are best observed in the presence of pharmacologic vasoconstriction. During PGF2α constriction, the normal vasomotor responses to hypercarbia and hypocarbia and to hypoxia are well preserved.

The in vitro pathologic consequences of endothelial dysfunction in the cerebral circulation include decreased microvascular blood flow and tissue oxygen delivery. Postreperfusion abnormalities in endothelium-dependent dilation may be more pronounced in the microcirculation than in larger vessels. Endothelial dysfunction also decreases the release of vasodilator/cytoprotective agents such as NO, prostacyclin, and adenosine, which normally inhibit the activation of leukocytes and prevent tissue injury.

This is the first report of hypoxia-induced endothelial dysfunction in intraparenchymal cerebral arterioles. Hypoxia specifically inhibited endothelium-dependent, but not endothelium-independent, vasodilation. Endothelial dysfunction and decreased endothelium-dependent dilation may decrease microvascular blood flow and oxygen delivery and play an important role in the pathogenesis of reperfusion injury. Our findings suggest that endogenous and exogenous endothelium-dependent dilators will have a reduced effect after cerebral hypoxia and reoxygenation. Future studies using this model will investigate the potential of other pharmacologic agents in preserving normal endothelial function during and after hypoxic insults.

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References
