CO₂ Reactivity Measured by Perfusion MRI During Transient Focal Cerebral Ischemia in Rats

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Background and Purpose—CO₂ response was examined in rats undergoing 60 minutes of middle cerebral artery occlusion (MCAO) and 4.5 hours of reperfusion. Because it is not clear whether the vasoreactivity improves during reperfusion in parallel with tissue recovery, CO₂ response was determined spatially resolved, sequentially in the initially ischemic but later recovered areas and in the permanently damaged areas.

Methods—Apparent diffusion coefficient (ADC) maps were calculated from diffusion-weighted images, whereas CO₂ reactivity maps were determined from the difference in perfusion signal intensity before and after CO₂ stimulation. CO₂ reactivity (administration of 6% CO₂ for 5 minutes) was expressed in % change of perfusion signal intensity/mm Hg of PCO₂ increase. ATP levels of tissue were used as a measure of outcome. The recovered and permanently damaged tissues were differentiated by combined use of end-ischemic ADC map and ATP image at the end of the experiment.

Results—The preischemic (control) CO₂ reactivity of 3.5±0.9%/mm Hg decreased dramatically during MCAO in the ischemic hemisphere. During reperfusion, it remained <1%/mm Hg in the region with end-ischemic ADC <80% of the preischemic control value, but showed gradual recovery in the region with end-ischemic ADC >80% of control. Although at the end of the experiment the CO₂ reactivity was significantly higher in the recovered tissue than in the permanently damaged tissue (1.15±0.44 and 0.13±0.47%/mm Hg, respectively; P<0.01), it still remained far below the normal control value (P<0.01).

Conclusions—The noninvasive perfusion-weighted MR imaging in combination with a CO₂ challenge permits the investigation of the spatially resolved vascular reactivity during a longitudinal study of cerebral ischemia. Our data suggest that severe ischemia is followed by a prolonged disturbance of CO₂ reactivity, despite already normalized energy metabolism. (Stroke. 2000;31:2236-2244.)

Key Words: carbon dioxide ■ cerebral ischemia, focal ■ cerebral ischemia, transient ■ magnetic resonance imaging ■ reperfusion ■ vasomotor reactivity ■ rats

Responsiveness of cerebral blood flow and blood volume to CO₂ is an established test of cerebrovascular reactivity, which is an important hemodynamic index in cerebrovascular diseases. In intact brain, hypercapnia results in vasodilation, causing an increase in cerebral blood flow. This capacity of cerebral vessels has been extensively investigated during permanent middle cerebral artery occlusion (MCAO)1–4 as well as during temporary global cerebral ischemia.5–8 These studies observed abolished or even reversed CO₂ response during focal ischemia, but it was also found to be severely impaired during the reperfusion period after global cerebral ischemia. However, only 2 studies investigated the sequential changes of CO₂ reactivity in the recirculation period following transient focal cerebral ischemia.9,10 Although both of these investigations reported impaired reactivity in tissues with histological injury, little is known about the relationship between cerebrovascular reactivity after reperfusion and the severity of ischemic injury during focal cerebral ischemia, and even less is known about the relationship between vasoreactivity and tissue recovery during recirculation.

The development of high-resolution MRI allowed to assess the cerebrovascular reactivity using T₁*-weighted images. Because of the change in blood oxygenation levels during CO₂ administration, these images indirectly reflect the CO₂-induced increase in cerebral blood flow (CBF).10–12 The other MRI method suitable for estimation of cerebral perfusion and thus cerebrovascular CO₂ reactivity is perfusion-weighted MRI (PWI) using arterial spin labeling,12 because the perfusion signal intensity is linearly related to CBF.13,14

The great advantage of the noninvasive NMR technique is that it allows repetitive assessment of perfusion level and cerebrovascular reactivity in a single animal. When combined with diffusion-weighted imaging (DWI) or even quantitative maps of the apparent diffusion coefficient (ADC) of water, which are sensitive to the energy state of the tissue,15–17 the...
severity of ischemic damage can be assessed during ischemia, and the alteration of energy metabolism can also be estimated after reperfusion. Thus, this combination of perfusion- and diffusion-weighted images allows the simultaneous investigation of both hemodynamic and metabolic consequences of ischemia and recirculation.

In the present investigation, perfusion signal intensity, CO₂ reactivity, and ADC values were measured during 1 hour of MCAO and 4.5 hours of reperfusion, at the level of the caudate-putamen. Additionally, the ATP content was determined at the end of the experiment to assess the outcome. Our aim was to examine the temporal evolution of vasoreactivity during the reperfusion period (1) in tissues with varying severity of ischemic injury, defined and graded by the end-ischemic relative ADC, and (2) in tissues with or without recovery of energy metabolism. We investigated whether the CO₂ reactivity improves in parallel with the recovery of the energy metabolism during reperfusion or whether it shows a prolonged disturbance even in metabolically recovered tissue.

Materials and Methods

Animal Model

All experiments were performed in accordance with NIH animal protection guidelines and approved by the governmental authorities. Male Wistar rats (n = 5; body weight 300 to 350 g) were anesthetized with 1.5% halothane in a 70%/30% mixture of N₂O/O₂. Rectal temperature was monitored throughout the experiment and maintained at ~37°C with a feedback-controlled heating pad. Animals were tracheotomized, mechanically ventilated, and immobilized with pancuronium bromide (0.3 mg · kg⁻¹ · h⁻¹). Once mechanical ventilation had begun, halothane concentration was reduced to 0.8%. Arterial and venous catheters were inserted into the femoral vessels for injection of drugs, monitoring of systemic blood pressure, and blood sampling. Blood gases were measured repeatedly and kept within physiological limits by appropriate settings of the respirator. The animals were placed in a nonmagnetic stereotactic headholder for accurate positioning in the magnet.

Focal ischemia was produced by intraluminal suture occlusion of the right middle cerebral artery (MCA) using a previously described, remotely controlled occluding device. Briefly, a monofilament nylon thread (4-0 Prolene; Ethicon Co), with its distal end thickened to 0.28 to 0.30 mm in diameter with silicone, was connected to an extension catheter and passed through a guide sheath that was fixed to the neck of the animal. The right common carotid artery was ligated, and the filament was introduced into the right internal carotid artery via the proximal end of the isolated external carotid artery until the tip reached the carotid canal at the base of the skull. This arrangement permitted the manipulation of the thread position from outside the magnet to allow measurements during the preischemic control phase, during MCAO, and after retraction of the thread without the need to repositaion the animal. The success of the occlusion was confirmed by the drop of perfusion signal intensity in PWIs. After 1 hour of MCAO, reperfusion was induced by retraction of the thread.

MRI

NMR measurements were performed at 200 MHz using a BIOSPEC system (Bruker Medical) with a 4.7-T magnet of 30-cm clear bore. The system was equipped with actively shielded gradient coils (maximum gradient strength 100 mT/m; gradient rise time <250 μs). A 12-cm-diameter Helmholz coil was used for radiofrequency transmission, and a 16-mm-diameter surface coil with inductive coupling was placed over the skull of the animal for signal reception. The 2 coils were positioned orthogonally to each other to minimize coupling. The transmitter coil used active decoupling via a pin diode switch to further reduce coupling, whereas passive decoupling by crossed diodes was used for the surface coil.

Sagittal scout scans, with a gradient echo imaging sequence (echo time [TE]=8.3 ms, [repetition time] TR = 300 ms), were performed for correct positioning of the animal’s head in the magnet. DWI was performed with a multislice Stejskal-Tanner-type spin-echo sequence. The sequence parameters were TE=32.5 ms, TR=2325 ms, matrix = 128×128. Six coronal slices with thicknesses of 1.21 mm and 0.54 mm interacine gap were recorded with a field of view (FOV) of 4 cm × 4 cm. For quantitative determination of the ADC, DWIs with different diffusion-sensitizing gradient strengths (b factor: 30, 1500 s/mm²; gradient along the ventral-dorsal direction, ie, y direction in magnet reference system) were recorded before MCAO (control phase), at the beginning and at the end of ischemia, and once an hour during 4.5 hours of reperfusion. This resulted in 10 minutes experimental time for 1 complete ADC data set. To minimize instrumental errors in ADC determination, extensive data postprocessing, including correction for image-specific background noise and gradient cross-talk, was performed, as described elsewhere. ADC was calculated pixelwise by solving the monoexponential intravoxel incoherent motion (IVIM) model of Le Bihan. For this purpose the MEMRIS software package, written in interactive data language (IDL, Research Systems Inc), was used.

Single-slice PWIs through the center of the MCA territory (at the level of the caudate-putamen) were obtained with the arterial spin tagging technique. This slice position was set to correspond with slice 4 of the DWI multislice data set. The P1I sequence consisted of 2 similar image acquisition intervals separated by a recovery time of 10 seconds, each of which comprised a magnetization preparation step of 3 seconds' duration followed by snapshot fast low-angle shot [FLASH] imaging (TE=3.9 ms, TR=7.4 ms, FOV = 4 cm × 4 cm, slice thickness=2 mm, matrix=128×64). During the first image acquisition (perfusion-sensitive image), spins of blood flowing through the neck vessels were inverted adiabatically through the combination of a magnetic field gradient applied in the z direction and a B1 field set off-resonance to excite a slice through the neck ~2 cm upstream from the imaging plane. During the second acquisition (control image), the sign of the frequency offset of the B1 field was inverted so that inflowing spins were left undisturbed. PWIs were obtained by subtraction of both acquisitions. Eight subtraction images were averaged to improve signal to noise, resulting in a total scan time of 56 seconds for P1I. PWIs were normalized to the control snapshot FLASH images without arterial spin labeling to compensate for signal loss in regions more distal to the receiver surface coil.

Measurement Protocol

One MCA multislice set and 2 PWIs (1 before the CO₂ reactivity test and 1 at the end of the 5-minute CO₂ addition) were obtained before MCAO (control), at the end of 1 hour of MCAO (ischemia), and after 30, 90, 150, 210, and 270 minutes of reperfusion.

Cerebrovascular CO₂ reactivity, ie, the change of perfusion signal intensity during hypercapnia, was assessed by adding 6% CO₂ to the inhalation gas for 5 minutes. Arterial blood samples were taken for the measurement of arterial PCO₂ before and at the end of each hypercapnic period, as close as possible to the time when PWIs were obtained before and during CO₂ reactivity. At the end of the experiment, after 4.5 hours of reperfusion, animals were frozen in situ in liquid nitrogen for metabolic imaging (see below).

Image Analysis

ADC maps and normalized PWIs were transferred to a Macintosh Power PC 7200/66 (Apple). Image analysis was performed with the image processing software IMAGE (NIH). Data analysis was performed in individual voxels.

CO₂ reactivity index (CO₂R) values were calculated in every voxel by use of the perfusion signal intensity (SI) before and at the end of the CO₂ reactivity, according to the following equation:
Physiological Variables at Different Phases of the Experiment

<table>
<thead>
<tr>
<th>Phase of Experiment</th>
<th>pH Before</th>
<th>During</th>
<th>PCO₂ mm Hg Before</th>
<th>During</th>
<th>PO₂ mm Hg Before</th>
<th>During</th>
<th>MABP mm Hg Before</th>
<th>During</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.46±0.05</td>
<td>7.32±0.05</td>
<td>35.8±2.5</td>
<td>52.3±3.4</td>
<td>143±21</td>
<td>137±17</td>
<td>115±5</td>
<td>120±5†</td>
</tr>
<tr>
<td>Ischemia</td>
<td>7.46±0.08</td>
<td>7.35±0.04</td>
<td>35.2±2.5</td>
<td>55.5±2.3</td>
<td>138±26</td>
<td>125±17</td>
<td>120±15</td>
<td>125±16†</td>
</tr>
<tr>
<td>Reperfusion 30 min</td>
<td>7.45±0.06</td>
<td>7.36±0.07</td>
<td>33.4±3.2</td>
<td>53.9±2.6</td>
<td>130±19</td>
<td>124±16</td>
<td>116±6</td>
<td>120±3†</td>
</tr>
<tr>
<td>Reperfusion 90 min</td>
<td>7.45±0.07</td>
<td>7.35±0.04</td>
<td>32.7±2.4</td>
<td>55.7±5.9</td>
<td>145±30</td>
<td>129±14</td>
<td>113±10</td>
<td>120±7†</td>
</tr>
<tr>
<td>Reperfusion 150 min</td>
<td>7.44±0.06</td>
<td>7.33±0.11</td>
<td>33.4±2.5</td>
<td>53.6±4.7</td>
<td>134±21</td>
<td>121±15</td>
<td>110±11</td>
<td>114±9†</td>
</tr>
<tr>
<td>Reperfusion 210 min</td>
<td>7.48±0.05</td>
<td>7.30±0.07</td>
<td>35.1±4.2</td>
<td>54.9±4.3</td>
<td>150±13</td>
<td>138±20</td>
<td>111±15</td>
<td>115±15†</td>
</tr>
<tr>
<td>Reperfusion 270 min</td>
<td>7.42±0.03</td>
<td>7.32±0.08</td>
<td>34.6±2.3</td>
<td>55.4±6.6</td>
<td>140±20</td>
<td>128±16</td>
<td>100±8</td>
<td>106±7†</td>
</tr>
</tbody>
</table>

In the column headings, Before and During indicate before and during CO₂ test. Significance levels are indicated for comparison with the corresponding values before CO₂ test. *P<0.05; †P<0.01; §P<0.001.


(1) \[ \text{CO₂ R} = \frac{(\text{Perfusion SI during CO₂ test} - \text{Perfusion SI before CO₂ test})}{\Delta P\text{CO₂}} \times 100\% \]

Here, PCO₂ represents the CO₂ systemic partial pressure, and \( \Delta P\text{CO₂} \) is the difference in partial pressure between control and hypercapnic states. With the equation, the CO₂ reactivity index (CO₂ R) was expressed in [%/mm Hg (percent of change of perfusion SI/1 mm Hg change of arterial PCO₂)].

Relative ADC maps were calculated at the level of the caudate putamen by pixelwise division of the ADC maps during ischemia and reperfusion by the control ADC map before MCAO. To investigate the CO₂ reactivity in areas with different degrees of ischemic damage, pixels on the relative ADC map at the end of MCAO were divided into 5 subgroups depending on their relative ADC (<70%, 70% to 80%, 80% to 90%, 90% to 100%, and >100%, respectively, of control value). In these subgroups, the CO₂ reactivity values were determined before MCAO (control status), at the end of ischemia, and at different time points during reperfusion. Relative perfusion signal intensities were also calculated at each time point in the different end-ischemic subgroups and expressed as a percent of the values obtained from the homologous contralateral areas.

The ischemic tissue area at the end of MCAO was estimated by summing up all pixels with a relative ADC <80% of control, because this degree of relative ADC reduction has been described as correlating well with ATP depletion in the acute phase of permanent ischemia. The outcome at the end of the experiment was assessed from ATP images at the level of the caudate-putamen. Damaged tissue was defined as the brain area with ATP depletion, whereas the remaining tissue with normal energy state was considered vital at the end of the experiment. ATP depletion was defined as that ATP remaining tissue with normal energy state was considered vital at the end of the experiment. ATP depletion was defined as that ATP remaining tissue with normal energy state was considered vital at the end of the experiment. ATP depletion was defined as that ATP remaining tissue with normal energy state was considered vital at the end of the experiment.

### Biochemical Imaging

Brains were removed from the skull in a cold box at −20°C, and sliced at the same temperature into 20-µm thin sections with the use of a cryostat microtome. Coronal sections at the level of the caudate-putamen were processed for the regional distribution of ATP by evoking substrate-specific bioluminescence. Regional tissue pH was measured with the umbelliferone fluorescence technique of Csiba et al.

### Statistical Analysis

All values are given as mean±SD. The physiological parameters (PCO₂, PO₂, pH, mean arterial blood pressure) before and after the CO₂ reactivity test were compared by a paired t test. The preischemic CO₂ reactivity between the 2 hemispheres and the pH between the recovered and permanently damaged tissues were compared by ANOVA, followed by the Schefé test. Repeated measures ANOVA was used to detect differences in cerebrovascular CO₂ reactivity between the recovered and permanently damaged tissues. When repeated-measures ANOVA detected a statistically significant difference, the Schefé test was used to compare the CO₂ reactivity values at each time point. The relative perfusion signal intensities and CO₂ reactivity values during ischemia and at different time points of reperfusion were compared with the preischemic (control) period, using a paired t test. A difference of \( P<0.05 \) was considered statistically significant.

### Results

#### Physiological Variables

Except for the short periods of the CO₂ reactivity tests, all general physiological parameters remained within the normal range during all phases of the experiment (Table). Ventilation with 6% CO₂ for 5 minutes increased arterial PCO₂ by 17 to 23 mm Hg and led to a significant decrease of arterial pH and a slight but significant increase in systemic blood pressure.

These parameters normalized, however, within minutes after shut-off of the supplementary CO₂.

#### PWIs, ADC Images

Figure 1 shows a typical example, including the relative ADC maps, the PWIs before and after CO₂ stimulation, and the calculated CO₂ maps before MCA occlusion (control), during ischemia, and during 4.5 hours of reperfusion.

After advancing the thread = 11 mm from the bony canal, the perfusion signal intensity dropped in the ipsilateral hemisphere, indicating successful occlusion of the middle cerebral artery. One hour later, reperfusion was induced by retraction of the thread, resulting in an increase of the perfusion signal intensity (Figure 2b). It should be noted that secondary hyperperfusion occurred in only 1 animal, accompanied by the worst reactivity values within the whole group of animals. The ADC declined in the supplying territory of...
the occluded artery during 1 hour of ischemia in all animals and showed partial normalization during reperfusion. The proportion of pixels in the corresponding ADC ranges (70%, 70% to 80%, 80% to 90%, 90% to 100%, and >100%) were 48±2%, 19±6%, 15±2%, 12±2%, and 6±3%, respectively, of the hemisphere at the end of MCAO. The lesion area, defined by relative ADC <80%, encompassed 67±6% of the hemisphere at the end of ischemia and decreased to 37±24% at the end of reperfusion. The lesion area defined by ATP depletion at the end of the experiment was 33±23%.

CO2 Reactivity Before Ischemia
The preischemic CO2 reactivity in the ipsilateral hemisphere was slightly lower (3.52±0.88%/mm Hg) than in the contralateral hemisphere (4.05±0.97%/mm Hg), but this difference was not statistically significant. In the nonischemic hemisphere the increase in perfusion signal intensity induced by hypercapnia before, during, and at 30, 90, 150, 210, and 270 minutes after MCA occlusion was 4.05±0.96, 3.23±0.70, 3.60±0.42, 3.14±0.65, 3.15±0.33, 3.89±0.84, and 3.26±0.70%/mm Hg, respectively.

CO2 Reactivity in the Ischemic Hemisphere as a Function of the End-Ischemic Relative ADC Value
During ischemia, an inverse CO2 response was observed in the area with relative end-ischemic ADC <90% (Figure 2a). However, even in areas with normal or only slightly decreased ADC (ie, relative end-ischemic ADC >90% of control), the CO2 reactivity decreased dramatically to below 1%/mm Hg. It should be noted that these areas also showed a perfusion deficit, even though this did not lead to any significant ADC change (Figure 2b).

After reperfusion, the CO2 reactivity remained below 1%/mm Hg in the area which had suffered severe ischemic injury during MCAO (relative end-ischemic ADC <80%), indicating that vasomotor reactivity failed to recover in 4.5 hours of recirculation. However, the CO2 response in the region with less-severe ischemic damage (end-ischemic relative ADC >80% of control) showed gradual improvement, and by the end of the reperfusion was no longer significantly different from the response during the control period (Figure 2a).

CO2 Response in the Recovered and the Permanently Damaged Tissues
To differentiate between the tissues that were damaged at the end of ischemia but recovered during reperfusion and
those that showed no recovery during recirculation, we used a combination of the end-ischemic relative ADC map and the ATP image at the end of the recirculation period. At first, the end-ischemic lesion was defined by the end-ischemic relative ADC <80% of control value. These pixels were then divided into 2 groups, depending on the ATP status at the end of the experiment: pixels with ATP depletion (permanently damaged tissue) and pixels with normal ATP content (recovered tissue). The drop of perfusion signal intensity during MCAO and the relative end-ischemic ADC were similar in these 2 regions, indicating an equal degree of ischemic injury (Figures 3b and 3c). After recirculation, the relative perfusion signal intensity (expressed as percentage of the contralateral homotopic area) returned to the control value and showed slight, but not significant, hyperperfusion in both regions compared with the preischemic control value (Figure 3b). The relative ADC value improved significantly in both groups after reperfusion in relation to the end-ischemic value, but although it reached 90% in the recovered tissue and remained above 80% during reperfusion, it declined significantly in the permanently damaged group and had reached the end-ischemic value by the end of the experiment (Figure 3c). A dramatic drop in CO2 reactivity was observed in both regions during MCAO, which slightly increased during the first 2 hours of reperfusion (Figure 3a). However, in the second half of the recirculation period, the vasoreactivity declined in the permanently damaged tissue and reached ≈0, whereas it continued to increase in the finally recovered tissue. Although the difference between the CO2 reactivities of these groups was significant at the end of the experiment, the CO2
response remained clearly below the control value ($P<0.01$) in the recovered tissue as well, which indicated still-impaired cerebrovascular reactivity after transient focal cerebral ischemia despite normalized ATP levels. The tissue pH at this time was 7.02 ± 0.13 and 6.33 ± 0.23 in the recovered and the permanently damaged tissues, respectively ($P<0.02$).

**Discussion**

The present study demonstrates a relationship between the severity of ischemic damage during 1 hour of MCAO and the disturbance of the CO$_2$ response after reperfusion. A prolonged disturbance of CO$_2$ reactivity was observed after severe ischemic injury not only in the ATP depleted area but also in the region with normal ATP content, which shows a dissociation between the recovery of energy metabolism and the improvement of vasoreactivity.

As the hypercapnia-induced blood flow change can be estimated from the difference in perfusion signal intensities before and after CO$_2$ stimulation, we used the PWI to construct the CO$_2$ reactivity map. The CO$_2$ response in the healthy hemisphere was between 3% and 4%/mm Hg throughout the observation time, a finding that corresponds with the results obtained by various methods in both human and animal studies. $^{9,12,27,28}$

**CO$_2$ Reactivity During Ischemia**

During MCAO, the cerebrovascular reactivity showed an inverse response to CO$_2$ stimulation in the severely damaged tissue (relative ADC <80% of control value), indicating the “steal” phenomenon. However, although positive, the vasoactivity was also seriously impaired in the brain area with normal or only slightly decreased relative ADC (relative ADC >90% of control value), where the disturbance of water and ion homeostasis could only be mild or negligible. The seriously impaired or inverse CO$_2$ response during ischemia is well known from earlier studies. $^{4,9,10,29}$ Seki et al. $^9$ found a negative or very low CO$_2$ response in severe (rCBF <40% of the control value) or moderate (40%<rCBF<70% of the control value) ischemia but only slightly impaired reactivity during mild ischemia (rCBF >70% of the control value). Dirnagl and Pulsinelli $^3$ reported similar results. Our findings are in good agreement with those of Dettmers et al. $^4$ who reported exhausted CO$_2$ reactivity during MCAO in baboons not only in infarcted or penumbral tissue but also in the remaining region of the ipsilateral hemisphere. Symon et al. $^{29}$ reported reduced CO$_2$ reactivity values in the ischemic hemisphere of baboon brains even where histological examination revealed normal tissue. The severely depressed reactivity and the inverse CO$_2$ response in the ischemic core and penumbra are usually explained by the accumulation of metabolites and development of tissue acidosis, which leads to maximal vasodilation of cerebral vessels, preventing any further response to hypercapnia.

There are other possible explanations for the fact that CO$_2$ reactivity was found to be impaired in the area with normal ADC. According to the definition of autoregulation, decreased perfusion pressure causes cerebral vasodilation, thus serving as a compensatory mechanism for maintaining constant cerebral blood flow. However, below a threshold, where the vessels are maximally dilated, the decrease of blood flow is paralleled by a decrease of perfusion pressure, indicating that the cerebrovascular reserve capacity has been exhausted. Regional CBF, on the other hand, can be decreased far below the control value without affecting ADC. $^{17,30}$ This means that the decreased flow induced by the decreased perfusion pressure usually indicates maximally dilated vessels but does not necessarily lead to ADC reduction. A similar example with decreased perfusion pressure and disturbed CO$_2$ reactivity is frequently seen in asymptomatic internal carotid artery occlusion in humans. The other possible explanation relates to spreading depression, which occurs shortly after ischemia $^{31}$ and results in a long-lasting reduction of cortical blood flow with impaired CO$_2$ response. $^{27,32}$

There was no difference in CO$_2$ response during MCAO between areas that were permanently damaged and those that recovered. This means that the CO$_2$ reactivity during MCAO cannot be used to predict the outcome after 4.5 hours of reperfusion.

**CO$_2$ Reactivity During Reperfusion**

Analysis of vasoreactivity in the different end-ischemic subgroups demonstrated a better and faster recovery of CO$_2$ reactivity after less severe ischemic injury (end-ischemic relative ADC >80%) but showed lack of recovery after severe ischemic damage (end-ischemic relative ADC <80%) (Figure 2a).

We found that after a comparable ischemic injury (graded by means of amount of change of the relative end-ischemic ADC), the CO$_2$ response was abolished in the permanently damaged tissue but was also impaired in the recovered tissue (Figure 3a) with normal ATP. This result indicates a prolonged disturbance of cerebrovascular reactivity after reperfusion despite recovery of energy metabolism. At first sight, our findings seem to contradict the earlier observations of Ono et al $^{10}$ and Seki et al. $^9$ who reported recovered or unimpaired CO$_2$ reactivity in regions without histological damage. However, the ischemia was mild or only moderate in those regions during MCAO, as emphasized by Seki et al and also shown by Ono et al. This means that their investigations failed to establish whether the observed recovery of the CO$_2$ response should be attributed to tissue recovery after reperfusion or to the less-severe ischemia during MCAO. Our data support the latter possibility, because the vasoreactivity recovered better and faster after less severe ischemic damage (end-ischemic relative ADC >80%; Figure 2a), but remained impaired during recirculation after severe ischemic injury (end-ischemic relative ADC <80%), even in the recovered tissue with normal ATP content (Figure 3a).

In agreement with our results, prolonged suppression of CO$_2$ reactivity was reported after 30 minutes of near-complete forebrain ischemia in rats $^{33}$ or after 60 minutes of complete brain ischemia in cats, $^3$ despite progressive recovery of electrophysiological and neurological functions and despite complete recovery of ADC and ATP. $^{16,34}$ However, as long as the duration of cardiac arrest did not exceed 10 minutes, the CO$_2$ response returned to the control value within 5 hours. $^6$ According to our findings, the recovery of...
vasoreactivity in this case is probably due to the shorter duration of the cerebrocirculatory arrest, which leads to less-severe ischemic damage.

The loss of vasoreactivity in the ATP-depleted area after reperfusion is not surprising, because energy failure leads to anaerobic metabolism, producing severe acidosis, as confirmed by the data presented here. Severe acidosis may result in lack of reactivity of cerebral vessels to vasodilatory stimuli35 but cannot account for the impaired CO2 response in the recovered tissue, as the brain pH was found to be normal there. Since Harder and Madden36 reported that the CO2 molecule also has a pH-independent influence on the membrane potential of the cerebrovascular smooth muscle cells, various other mechanisms have been shown to influence cerebrovascular resistance. Recent studies have suggested that cerebral vasodilatation in response to hypercapnia largely depends on formation of nitric oxide and vasodilator prostanoids.37,38 Inhibition of neuronal nitric oxide synthase or of cyclooxigenase in normal rats led to a decrease of CO2-induced arteriolar dilation by 77% and 83%, respectively.39 Several studies reported decreased vasoreactivity after transient cerebral ischemia, and suggested that either the hypercapnia-induced vasodilator prostanoid40 or neuronal nitric oxide synthesis41,42 might be hindered during the recirculation phase, whereas other results concluded that the action of these molecules at their effector sites in the vascular smooth muscle could be disturbed,43,44 leading to an impaired CO2 response.

In the present study, tissue viability was defined by the presence of ATP, but this definition has to be used with caution. Dissociation of the fast restoration of high-energy phosphates from the very slow or absent improvement of protein synthesis45–47 suggests that restoration of energy metabolism is essential for neuron revival after reperfusion, but that this is only 1 of numerous processes involved in the maintenance and control of neuronal and vascular functions. In other words, the normalization of energy metabolism is a necessary but not sufficient criterion for tissue recovery. The dissociation of the recovery of energy metabolism from the persistent disturbance of vasoreactivity further supports this notion. It could be speculated that oxygen requirements and consumption increase after recovery of energy metabolism; however, due to the disturbed vasoreactivity, this increased metabolism is not coupled to a parallel rise of blood flow. This then results in an increase of oxygen extraction and may, in critical cases, lead to hypoxia and stimulation of anaerobic glycolysis. Consequently, the prolonged suppression of the CO2 reactivity in the tissue with recovered ATP content could result in secondary energy failure. However, several other factors may be responsible for the secondary energy failure, and we feel that although the above mentioned hypothesis could be of a significance in cases of impaired recirculation or delayed hyperperfusion, but it is probably not relevant to the present experiments, where adequate reperfusion was observed (Figure 3b). Certainly, we cannot exclude the possibility that the observed ATP and ADC normalizations reflect only transient improvement in the recovered tissue, because secondary energy failure could occur later47,48 and the ADC showed a tendency to decline again within the observation period of the present study (Figure 3c).

Studies that use longer reperfusion periods will be needed to address the question of whether the CO2 reactivity remains low in the recovered tissue area or whether recovery of functional vasoreactivity lags behind the recovery of energy metabolism.

References

Diffusion-perfusion MRI is currently being used with increasing frequency for both clinical and experimental investigations. Clinically, these MRI technologies are used to establish the location and extent of focal ischemic injury rapidly after stroke onset. The presence of a diffusion-perfusion mismatch shortly after stroke onset appears to afford the possibility to predict a response to thrombolytic treatment. Clinical trials of new acute stroke therapies are using diffusion-perfusion MRI as part of the drug development process to document treatment effects on ischemic lesion evolution. Preclinically, diffusion-perfusion MRI is used to evaluate the temporal and spatial evolution of focal brain ischemia, to enhance understanding of the pathophysiology of focal ischemic injury, to determine the presence of secondary events after successful reperfusion, and to follow in vivo the effects of a variety of neuroprotective and thrombolytic interventions on ischemic brain injury.

Applying these MRI techniques to study physiological responses in vivo in both normal and ischemic brain is relatively novel. In the experiment reported by Olah and colleagues, perfusion imaging was used to measure CO$_2$ reactivity in normal and ischemic brain of rats undergoing temporary middle cerebral arterial occlusion. They observed a significant increase of CBF in non-ischemic cerebral cortex. Stroke 2017;5057–5061.


**Editorial Comment**
also demonstrated poor vasoreactivity at the end of the observation period.

This elegant study confirms observations about vasoreactivity demonstrated by other techniques but also provides much new information. This group of investigators and others will presumably use diffusion-perfusion MRI to extend these observations and to investigate other important physiological consequences of focal ischemic brain injury.

Marc Fisher, MD, Guest Editor
University of Massachusetts Medical School
Worcester, Massachusetts

References
CO<sub>2</sub> Reactivity Measured by Perfusion MRI During Transient Focal Cerebral Ischemia in Rats
Laszlo Olah, Claudia Franke, Wolfram Schwindt and Mathias Hoehn

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