Dynamics of Cerebral Tissue Injury and Perfusion After Temporary Hypoxia–Ischemia in the Rat

Evidence for Region-Specific Sensitivity and Delayed Damage

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Background and Purpose—Selective regional sensitivity and delayed damage in cerebral ischemia provide opportunities for directed and late therapy for stroke. Our aim was to characterize the spatial and temporal profile of ischemia-induced changes in cerebral perfusion and tissue status, with the use of noninvasive MRI techniques, to gain more insight in region-specific vulnerability and delayed damage.

Methods—Rats underwent 20 minutes of unilateral cerebral hypoxia-ischemia (HI). We performed combined repetitive quantitative diffusion-weighted, T2-weighted, and dynamic susceptibility contrast-enhanced MRI from before HI to 5 hours after HI. Data were correlated with parallel blood oxygenation level–dependent MRI and laser-Doppler flowmetry. Finally, MRI and histology were done 24 and 72 hours after HI.

Results—Severe hypoperfusion during HI caused acute reductions of the apparent diffusion coefficient (ADC) of tissue water in the ipsilateral hemisphere. Reperfusion resulted in dynamic perfusion alterations that varied spatially. The ADC recovered completely within 1 hour in the hippocampus (from 0.68±0.07 to 0.83±0.09×10^{-3} mm²/s), cortex (from 0.56±0.06 to 0.77±0.07×10^{-3} mm²/s), and caudate putamen (from 0.58±0.06 to 0.75±0.06×10^{-3} mm²/s) but only partially or not at all in the thalamus (from 0.65±0.07 to 0.68±0.12×10^{-3} mm²/s) and substantia nigra (from 0.80±0.08 to 0.76±0.10×10^{-3} mm²/s). Secondary ADC reductions, accompanied by significant T2 elevations and histological damage, were observed after 24 hours. Initial and secondary ADC decreases were observed invariably in the hippocampus, cortex, and caudate putamen and in approximately 70% of the animals in the thalamus and substantia nigra.

Conclusions—Region-specific responses and delayed ischemic damage after transient HI were demonstrated by MRI. Acute reperfusion-induced normalization of ADCs appeared to poorly predict ultimate tissue recovery since secondary, irreversible damage developed eventually. (Stroke. 1998;29:695–704.)

Key Words: cerebral ischemia, transient ■ magnetic resonance imaging ■ selective vulnerability ■ rats
viable for longer times and could therefore be responsive to delayed therapeutic interventions. In this view, early recognition and differentiation of these regions are crucial. The aim of our study was to evaluate the concepts of region-specific ischemic sensitivity and delayed tissue damage in association with brain perfusion dynamics in a rat model of transient HI. We were especially interested in the hyperacute region-specific tissue response in relation to long-term postischemic injury since this could provide prognostic information on the development of damage. We used noninvasive MRI techniques that allowed longitudinal and multiparametric measurements and combined these with parallel LDF measurements and histology.

Materials and Methods

Animal Model

Experimental protocols were approved by the Groningen Medical School Animal Experiment Committee. We used a modified Levine model (global hemispheric ischemia induced by unilateral carotid artery ligation with hypoxic ventilation) in the rat.

Male Wistar rats (weight, 305 to 370 g) were anesthetized with subcutaneous injections of a mixture of pentetate (Gd-DTPA) (0.5 mol/L). The left femoral artery was cannulated for continuous recording of the blood pressure (Datascope). After a neck incision the left CCA was cannulated for continuous recording of the blood pressure (Datascope). Inflation of the balloon inevitably accomplished unilateral occlusion of the CCA.

MRI Experiments

After surgery the animals were immobilized in a specially designed stereotaxic device and placed in an animal cradle. MRI was done on a 4.7-T SISCO/Varian Instruments NMR spectrometer (horizontal bore) with a 120-mm gradient insert (gradients up to 220 mT/m). A Helmholtz volume coil (85-mm diameter) and an inductively coupled surface coil (20-mm diameter) placed on the rat’s head, were used for signal transmission and detection, respectively. Brain coordinates were determined from a sagittal scout image, acquired with the use of a FLASH sequence.

Dynamic susceptibility contrast-enhanced (bolus track) MRI (FLASH, TR, 11 milliseconds; TE, 7 milliseconds; two acquisitions; 15° flip angle; FOV, 30 × 30 mm2; 64 × 64 data matrix; slice thickness, 1.7 mm; 120 consecutive images) was performed in combination with an intravenous bolus injection of Gd-DTPA (0.5 mmol/kg) given during the acquisition of the 13th MR image (time resolution, 0.7 second per image). Damage in our modified Levine model develops predominantly in areas served by the middle cerebral artery. Therefore, we performed bolus track MRI of a brain slice encompassing a relatively large middle cerebral artery territory, which was at the position 1 mm posterior to bregma according to Paxinos and Watson. The selected slice matched with the (single) slice in the BOLD MRI experiments and with slice number five in the multislice MRI experiments (see below).

Multislice DW MRI was performed with the use of a spin-echo sequence (TR, 2 seconds; TE, 33 milliseconds; two acquisitions; FOV, 30 × 30 mm2; 64 × 64 data matrix; eight contiguous 1.7-mm slices) with four different b values (0 to 1848 s/mm2), which allowed calculation of the tissue-water ADC. The measured ADC values in the brain are dependent on the direction of the diffusion-encoding gradient because of diffusion anisotropy, particularly in white matter. Nevertheless, the degree of ADC reduction in ischemic areas (arising predominantly in gray matter) is not influenced by changes in anisotropy. Besides, areas displaying a significant ADC decrease have been shown to be highly correlated with histological damage when the diffusion-sensitive gradient is applied along the z axis (ie, parallel to the long axis of the brain), which was done in the present study. Multislice T2-weighted MRI was also done with a spin-echo sequence, with three different echo times (30, 80, and 130 milliseconds).

BOLD MRI was done with a FLASH sequence (TR, 35 milliseconds; TE, 30 milliseconds; flip angle, 15°; FOV, 30 × 30 mm2; 64 × 64 data matrix; slice thickness, 1.7 mm). Images of a slice 1 mm posterior from bregma were collected during the onset and completion of HI (120 consecutive images; time resolution, 2.2 seconds per image).

MRI Protocol

A scheme explaining the timing of the experiments is given in Fig 1. MRI was done from approximately 1 hour before to 2 hours after HI (n = 10). In four of the animals MRI experiments were continued up to 5 hours after induction of HI. DW and T2-weighted MRI were performed repeatedly. Bolus track MRI was done on an hourly basis. It requires approximately 30 minutes to ensure essentially complete clearance of Gd-DTPA. Therefore, around the period of HI, bolus track MRI experiments were performed just before the end of HI (19 minutes after HI) in one group (n = 4) and just after the end of HI (25 minutes after HI) in another group of animals (n = 6). Since circulation of the contrast agent Gd-DTPA influences the tissue T2*, T2*-sensitive BOLD MRI during onset and completion of HI was performed in four separate animals that were not subjected to bolus track MRI experiments. DW, T2-weighted, and bolus track MRI were also performed 24 hours (n = 6) and 72 hours (n = 5) after HI in separate groups of animals.

Control experiments were performed in animals that underwent either 20 minutes of hypoxia without CCA occlusion (n = 4) or CCA occlusion under normoxic conditions (n = 4). The imaging protocol in these experiments was as described above, except that BOLD MRI was omitted.

Analysis of MR Data

Bolus track MRI data were processed as described previously. Briefly, contrast agent–induced SI changes were converted to changes...
in the transverse relaxation rate $1/T2^*$, ie, $\Delta R2^*$, $\Delta R2^*$ time plots were fitted to a $\gamma$-variate function that allowed calculation of the bolus peak time ($t_{\text{peak}}$), the maximal $\Delta R2^*$ ($\Delta R2^*_{\text{max}}$), and the area under the curve, which is proportional to the rCBV.25,26 Pixel-by-pixel fitting of the $\Delta R2^*$ time plots allowed generation of two-dimensional maps of the hemodynamic parameters. Brain maps of the ADC and T2 were generated from the DW and T2-weighted MRI measurements, respectively, by monexponential fitting on a pixel-by-pixel basis.25-27 Parametric images were analyzed in anatomic ROIs with use of the image analysis software package ImageBrowser (SISCO/Varian).

Laser-Doppler Flowmetry
In six rats, regional blood flow in the parietal cortex of both hemispheres was measured by means of LDF, essentially as described by De Wildt et al.28 from 2 minutes before (baseline) up to 2 hours after induction of HI (see also Fig 1). The parietal cortex was exposed on both the left and right hemispheres by a trepanation (approximately 6 mm2); the dura was left intact. Two LDF probes (PF 302, Perimed) on both the left and right hemispheres by a trepanation (approximately 6 mm2); the dura was left intact. Two LDF probes (PF 302, Perimed) were stereotaxically placed on the exposed dura at 1 mm posterior and 5 mm lateral to bregma. Regional LDF was expressed as a percentage of baseline.

Histology
After the MRI experiments at 24 and 72 hours after HI (see Fig 1), rats were killed by means of an overdose of anesthetic and directly after induction of HI (see also Fig 1). The parietal cortex was exposed on both the left and right hemispheres by a trepanation (approximately 6 mm2); the dura was left intact. Two LDF probes (PF 302, Perimed) were stereotaxically placed on the exposed dura at 1 mm posterior and 5 mm lateral to bregma. Regional LDF was expressed as a percentage of baseline.

Figure 1. Schematic representation of the sequence of experiments performed before, during, and after HI in the rat (number of animals between parentheses). The subdivision in horizontal bars reflects the three parallel studies. Time zero corresponds to the onset of HI. The shaded region corresponds to the 20-minute period of HI.

Results
Physiological Variables
During the period of HI, MABP and PaO2 dropped significantly (Table 1) which, in combination with occlusion of the left CCA, established global hemispheric ischemia. Although the PaCO2 was also lowered during HI, its value always remained in the physiological range. Reperfusion together with normoxic ventilation normalized all systemic variables.

BOLD MRI
Continuous rapid acquisition of BOLD MR images during the onset and cessation of HI was done to obtain information on the acute cerebral hemodynamic responses. The BOLD MR SI is affected by changes in the oxygenation level of blood as deoxyhemoglobin acts as an intrinsic (T2* shortening) contrast agent.30 As a result of the induction of hypoxia, the SI on the BOLD images significantly ($P<.05$, relative to pre-HI value) dropped to 80% to 85% of baseline in the ipsilateral and contralateral ROIs in the parietal cortex and caudate putamen in approximately 1 minute (Fig 2A and 2B). Subsequently, we detected an elevation of the ipsilateral BOLD SI starting approximately 2 minutes after induction of HI, which was, however, not statistically significant. At the end of the HI period, the BOLD SI had returned to 90% to 95% of pre-HI values in ipsilateral cortex and caudate putamen; contralateral it remained at approximately 80% to 90%. Reoxygenation with normoxic blood resulted in recovery of the SI in the contralateral ROIs; the overshoot of the SI reasonably reflected luxury perfusion (see also LDF results). However, in the ipsilateral cortex and caudate putamen the BOLD SI showed small changes within the examined time period, pointing toward a state of sustained hypoperfusion during the first minutes of reperfusion.

Bolus Track MRI
Repetitive T2*-sensitized MRI experiments performed in combination with intravenous bolus injections of the NMR

<table>
<thead>
<tr>
<th>Time After HI</th>
<th>MABP, mm Hg</th>
<th>PaCO2, mm Hg</th>
<th>PaO2, mm Hg</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre HI</td>
<td>88±12</td>
<td>43±7</td>
<td>140±27</td>
<td>7.42±0.05</td>
</tr>
<tr>
<td>Post HI</td>
<td>39±10*</td>
<td>35±6*</td>
<td>41±6*</td>
<td>7.39±0.06</td>
</tr>
<tr>
<td>Post HI</td>
<td>88±17</td>
<td>40±4</td>
<td>148±18</td>
<td>7.40±0.08</td>
</tr>
</tbody>
</table>

Values are mean±SD.
*P<.05 vs pre-HI.
contrast agent Gd-DTPA enabled the estimation of several hemodynamic parameters. Two-dimensional brain maps of hemodynamic parameters are shown in Fig 3. More specific analysis of regional perfusion differences was done by quantification of the hemodynamic parameters in ROIs in the parietal cortex and caudate putamen shown in Table 2, which presents the ipsilateral values relative to contralateral. A significantly affected brain circulation was revealed by a significantly altered ipsilateral $t_{\text{peak}}$ and $\Delta R2^*_{\text{max}}$ during HI (Table 2). Fig 3 demonstrates that 1 hour after the insult the perfusion status had strongly improved as a consequence of successful reperfusion. However, perfusion in the ipsilateral hemisphere was still impaired at this stage. The delayed ipsilateral $t_{\text{peak}}$ in particular reflected a diminished blood supply. Signs of hyperemia (short $t_{\text{peak}}$, elevated $r\text{CBV}$) were displayed in the ipsilateral caudate putamen at 24 and 72 hours after HI (Fig 3). Although longitudinal comparison of hemodynamic parameters estimated from separate bolus track MRI experiments is ambiguous (see “Materials and Methods”), the immense deviations during and directly after HI can definitely be ascribed to the severe perfusion alterations compared with pre-HI values in both the ipsilateral and contralateral hemispheres. All pre-HI ipsilateral and contralateral $t_{\text{peak}}$ values were in the range of 4.0 to 7.5 seconds, whereas during HI ipsilateral and contralateral $t_{\text{peak}}$ values were 38.6 ± 9.0 and 12.6 ± 3.9 seconds in the parietal cortex and 27.4 ± 8.3 and 13.1 ± 4.1 seconds in the caudate putamen, respectively. Strong vasodilatation was delineated by the major elevation of the $r\text{CBV}$ to 300% to 500% of pre-HI values in both the parietal cortex and caudate putamen. This is not evident from Table 2 because it occurred in both the ipsilateral and contralateral hemispheres. A few minutes after reperfusion, significant decreases of the individual $t_{\text{peak}}$ and increases of the $\Delta R2^*_{\text{max}}$ values were found in all contralateral areas (mean $t_{\text{peak}}$ decreases [relative to pre-HI value]: 1.2 ± 1.6 and 1.7 ± 1.8 seconds in contralateral parietal cortex and caudate putamen, respectively; mean $\Delta R2^*_{\text{max}}$ increase [relative to pre-HI value]: 40.2 ± 18.5 and 37.3 ± 8.8 s⁻¹ in contralateral parietal cortex and caudate putamen, respectively), suggestive of an increase in intravascular flow velocity. In four of six rats hemodynamic parameter values also pointed toward posts ischemic hyperperfusion in the ipsilateral hemisphere (short $t_{\text{peak}}$; increased $\Delta R2^*_{\text{max}}$), whereas in two rats ipsilateral perfusion was still compromised 5 minutes after HI. At this stage, comparison between the two hemispheres revealed that the mean ipsilateral blood supply was lower than contralateral (Table 2). After a few hours the hemodynamic parameters had largely returned to pre-HI values in the cortex, while the perfusion remained diminished in the ipsilateral caudate putamen. Interestingly, after 24 and 72 hours the ipsilateral $r\text{CBV}$ and $\Delta R2^*_{\text{max}}$ had clearly increased, particularly in the caudate putamen. In addition, the shorter ipsilateral $t_{\text{peak}}$ compared with contralateral denoted pronounced hyperperfusion in the caudate putamen. No significant changes in any of the above hemodynamic parameters were found in rats subjected to
concentration of the contrast agent during bolus passage is lower in the ipsilateral than in the contralateral ROI.

Caudate Putamen Before, During, and After HI Onset

Although the bolus track MRI provided detailed information on ischemia-induced perfusion changes, it does not allow calculation of the absolute or relative CBF.31 Furthermore, bolus track MRI experiments were only performed at hourly intervals because of the relatively slow washout of the contrast agent. To compare the hemodynamic parameters deduced from the MRI experiments with continuous relative flow measurements, we performed parallel LDF studies. During HI, LDF dropped to 12±6% of baseline values in the ipsilateral LDF to recover. In both hemispheres postischemic hyperperfusion occurred, peaking approximately 5 minutes after HI completion on the contralateral side and approximately 6 minutes later on the ipsilateral side. Approximately 1 hour after HI, cortical LDF had reached values of 70% to 80% of baseline in both hemispheres.

**TABLE 2. Relative Ipsilateral ΔR2*\text{max}, rCBV, and Differences in tpeak Between Ipsilateral and Contralateral Parietal Cortex and Caudate Putamen Before, During, and After HI Onset**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cx</th>
<th>CP</th>
<th>Cx</th>
<th>CP</th>
<th>Cx</th>
<th>CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>-36±8 min</td>
<td>0.46±0.43</td>
<td>0.66±0.46</td>
<td>98.2±17.3</td>
<td>101.1±10.7</td>
<td>98.2±17.3</td>
<td>101.1±10.7</td>
</tr>
<tr>
<td>19±0 min</td>
<td>26.82±13.02*</td>
<td>15.45±5.66*</td>
<td>15.7±5.4*</td>
<td>37.3±13.8*</td>
<td>15.7±5.4*</td>
<td>37.3±13.8*</td>
</tr>
<tr>
<td>25±1 min</td>
<td>3.83±7.47</td>
<td>1.41±2.03</td>
<td>60.1±40.8*</td>
<td>74.1±34.3*</td>
<td>60.1±40.8*</td>
<td>74.1±34.3*</td>
</tr>
<tr>
<td>86±6 min</td>
<td>1.00±0.92</td>
<td>1.40±0.46*</td>
<td>83.9±18.4*</td>
<td>74.0±14.0*</td>
<td>83.9±18.4*</td>
<td>74.0±14.0*</td>
</tr>
<tr>
<td>151±10 min</td>
<td>0.42±0.53</td>
<td>1.29±0.31*</td>
<td>87.6±6.7</td>
<td>70.1±14.8</td>
<td>87.6±6.7</td>
<td>70.1±14.8</td>
</tr>
<tr>
<td>245±10 min</td>
<td>0.64±0.49</td>
<td>1.22±0.60</td>
<td>80.9±3.1</td>
<td>76.0±18.1</td>
<td>80.9±3.1</td>
<td>76.0±18.1</td>
</tr>
<tr>
<td>24±3 h</td>
<td>0.04±0.37</td>
<td>-0.66±0.46*</td>
<td>123.8±9.2*</td>
<td>189.0±33.4*</td>
<td>123.8±9.2*</td>
<td>189.0±33.4*</td>
</tr>
<tr>
<td>70±3 h</td>
<td>0.29±0.85</td>
<td>-0.86±0.81*</td>
<td>108.2±33.1</td>
<td>167.9±39.0*</td>
<td>108.2±33.1</td>
<td>167.9±39.0*</td>
</tr>
</tbody>
</table>

Cx indicates parietal cortex; CP, caudate putamen. Percentages are percentages of contralateral value. Values are mean±SD. A positive Δtpeak indicates that the maximal concentration of the contrast agent during bolus passage is reached later in the ipsilateral than in the contralateral ROI. A relative ΔR2*\text{max} below 100% indicates that the maximal concentration of the contrast agent during bolus passage is lower in the ipsilateral than in the contralateral ROI.

*P<.05 vs pre-HI.

**D Laser-Doppler Flowmetry**

Although the bolus track MRI provided detailed information on ischemia-induced perfusion changes, it does not allow calculation of the absolute or relative CBF.31 Furthermore, bolus track MRI experiments were only performed at hourly intervals because of the relatively slow washout of the contrast agent. To compare the hemodynamic parameters deduced from the MRI experiments with continuous relative flow measurements, we performed parallel LDF studies. During HI, LDF dropped to 12±3% and 37±13% of baseline values in the ipsilateral and contralateral parietal cortex, respectively (Fig 4). On induction of reperfusion the contralateral LDF improved almost immediately, whereas it took a few minutes longer for the ipsilateral LDF to recover. In both hemispheres postischemic hyperperfusion occurred, peaking approximately 5 minutes after HI completion on the contralateral side and approximately 6 minutes later on the ipsilateral side. Approximately 1 hour after HI, cortical LDF had reached values of 70% to 80% of baseline in both hemispheres.

**Figure 4.** Relative LDF (mean±SD) in the ipsilateral (●) and contralateral parietal cortex (□) as a function of time after HI in rat brain. *P<.05 vs pre-HI; #P<.05 vs contralateral.

**Figure 5.** Two-dimensional brain tissue water ADC maps of four adjacent coronal slices obtained before and 10, 40, and 80 minutes after HI. ROIs in the ipsilateral substantia nigra (SN), dorsal hippocampus (Hc), thalamus (Th), parietal cortex (Cx), and caudate putamen (CP) are depicted on the pre-HI slices. Note the widespread ADC reduction in the ipsilateral hemisphere during HI (10 minutes after induction). At 40 and 80 minutes after HI the ipsilateral ADC had recovered in most brain areas; however, a reduced ADC was still evident in the substantia nigra and thalamus.

**DW and T2-Weighted MRI**

Multislice quantitative DW and T2-weighted MRI were performed at regular intervals during the entire experimental protocol. DW MRI can monitor the development of acute cytotoxic edema from the associated reduction of tissue water ADC.32–34 T2 prolongation of brain tissue water, measured by T2-weighted MRI, is known to be due to vasogenic edema35 and is indicative of irreversible tissue damage. Fig 5 shows typical ADC maps of coronal brain slices obtained before, during, and acutely after HI. The results of region-specific quantitative analysis of the ADC maps are given in Fig 6. During HI, the ADC was significantly reduced in major parts of the ipsilateral hemisphere. ADC declines relative to pre-HI values were found in all animals in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen and in hypoxia without CCA occlusion or to CCA occlusion without hypoxia (data not shown).
eight and seven of 10 rats in the ipsilateral thalamus and substantia nigra, respectively. Within 1 hour after reperfusion in combination with normoxia, the ADC normalized completely in the hippocampus, parietal cortex, and caudate putamen followed by a small temporary overshoot (see Fig 6). However, in some regions, typically the ipsilateral substantia nigra and thalamus, the mean ADC continued to progressively decline directly after HI and only partially recovered at later time points. There were no significant T2 changes in the brain during the first hours after HI; nevertheless, the T2 in the substantia nigra and thalamus showed a tendency to increase during these acute stages (Fig 7). ADC (and T2) maps revealed no significant alterations in these parameters in the brains of sham-operated rats (data not shown).

Fig 8 demonstrates that 24 and 72 hours after the HI period all examined ipsilateral areas exhibited ADC and T2 changes (see also Figs 6 and 7). The brain water ADC was significantly reduced in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen 24 hours after HI. In addition, the T2 was significantly prolonged in these areas (see also Fig 7). In the substantia nigra and thalamus these alterations were less evident; in 27% of the animals at 24 and 72 hours after HI explicit changes were even absent in these areas. After 72 hours the tissue changes on the ADC (Figs 6 and 8) and T2 maps (Fig 7 and 8) were more pronounced, and the T2 values in particular had further increased. Interestingly, at this stage aberrations were also seen in white matter areas (typically the corpus callosum and external capsule). These areas were characterized by very high ADC and long T2 values, presumably as a result of vasogenic edema. Ventricular dilatation and small but significant ADC reductions and T2 elevations were also detected in the contralateral hemisphere during these stages (Figs 6, 7, and 8).

**Histology**

Histological analysis was done after the MRI experiments at 24 and 72 hours after HI and clearly revealed ipsilateral tissue damage. Silver-stained areas, indicative of neuronal degeneration, matched with areas with ADC and T2 abnormalities (Fig 9). Silver staining was most pronounced in the areas with highly increased T2 values, typically the dorsal hippocampus, parietal cortex, and caudate putamen. The silver staining was less intense in the substantia nigra and thalamus.

**Discussion**

In this study we assessed the ongoing brain perfusion and tissue response after 20 minutes of HI in rats to gain a better understanding of region-specific tissue susceptibility and delayed damage after a transient ischemic episode. Multiparametric MRI, LDF, and histological data revealed a sequence of alterations that dynamically varied with time and between different brain structures.
Hemodynamics

Critical flow alterations in the ipsilateral hemisphere were clearly demonstrated by the bolus track MRI data and LDF measurements. Unilateral occlusion of the CCA in combination with hypoxic ventilation gave rise to a severe ipsilateral perfusion reduction. In the contralateral hemisphere we observed moderate hypoperfusion. Immediate tissue deoxygenation was evident from the rapid BOLD MRI experiments. Interestingly, we detected a rapid partial recovery of the ischemia-induced BOLD SI reductions, which has also been found during acute focal ischemia in the rat.\(^{36,37}\) Although the exact mechanism responsible for this is unknown, factors involved could be secondary changes in the microcirculation, a decline in the oxygen extraction fraction, or vasodilatation, which all decrease the local amount of deoxygenated hemoglobin. In addition, the regional decrease in paramagnetic tissue oxygen could counterbalance the increase in paramagnetic deoxyhemoglobin, as suggested by Hossmann and Hoehn-Berlage.\(^ {38}\) After induction of reperfusion, recovery of the BOLD MR SI reflected reoxygenation. Luxury perfusion emerged readily in the first 10 to 20 minutes of reperfusion in both hemispheres. Thereafter, a state of mild hypoperfusion was evident in cortical and striatal areas, which persisted for several hours and was most pronounced in the caudate putamen. These findings are in agreement with those from other studies that also described posts ischemic early reactive hyperemia and delayed hypoperfusion and that were associated with changes in metabolic activity.\(^{15,16,30}\)

Tissue Parameter Changes

The drastic HI-induced perfusion deficit was accompanied by a drop of the tissue water ADC in all investigated ipsilateral brain regions. Acute ADC reductions have been described in several experimental ischemia models as well as in human brain ischemia and are typically associated with the development of cytotoxic edema.\(^ {35–37,41}\) Cytotoxic edema or cellular swelling is a direct result of the loss of ionic gradients due to ischemia-induced energy failure that develops below a CBF threshold level of approximately 20% to 25% of the baseline value in acute ischemia.\(^ {42}\) Accordingly, similar CBF threshold levels have been reported for ADC reductions in early ischemia.\(^ {35–37,41}\) Perfusion reduction in the contralateral hemisphere was insufficient to result in significant ADC changes. Reperfusion of the brain completely normalized the ADC in the ipsilateral dorsal hippocampus, parietal cortex, and caudate putamen within 1 hour. In the substantia nigra and thalamus, however, the mean ADC remained below baseline values, suggesting irreversible tissue impairment. This was confirmed by significant ADC reductions, T2 elevations, and silver staining in these areas after 24 and 72 hours. Still, also in

Figure 7. T2 in the ipsilateral (●) and contralateral (○) substantia nigra, dorsal hippocampus, thalamus, parietal cortex, and caudate putamen as a function of time after HI. *\(^ P<.05\) vs pre-HI. Positioning of the ROIs is exemplified on T2 maps that were obtained before HI.
regions where the ADC initially normalized, severe tissue injury was demonstrated after 24 and 72 hours. At these time points, small but significant ADC decreases and T2 elevations and ventricular dilatation occurred in the contralateral hemisphere. Whether this was directly caused by the moderate contralateral hemodynamic changes or was an indirect result of the comprehensive ipsilateral changes, such as hemispheric swelling, remains unclear.

Delayed Brain Damage

Delayed neuronal damage and/or reperfusion-induced secondary injury have been frequently observed in both experimental and clinical studies of cerebral ischemia. The exact mechanisms underlying delayed/secondary damage, however, are still unclarified. Possible factors involved include sustained impairment of calcium homeostasis, long-term glutamate accumulation, reflow-induced formation of free radicals, secondary hyperperfusion, secondary impairment of mitochondrial function, and apoptosis. The reappearance of reduced tissue water ADCs after 24 and 72 hours in our study suggested cytotoxic edema due to secondary energy depletion and dissipation of ion gradients. Accordingly, Ordidge et al recently found a correlation between delayed ADC reductions and secondary loss of high-energy phosphates in a neonatal model of HI. Secondary energy failure is a well-known phenomenon in perinatal HI encephalopathy, and the extent of the delayed impairment of energy metabolism has been shown to correlate with the magnitude of the cerebral infarction. Despite the initial recovery of mitochondrial function and high-energy phosphate levels on early reperfusion, secondary energy failure could definitely also be a major factor responsible for delayed damage in adult brain. In our study, posts ischemic hypoperfusion did not reach threshold levels known to cause energy failure in normal, nondisturbed brain tissue. However, a mismatch between CBF and metabolic rate could arise in hypermetabolic postischemic brain. Secondary processes, such as the protective mechanisms against the massive loss of calcium homeostasis, activation of free radical scavenging enzymes, and apoptosis-related protein synthesis, possibly lead to high glucose and oxygen demands that may not be met by the compromised perfusion status.

Region-Specific Sensitivity

Certain brain regions appeared more susceptible to an ischemic insult than other areas. In the hippocampus, cortex, and caudate putamen, the frequency of the occurrence of primary ischemia-induced ADC changes was higher than in the substantia nigra and thalamus. The manifestation of delayed damage was also more prominent in the former areas. Since ADC reductions in ischemia only develop below a certain CBF threshold, the above suggests that in the substantia nigra and thalamus perfusion levels occasionally would not be reduced to below threshold levels for ischemic damage. Selective vulnerability has been associated with heterogeneities in local CBF.

In fact, our results demonstrated differences in ischemic and postischemic perfusion between cortical and striatal areas. Unfortunately, in our study we could not compare the perfusion status in these areas to those in the hippocampus, thalamus, and substantia nigra. However, previous studies have shown that CBF reductions during global ischemia are less drastic in the diencephalic thalamus and substantia nigra than in forebrain regions (dorsal hippocampus, thalamus, and substantia nigra). We believe that the region-specific response to ischemia is associated with heterogeneities in local ischemic and postischemic hemodynamics. However, other factors such as differences in postsynaptic organization, differences in time needed for exposure to cumulative threshold levels of glutamate, and variations in mitochondrial capacity and in the activity of free radical defense systems.

Delayed neuronal damage and/or reperfusion-induced secondary injury have been frequently observed in both experimental and clinical studies of cerebral ischemia. The exact mechanisms underlying delayed/secondary damage, however, are still unclarified. Possible factors involved include sustained impairment of calcium homeostasis, long-term glutamate accumulation, reflow-induced formation of free radicals, secondary hyperperfusion, secondary impairment of mitochondrial function, and apoptosis. The reappearance of reduced tissue water ADCs after 24 and 72 hours in our study suggested cytotoxic edema due to secondary energy depletion and dissipation of ion gradients. Accordingly, Ordidge et al recently found a correlation between delayed ADC reductions and secondary loss of high-energy phosphates in a neonatal model of HI. Secondary energy failure is a well-known phenomenon in perinatal HI encephalopathy, and the extent of the delayed impairment of energy metabolism has been shown to correlate with the magnitude of the cerebral infarction. Despite the initial recovery of mitochondrial function and high-energy phosphate levels on early reperfusion, secondary energy failure could definitely also be a major factor responsible for delayed damage in adult brain. In our study, posts ischemic hypoperfusion did not reach threshold levels known to cause energy failure in normal, nondisturbed brain tissue. However, a mismatch between CBF and metabolic rate could arise in hypermetabolic postischemic brain. Secondary processes, such as the protective mechanisms against the massive loss of calcium homeostasis, activation of free radical scavenging enzymes, and apoptosis-related protein synthesis, possibly lead to high glucose and oxygen demands that may not be met by the compromised perfusion status.

Region-Specific Sensitivity

Certain brain regions appeared more susceptible to an ischemic insult than other areas. In the hippocampus, cortex, and caudate putamen, the frequency of the occurrence of primary ischemia-induced ADC changes was higher than in the substantia nigra and thalamus. The manifestation of delayed damage was also more prominent in the former areas. Since ADC reductions in ischemia only develop below a certain CBF threshold, the above suggests that in the substantia nigra and thalamus perfusion levels occasionally would not be reduced to below threshold levels for ischemic damage. Selective vulnerability has been associated with heterogeneities in local CBF. In fact, our results demonstrated differences in ischemic and postischemic perfusion between cortical and striatal areas. Unfortunately, in our study we could not compare the perfusion status in these areas to those in the hippocampus, thalamus, and substantia nigra. However, previous studies have shown that CBF reductions during global ischemia are less drastic in the diencephalic thalamus and substantia nigra than in forebrain regions (dorsal hippocampus, cortex, and caudate putamen). Nevertheless, if an early ADC drop occurred in the substantia nigra or thalamus, the reduction was profound and irreversible, in contrast to the reversible changes in the other investigated regions. This may imply a higher intrinsic susceptibility to ischemia for the thalamus and substantia nigra. We believe that the region-specific response to ischemia is associated with heterogeneities in local ischemic and postischemic hemodynamics. However, other factors such as differences in postsynaptic organization, differences in time needed for exposure to cumulative threshold levels of glutamate, and variations in mitochondrial capacity and in the activity of free radical defense systems.
may also be involved. Such factors could be the cause of the differences in intrinsic susceptibility between brain regions apart from the hemodynamic component.

In conclusion, in this study heterogeneous tissue susceptibility and delayed damage after transient HI in rat brain were demonstrated by means of in vivo MRI. Areas exhibiting ADC reductions during the ischemic episode were invariably injured in the chronic phase, despite early postischemic recovery on reperfusion. For the clinical situation, this may imply that early restoration of the blood supply in acute stroke (eg, by thrombolysis) should be combined with cytoprotective therapy to reduce postischemic secondary damage. Clinical studies evaluating our findings, in which (DW) MRI could play an essential role, are eagerly awaited.

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References

It is well recognized that brief periods of cerebral ischemia resulting from either a transient disruption of the blood supply or a period of hypoxia can lead to delayed tissue damage. This delayed infarction may occur days or even weeks after the initial insult, depending upon the nature and the site of the insult. With the advent of therapies that are efficacious in acute stroke (especially thrombolytics), there has been a resurgence of interest in secondary or delayed damage as a potential therapeutic target. Indeed it seems likely that protection against 'secondary energy failure' in the newborn piglet. In: Proceedings of the Fifth Scientific Meeting and Exhibition of the International Society for Magnetic Resonance in Medicine; April 12–18, 1997; Vancouver, BC, Canada. 1997:395. Abstract.

It needs to be stated that reversal of the acute response seen from the diffusion-weighted image that may occur upon successful reperfusion of the ischemic territory or after pharmacological intervention is not in itself indicative of eventual tissue recovery. As this study clearly demonstrates, normalization of the acute ADC is not necessarily associated with tissue salvage and in isolation is a poor predictor of long-term outcome. The combination of perfusion-weighted imaging with diffusion-weighted MRI should provide a more accurate assessment of the nature of the acute pathology. The underlying mechanisms responsible for the slow evolution of the tissue injury remain uncertain, and further studies are clearly required to identify the relationship between the perfusion deficit, selective vulnerability, and delayed infarction. It is, however, clear that caution should be applied to the interpretation of the acute changes in ADC as a predictor of eventual infarction.

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Dynamics of Cerebral Tissue Injury and Perfusion After Temporary Hypoxia-Ischemia in the Rat: Evidence for Region-Specific Sensitivity and Delayed Damage

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