Proton T_2 Relaxation of Cerebral Metabolites During Transient Global Ischemia in Rat Brain

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Putative changes of metabolite T_2 relaxation times were investigated before and after a 20-min period of global ischemia in rat brain *in vivo* (n = 10) using localized proton MRS at different echo times (2.35 T). Neither absolute T_2 relaxation times (TE = 20–270 ms) nor time courses of T_2-weighted metabolite signals (TE = 135 ms) revealed statistically significant changes during the occlusion or early reperfusion relative to pre-ischemic baseline. These findings are in line with reports of relaxation changes at much later stages and further demonstrate that altered T_2 relaxation is not a confounding factor in diffusion-weighted long-TE proton MRS during early ischemic events.

Key words: brain ischemia; proton MRS; T_2 measurement; brain metabolites.

INTRODUCTION

Apart from a depletion of brain glucose pools and the accumulation of lactic acid (Lac), the concentrations of major metabolites in rat brain are largely unaffected during brief periods of transient global ischemia (1, 2). This particularly applies to *N*-acetylaspartate (NAA), total creatine (Cr), choline-containing compounds (Cho), and *myo*-inositol (Ins), which are conveniently detected by short TE, localized proton MRS *in vivo*. Extending such work, the use of diffusion-weighted proton MRS revealed an intracellular component of the well-known ischemia-related alterations of water diffusion by showing that metabolite apparent diffusion coefficients (ADCs) decrease after occlusion (3, 4). Because limitations in signal-to-noise ratio preclude metabolite ADC determinations at high temporal resolution, metabolite diffusion during occlusion and early reperfusion has been studied by diffusion-weighted spectra that result in relative resonance signal increases for reduced ADC values (3). A potential drawback stems from the fact that pertinent MRS studies have to be carried out at long TEs to achieve a sufficiently strong diffusion weighting. Thus, unrecognized prolongations of metabolite T_2 relaxation times might contribute to the observed signal changes. Although delayed prolongation of water proton T_2 relaxation times has been demonstrated by MRI, e.g., see refs. 5–7, only one MRS study reported that metabolite T_1 and T_2 relaxation times are similar to controls at 3–6 h after focal ischemia, whereas the T_2 value of Cr increased by about 10% after 24 h (8). The purpose of this study was to investigate whether metabolite T_2 relaxation times change during the acute phase of transient global ischemia in rat brain and thus complicate the interpretation of diffusion-weighted MRS signal alterations.

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METHODS

Transient global ischemia was induced in male Wistar rats (n = 10, 448 ± 48 g) by a modification of the four-vessel occlusion model (9). Twenty-four hours before the MRS experiment, both vertebral arteries were occluded by electrocauterization under halothane anesthesia (1–1.5% in 70:30 N_2O:O_2). After surgery, the animals had free access to food and water. For MRS investigations, the animals were anesthetized, tracheotomized, artificially ventilated, and immobilized with a constant injection of pancuronium bromide (0.3 mg/kg/h). They were placed in a supine position with their heads firmly fixed between a bite bar and a surface coil under the head used for signal reception. Transient global ischemia (20 min) was induced inside the magnet bore using inflatable occluders (In Vivo Metric, Healdsburg, CA) fixed around the common carotid arteries bilaterally. Catheterization of the femoral artery was used to determine arterial blood pressure (Siemens, Erlangen, Germany), blood gas and pH (AVL LIST, Graz, Austria), and plasma glucose (Home Diagnostics, Fort Lauderdale, FL). Respiratory motion of the rats was monitored by a water-filled balloon attached to the chest. The body temperature was maintained constant using a heated water blanket.

Localized proton MRS was performed at 2.35 T (Bruker Biospec, Karlsruhe, Germany) using a 14-cm Helmholtz coil for RF excitation and a 2-cm surface coil for signal reception. Volumes of interest (VOIs) of 0.245 ml (7 × 5 × 7 mm^3) were selected from multislice gradient-echo images (RF-spoiled fast low angle shot; TR = 150 ms; TE = 5 ms; $\alpha = 20°$; slice thickness = 1 mm) and covered the central rat brain extending over both hemispheres. Spectra were acquired with use of a STEAM localization sequence (TR = 3000 ms; TM = 10 ms) at variable echo times (TE = 20, 68, 135, 206, 270 ms) and 64–128 accumulations. To minimize temporal differences in the T_2 determinations, individual T_2-weighted scans were obtained in an interleaved mode. Absolute T_2 relaxation times of cerebral metabolites were determined before ischemia as well as during reperfusion and 90 and 120 min after the end of ischemia.

During the acute phase immediately before, during, and after ischemia, putative effects on T_2 relaxation were monitored by T_2-weighted signal intensity ratios of metabolite resonances acquired at TEs of 20 and 135 ms in alternate scans. Absolute metabolite signal levels and

relative changes therefrom were obtained from short TE spectra. An on-line display of a scan-to-scan sliding average of 16 short-TE spectra was used to confirm a successful occlusion of the carotid arteries as evidenced by a corresponding accumulation of Lac. Rats with a transient decrease of the elevated brain Lac signal during ischemia were excluded from the study.

Fully automated and user-independent spectral evaluation was accomplished by LCModel (S.W. Proveucher, Göttingen, Germany), which takes advantage of a linear combination of model metabolite spectra obtained under identical experimental conditions (10). The analysis of T_2-weighted resonance signal ratios was based on two independent sets of model spectra with TEs of 20 and 135 ms, respectively. Absolute T_2 relaxation times were obtained by fitting only the most prominent metabolite resonances to a mono-exponential decay function for five TEs ranging from 20 to 270 ms. Values with a correlation coefficient of less than 0.9 were discarded from further analysis. This particularly applied to the determination of T_2 relaxation times for Cho and Ins. Statistically significant differences between pre- and postischemic groups were analyzed by a paired t test ($P < 0.05$). Statistical analyses were based not on sliding averages but on independent data sets.

RESULTS AND DISCUSSION

The mean physiologic parameters before and after transient global ischemia for the investigated group of animals are summarized in Table 1. None of these parameters revealed a statistically significant difference. Representative localized proton spectra of pre-ischemic rat brain are shown in Fig. 1 as a function of TE. Although metabolites with singlet resonances provide sufficient signal strength, even at the longest TE of 270 ms, Ins allows a reasonable T_2 estimate only for TE $\leq$ 135 ms due to a shorter T_2 and J-modulation of its strongly coupled resonance.

The mean time courses of relative concentration changes for NAA, Cr, Cho, Ins, and Lac before and during global ischemia as well as in the early reperfusion phase are shown in Fig. 2. The values are derived from absolute although mildly T_1-weighted concentrations (TR = 3000 ms; TE = 20 ms) and normalized to pre-ischemic conditions. In agreement with results for a 10-min period of global ischemia (1, 2), postocclusion levels of major metabolites remain constant except for a mild, statistically insignificant increase of Ins, a strong elevation of Lac

Table 1
Mean Physiologic Parameters of the Investigated Animals (mean ± SD, n = 10) Before and 60 min After a 20-min Period of Global Ischemia

	Before Ischemia	After Ischemia
Arterial Po_2/mm Hg	151 ± 20	149 ± 29
Arterial Pco_2/mm Hg	43 ± 7	44 ± 9
Arterial Blood pH	7.37 ± 0.06	7.30 ± 0.08
Plasma Glucose/mg · dl^{-1}	120 ± 24	104 ± 18
Mean Arterial Blood Pressure/mm Hg	89 ± 13	81 ± 16

No statistically significant changes were observed ($p < 0.05$, paired t test).

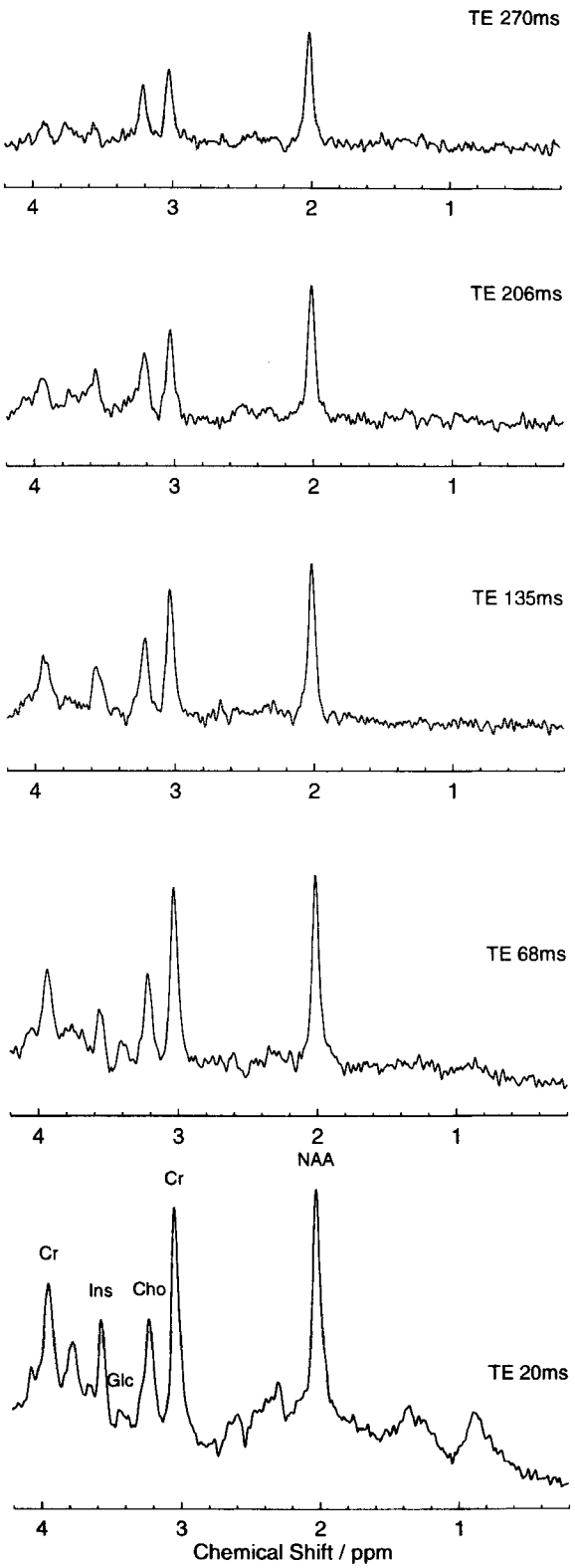

FIG. 1. Localized proton spectra (STEAM; TR = 3000 ms; TM = 10 ms; 0.245-ml VOI; 128 accumulations) of pre-ischemic rat brain *in vivo* obtained at TEs of 20, 68, 135, 206, and 270 ms. Metabolite resonances include NAA, Cr, Cho, Ins, and glucose (Glc).

($P < 0.0001$, pre-occlusion baseline versus last 12.8 min of occlusion), and a decrease of glucose ($P < 0.001$, similar to Figs. 2–4 of ref. 1). In fact, Lac accumulation

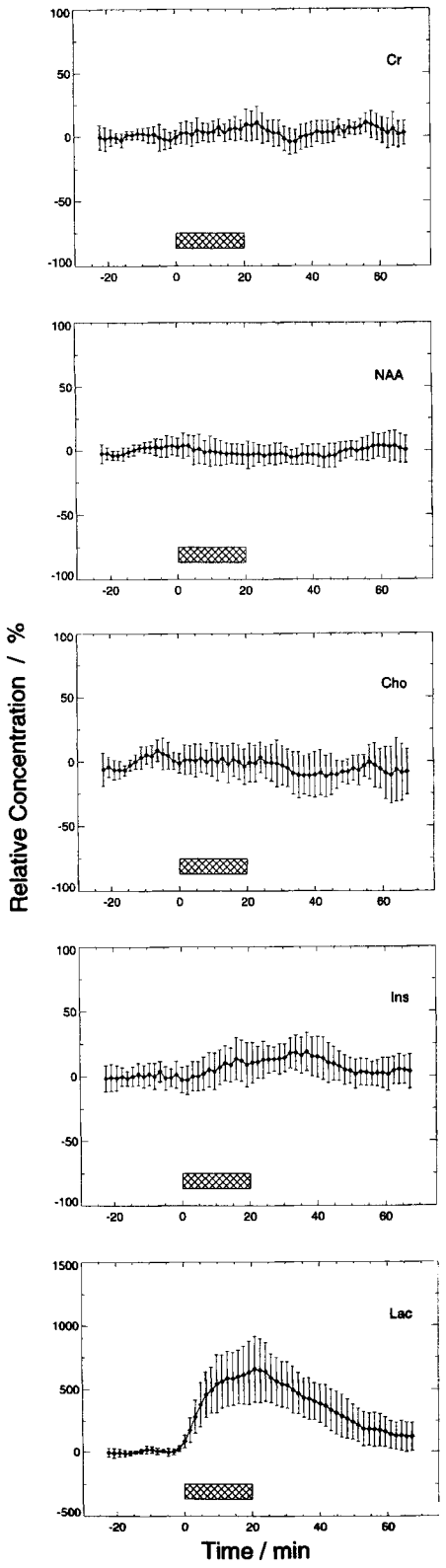

FIG. 2. Mean time courses (mean ± SD, n = 10) of relative concentration changes of NAA, Cr, Cho, Ins, and Lac before, during (hashed bar), and after a 20-min period of global ischemia. The individual data points represent the average of 64 accumulations (6 min 24 s) time-shifted by 16 accumulations (1 min 36 s) and normalized to pre-occlusion baseline.

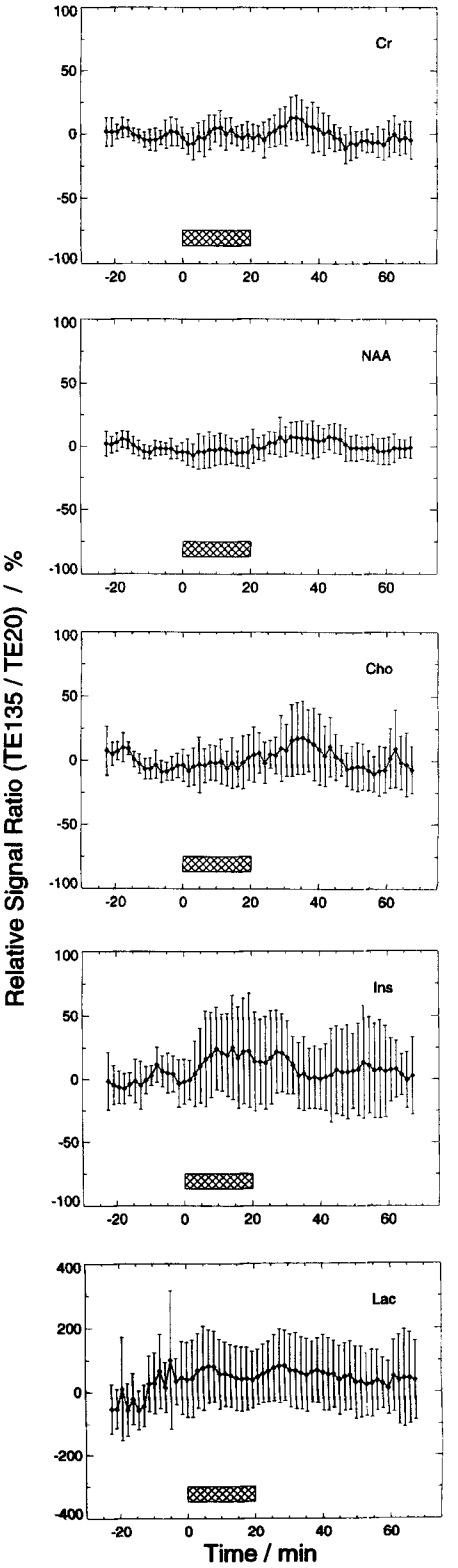

FIG. 3. Mean time courses (mean ± SD, n = 10) of relative changes of T_2-weighted signal ratios (135 ms/20 ms) for NAA, Cr, Cho, Ins, and Lac before, during (hashed bar), and after a 20-min period of global ischemia. The individual data points represent the average of 64 accumulations (6 min 24 s) time-shifted by 16 accumulations (1 min 36 s) and normalized to pre-occlusion baseline.

was taken as an index for a successful vascular occlusion and the development of comparable global ischemia between animals. Decreased concentrations of, for example, NAA and Cr have been reported several hours after *focal* cerebral ischemia in cats (11) and rats (12, 13), whereas similarly reduced metabolite levels in transient *global* ischemia in rats were reported after 24 h of reperfusion (14).

The mean time courses of relative changes of T_2-weighted dual-echo signal ratios (135 ms/20 ms) as a measure of metabolite T_2 relaxation during the acute phase of a 20-min period of global ischemia are shown in Fig. 3. No changes are observed as mean values remain within one standard deviation for all metabolites including Lac. Because ratios of metabolite resonances at two different TEs exclude potentially confounding effects from alterations in concentration or T_1 relaxation, these findings may be taken as evidence for the absence of T_2 relaxation time changes during the acute phase of cerebral ischemia. Of course, as the present model does not cause changes of metabolite concentrations (Fig. 2), also the T_2-weighted metabolite signals at TE = 135 ms do not reveal any alterations during or after ischemia (not shown).

The results of the T_2-weighted acquisitions are further confirmed by a comparison of absolute T_2 relaxation times for NAA, Cr, Cho, and Ins before ischemia and during reperfusion as summarized in Table 2. No statistically significant differences are observed between groups. Comparisons with data from the literature (8, 15–18) are hampered because of large variations due to differences in species (e.g., rat, gerbil, dog, piglet), field strength (e.g., 2.35–9.4 T), and model assumption (e.g., mono- versus biexponential decay curves).

In summary, the most relevant finding of this work is the fact that T_2 relaxation times of cerebral metabolites remain constant for at least 2 hours after a 20-min period of global ischemia. This result supplements MRS studies of focal ischemia reporting significant changes of relaxation times not before 24 h after the ischemic event (8). It may therefore be concluded that T_2 relaxation does not contribute to signal alterations of cerebral metabolites detected by diffusion-weighted long-TE proton MRS during early ischemia.

Table 2
Mean T2 Relaxation Times (ms, mean ± SD, *n* given in brackets) of Rat Brain Metabolites Before and After a 20-min Period of Global Ischemia

Metabolite	Before Ischemia	90 Min After Ischemia	120 Min After Ischemia
NAA (CH_3)	406 ± 82 (10)	339 ± 35 (10)	359 ± 71 (10)
Cr (CH_3)	203 ± 19 (10)	198 ± 14 (10)	193 ± 27 (10)
Cr (CH_2)	136 ± 24 (10)	135 ± 18 (9)	115 ± 23 (9)
Cho	350 ± 67 (8)	333 ± 34 (9)	322 ± 100 (8)
Ins	125 ± 23 (5)	126 ± 15 (5)	135 ± 15 (3)

No statistically significant changes were observed between groups ($p < 0.05$, paired *t* test).

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