Journal of Neuroscience Research 66:891–898 (2001)

Metabolic Changes in Quinolinic Acid-Lesioned Rat Striatum Detected Non-Invasively by In Vivo ^{1}H NMR Spectroscopy

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Intrastriatal injection of quinolinic acid (QA) provides an animal model of Huntington disease. In vivo ^{1}H NMR spectroscopy was used to measure the neurochemical profile non-invasively in seven animals 5 days after unilateral injection of 150 nmol of QA. Concentration changes of 16 metabolites were measured from 22 μl volume at 9.4 T. The increase of glutamine ((+25 ± 14)%, mean ± SD, n = 7) and decrease of glutamate (−12 ± 5)%, *N*-acetylaspartate (−17 ± 6)%, taurine (−14 ± 6)% and total creatine (−9 ± 3%) were discernible in each individual animal ($P < 0.005$, paired *t*-test). Metabolite concentrations in control striata were in excellent agreement with biochemical literature. The change in glutamate plus glutamine was not significant, implying a shift in the glutamate-glutamine interconversion, consistent with a metabolic defect at the level of neuronal-glial metabolic trafficking. The most significant indicator of the lesion, however, were the changes in glutathione ((−19 ± 9)%, $P < 0.002$)), consistent with oxidative stress. From a comparison with biochemical literature we conclude that high-resolution in vivo ^{1}H NMR spectroscopy accurately reflects the neurochemical changes induced by a relatively modest dose of QA, which permits one to longitudinally follow mitochondrial function, oxidative stress and glial-neuronal metabolic trafficking as well as the effects of treatment in this model of Huntington disease. J. Neurosci. Res. 66:891–898, 2001.
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Key words: quinolinic acid lesion; rat striatum; ^{1}H NMR spectroscopy; quantification; brain metabolites

Huntington disease (HD) is an autosomal dominant neurodegenerative disorder characterized by dyskinesia, cognitive impairment, and emotional disturbances (Sharp and Ross, 1996; Haque et al., 1997). Among the pathogenic theories of Huntington disease is the presence of a defect in oxidative phosphorylation, which is supported by the observation of elevated lactate concentrations in the occipital cortex and basal ganglia of HD patients (Jenkins et al., 1993, 1998), decreased cerebral glucose utilization (Greene and Greenamyre, 1996), and a reduced mitochondrial complex II–III activity found in postmortem brain tissue (Gu et al., 1996; Beal, 2000). Moreover, treatment of HD patients with the anti-oxidant coenzyme Q_{10} (ubiquinone) was reported to significantly lower cortical lactate concentration in patients (Koroshetz et al., 1997). Furthermore, neuroprotective effects of coenzyme Q_{10} (Matthews et al., 1998a) as well as of creatine (Ferrante et al., 2000) were described in a transgenic mouse model of HD. Recent studies have suggested that oxidative stress may also be involved in Huntington disease as judged from alterations in the metabolism of glutathione and related enzymes (Tabrizi et al., 1999).

An accepted animal model of HD is based on the intrastriatal administration of quinolinic acid (Schwarcz et al., 1983; Beal et al., 1986, 1991), an endogenous metabolite of L-tryptophan and agonist of the NMDA-receptor. In this model, neuronal damage is apparently caused by overstimulation of glutamate receptors, which can be prevented with NMDA receptor antagonists such as MK-801 (Beal et al., 1988) or kynurenate (Ceresoli-Borroni et al., 1999). Excessive receptor stimulation results in a loss of Ca^{2+} homeostasis, which can lead to mitochondrial dysfunction and formation of nitric oxide and other free radicals (Greene and Greenamyre, 1996) leading to neuronal death via apoptotic and necrotic mechanisms (Ferrer et al., 1995; Portera-Cailliau et al., 1995). The QA model produces behavioral and neuroanatomical effects mimicking some of the earlier symptoms of HD (Shear et al., 1998).

Methods used to assess metabolite concentration changes in excitotoxic lesions include immunohistochemistry (Nakao et al., 1996), radioimmunoassay (Beal et al., 1986), freeze clamp technique followed by tissue extrac-

This work was part of the thesis requirement for C. Dirk Keene, who shared the experimental responsibility in executing this study with the first author.

Contract grant sponsor: NIH; Contract grant number: P41RR08079, R01NS38672; Contract grant sponsor: Lyle French Fund; Contract grant sponsor: Whitaker Foundation.

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Received 15 June 2001; Revised 3 August 2001; Accepted 7 August 2001

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tion and HPLC analysis (Beal et al., 1986; Bordelon et al., 1997), microelectrodes (Bordelon et al., 1998), and microdialysis (Reynolds et al., 1997), all of which are invasive and thus not very well suited for longitudinal studies. Non-invasive microPET imaging was used to assess time course of alternations in energy utilization and dopamine receptors in QA-lesioned rat striatum (Araujo et al., 2000). In vivo NMR spectroscopy is a non-invasive method, which does not require substrates with isotope labeling. Only a limited number of metabolites, such as *N*-acetylaspertate, creatine, and lactate, however, has been quantified with in vivo NMR spectroscopic techniques in excitotoxic lesions (Jenkins et al., 1996; Strauss et al., 1997; Matthews et al., 1998b; Lee et al., 2000). Recent developments in high field in vivo ^{1}H NMR spectroscopy (Gruetter et al., 1998b) have resulted in the ability to quantify up to 18 metabolites simultaneously and non-invasively (Pfeuffer et al., 1999a; Tkáč, et al., 1999). The aim of our study was to quantify the concentration changes of at least 15 metabolites simultaneously in QA-lesioned and contralateral rat striatum and to test the potential to detect QA induced changes in individual animals.

Previous biochemical, histological, and behavioral studies of the QA model of HD have covered wide ranges in dosage (from 30–500 nmol QA) and time scale (minutes to several months). Recent studies focused mainly on the early post-injection effects (Bordelon et al., 1998; Bordelon and Chesselet, 1999; Ceresoli-Borroni et al., 1999; Mena et al., 2000; Rodriguez-Martinez et al., 2000). We chose a protocol (5 days post-injection, 150 nmol QA dose) because this dose has been shown to induce significant neuropathology and behavioral deficits that are amenable to treatment. Because the goal of this study was to evaluate the usefulness of a new non-invasive modality that was potentially suitable for longitudinal monitoring, we focused on a time point (5 days post-injection) where cell death and degeneration were fairly well resolved, yet acute enough to allow treatment. In addition, the QA lesion model provides us with an internal control: the contralateral striatum, allowing a paired comparison between lesioned and control striata in the same animal, with the potential to follow the effect and progression of cell transplantation.

MATERIALS AND METHODS

Animals

Experiments were performed according to procedures approved by the Institutional Review Board's animal care and use committee. Ten female Fischer 344 rats (150–200 g) were used for the study because animal growth in this strain is limited. Animals were anesthetized with ketamine-xylazine and placed in a stereotactic frame. Excitotoxic lesions were induced by injection of buffered solution of quinolinic acid (1 μl, 150 nmol/μl) into the right anterior striatum of seven rats 5 days before the NMR measurement. Three animals received a larger QA dose (1 μl, 300 or 450 nmol/μl). During the NMR experiments, animals were anesthetized by a gas mixture of O_2:N_2O = 3:2 with 1.5% isoflurane. The rats were ventilated at physiological conditions by a pressure-controlled respirator (Kent Scientific Corp., Litchfield, CT). Oxygen saturation was maintained above 95% and was continuously monitored by a pulse oximeter attached to the tail (Nonin Medical, Inc., Minneapolis, MN). Body temperature was maintained at 37°C by warm water circulation and verified by a rectal thermosensor (Cole Parmer, Vernon Hills, IL).

^{1}H NMR Spectroscopy

In vivo ^{1}H NMR spectra were measured in the same session from the QA-lesioned as well as from the contralateral (control) striatum 5 days after QA was injected into one striatum. ^{1}H NMR spectra were measured at 9.4 T from a 22 μl volume using a Varian INOVA spectrometer (Varian, Palo Alto, CA) interfaced to a 9.4 T magnet with 31 cm horizontal bore size (Magnex Scientific, Abingdon, UK). The actively shielded gradient coil insert of 12 cm inner diameter (Magnex) was capable of switching to 300 mT/m within 500 μsec. A quadrature surface RF coil consisting of two geometrically decoupled single turn ^{1}H coils with 14 mm diameter, constructed according to a previously described design (Adriany and Gruetter, 1997), was used for transmitting and receiving. A stimulated echo acquisition mode (STEAM) localization sequence with very short echo time (TE = 2 msec, TM = 5 msec) combined with outer volume saturation was used (Tkáč, et al., 1999). The bandwidth of the slice-selective RF pulses was 13.5 kHz, which ensured that over a 3 ppm range, the chemical shift displacement error was less than 10%. T_1 was assessed to be constant as judged from the measured metabolite-nulled spectra acquired in each animal for each striatum using an inversion time on 0.95 sec, which implies that T_1 of metabolites was approximately 1.4 sec. The repetition time was 4 sec, which minimized effects due to potential T_1 variation. As in our previous study (Pfeuffer et al., 1999a), metabolite-nulled spectra were used to assess the macromolecule background, which was found to be highly consistent between animals (data not shown). The water signal was suppressed by variable power RF pulses with optimized relaxation delays (VAPOR) as described recently (Tkáč, et al., 1999). First- and second-order-shimming was performed by a fully adiabatic version of FASTMAP (fast, automatic shimming technique by mapping along projections) (Gruetter, 1993; Gruetter et al., 1998b; Gruetter and Tkáč, 2000) resulting typically in an 11–12 Hz linewidth of the water signal in vivo. T_2-weighted spin echo MR images were obtained for positioning of the localized volume.

NMR Quantification of Metabolite Concentrations

Metabolite concentrations were quantified by LCModel (Linear Combination of Model spectra of metabolite solutions) (Provencher, 1993) as described recently (Pfeuffer et al., 1999a) by including macromolecule spectra in the fitting procedure and by using the water signal as an internal reference (assuming a water content of 0.83 ml/g). Due to the short echo-time of 2 msec, T_2 relaxation effects on NMR signal intensity were assessed to be below 2% and, therefore, neglected. The LCModel method of quantifying GABA in human brain at 7 T (Tkáč, et al., 2001) was compared to homonuclear editing methods of measuring GABA (Terpstra et al., 2001), which

provided results that were in excellent agreement within the experimental error. Sixteen metabolites were quantified from 22 μ volume: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (GSH), glycerophosphorylcholine plus phosphorylcholine (GPC+PCho), *myo*-inositol (Ins), lactate (Lac), *N*-acetylaspartate (NAA), *N*-acetylaspartylglutamate (NAAG), phosphocreatine (PCr), phosphorylethanolamine (PE), and taurine (Tau). The strongly represented signals of Cr, PCr, Gln, Glu, Ins, NAA, and Tau were quantified with Cramér-Rao lower bounds below 6%. Lac, PE, and the sum of PCho and GPC were quantified with Cramér-Rao lower bounds below 10%, and those of GABA, Glc, and GSH were below 20%. Metabolite concentrations QA-lesioned striatum were compared to concentrations in contralateral striatum using the paired *t*-test.

RESULTS

^{1}H NMR spectra from the lower dose QA striatal lesion and from contralateral position are shown in Figure 1. Due to the high magnetic field and very good shimming, characteristic multiples of several metabolites, e.g., *myo*-inositol (3.53 ppm, 3.62 ppm) and taurine (3.25 ppm, 3.42 ppm), were resolved. Signals of protons bound to the C4 of glutamate (2.35 ppm) and glutamine (2.45 ppm) were clearly distinguishable. Due to a small chemical shift difference of 0.002 ppm, the methylene resonances of creatine and phosphocreatine were partially resolved at 3.9 ppm as in our previous studies (Pfeuffer et al., 2000, 1999a,b; Tkáč, et al., 1999). The differences between the spectra measured from the QA lesion and contralateral striatum, highlighted by the arrows in Figure 1, were clearly discernible in each animal.

The mean values of metabolite concentrations measured in striatal QA lesions and contralateral positions are shown in Figure 2. The most significant changes ($P < 0.005$, paired *t*-test) between QA-lesioned and contralateral striata were increased concentration of glutamine ((by +25 ± 14)% of control striatum, mean ± SD)) and decreased concentrations of glutamate (−12 ± 5)%, *N*-acetylaspartate (−17 ± 6)%, taurine (−14 ± 6)%, glutathione (−19 ± 9)%, and creatine (−11 ± 6)%. Total creatine concentration ([Cr] + [PCr]) was decreased by (−9 ± 3%). Inter-individual coefficients of variation of the measured concentrations were small, e.g., below 4% for total Cr in contralateral striatum, such that the unpaired *t*-test also returned statistical significance for all these changes ($P < 0.02$). The relative changes in metabolite concentrations between QA-lesioned and contralateral striata from each study ($n = 7$) are shown in Figure 3. Increased concentrations of Gln and decreased concentrations of Glu, Cr, GSH, NAA, and Tau in QA-lesioned relative to contralateral striatum were detected in all seven rats (Fig. 3). Phosphocreatine was decreased in five of seven rats (−6 ± 9%). Glucose concentrations in QA-lesioned striatum were decreased in six of seven rats and the concentrations of lactate were increased in five of seven rats. The intensities of the short T_1 background

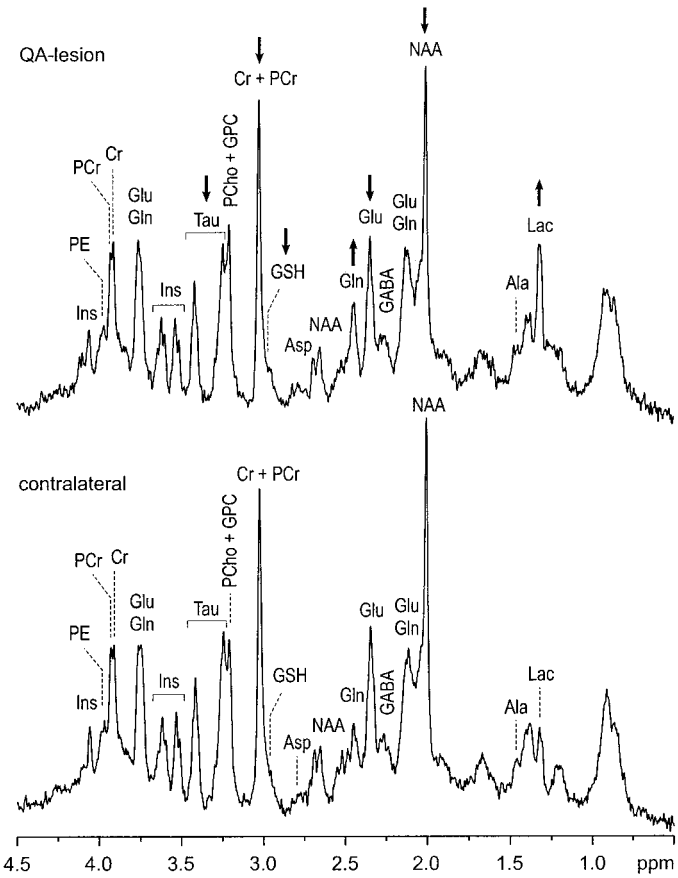

Fig. 1. In vivo ^{1}H NMR spectra from ipsi- and contralateral striata of QA-lesioned rat brain (150 nmol dose). STEAM, echo time (TE) = 2 msec, middle interval (TM) = 5 msec, repetition time (TR) = 4 sec, volume of interest (VOI) = 22 μl, number of transients (NT) = 640, Gaussian line broadening of 1 Hz. Arrows indicate the most significant changes between the spectra. Assignment of signals: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glutamate (Glu), glutamine (Gln), glycerophosphorylcholine (GPC), myo-inositol (Ins), lactate (Lac), *N*-acetylaspartate (NAA), phosphocreatine (PCr), phosphorylcholine (PCho), phosphorylethanolamine (PE), and taurine (Tau).

signals ("macromolecules") were very stable and the coefficient of variation of all fitted values from the spectra of control striata was 4%. In the lesioned striatum, the macromolecule signal was reduced by (−7 ± 5)%. None of the other metabolite changes were strong enough to reach statistical significance (Table I).

A ^{1}H NMR spectrum measured from a control striatum and spectra from striatal lesions induced with increasing QA dose are compared in Figure 4. A higher dose of quinolinic acid (300 nmol) resulted in more pronounced changes (Fig. 4C) than observed in lesions induced by the smaller QA dose (150 nmol, Fig. 4B) and, in addition, lipid resonances were detected. When the QA dose was increased even more (450 nmol), the necrotic part of the lesion caused strong field inhomogeneity, impossible to correct by shimming, which deteriorated the

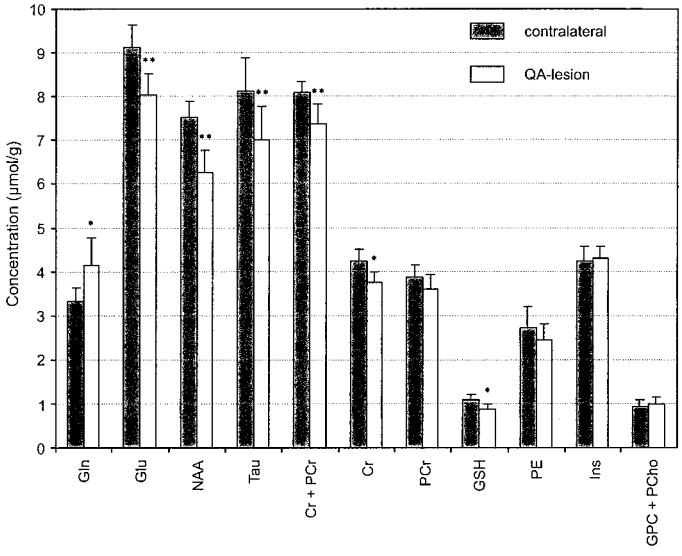

Fig. 2. Averaged values of metabolite concentrations in low dose (150 nmol) QA lesions and contralateral striata ($n = 7$). Error bars indicate SD, $\star P < 0.005$, $\star\star P < 0.001$.

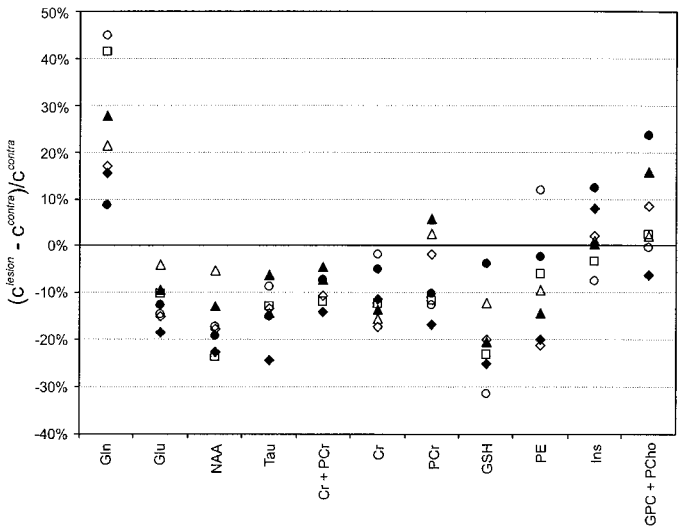

Fig. 3. Relative concentration differences of the metabolites between ipsi- and contralateral QA-lesioned rat striatum for each measured rat ($n = 7$) indicated by different symbol (QA dose: 150 nmol).

quality of ^{1}H NMR spectra (Fig. 4D) and thus decreased the accuracy and reliability of quantification. Nevertheless, it was possible to determine that the concentration of NAA in Figure 4D was decreased to 1.9 μmol/g, total creatine to 2.3 μmol/g, taurine to 3.4 μmol/g, glutamate to 5.5 μmol/g, and the lactate concentration was increased to 12.7 μmol/g, consistent with necrosis evident from the increased hyper-intensity on T_2-weighted MRI (not shown).

The average metabolite concentrations in the rat striatum were calculated using all data from contralateral striata and striata of healthy rats (first column in Table I). In addition, average concentration changes between QA-lesioned and contralateral striatum are presented in the second column in Table I.

TABLE I. Metabolite Concentrations in Rat Striatum and the Differences Between QA-Lesioned and Contralateral Striata Determined by LCModel Analysis of In Vivo ^{1}H NMR Spectra

Metabolite	Contralateral striatum Mean ± SD (μmol/g wet weight)	Differences between QA-lesioned and contralateral striata Mean ± SD (μmol/g wet weight)
Ala	0.8 ± 0.2	−0.1 ± 0.2
Cr	4.2 ± 0.3	−0.5 ± 0.3★
PCr	3.9 ± 0.3	−0.3 ± 0.4
Glc	2.9 ± 0.3	−0.3 ± 0.5
Gln	3.3 ± 0.3	0.8 ± 0.5★
Glu	9.1 ± 0.5	−1.1 ± 0.4★
GSH	1.1 ± 0.1	−0.2 ± 0.1★
Ins	4.2 ± 0.3	0.1 ± 0.3
Lac	3.1 ± 1.2	1.0 ± 1.5
NAA	7.5 ± 0.4	−1.3 ± 0.5★
NAAG	0.3 ± 0.1	0.0 ± 0.1
PE	2.7 ± 0.5	−0.3 ± 0.3
Tau	8.1 ± 0.8	−1.1 ± 0.5★
NAA + NAAG	7.8 ± 0.4	−1.4 ± 0.4★
GPC + PCho	0.9 ± 0.1	0.1 ± 0.1
Cr + PCr	8.1 ± 0.3	−0.7 ± 0.3★

★Paired t-test, $P < 0.005$.

DISCUSSION

Total creatine concentration of (8.1 ± 0.3) μmol/g was in excellent agreement with published data (McIlwain and Bachelard, 1985). The normal striatal metabolite concentrations in Table I are in very good agreement with biochemical data (Perry, 1982; McIlwain and Bachelard, 1985; Clarke et al., 1989) and in good agreement with biochemical measurements from the striatum (Jenkins et al., 2000).

The changes we observed in the QA-lesioned striatum were highly consistent among animals and highly reproducible, yet rather subtle (on the order of 10%). The qualitative agreement of the results with previously published data emphasize the utility of ^{1}H NMR spectroscopy to follow disease progression and treatment in this and possibly other animal models, as well as in patients with Huntington disease. To achieve such a reproducible measurement, however, several aspects in NMR methodology need to be emphasized that we considered critical and that are currently not yet routinely available on commercial equipment. First, a high-magnetic field (9.4 T) wide-bore horizontal magnet with a 31 cm clear bore was used and the magnetic field inhomogeneities were corrected by a rapid quantitative shimming method (FASTMAP) (Gruetter, 1993; Gruetter and Tkáč, 2000) to obtain a high spectral resolution. In addition, the pulse sequence with ultra-short echo-time of 2 msec was used to minimize

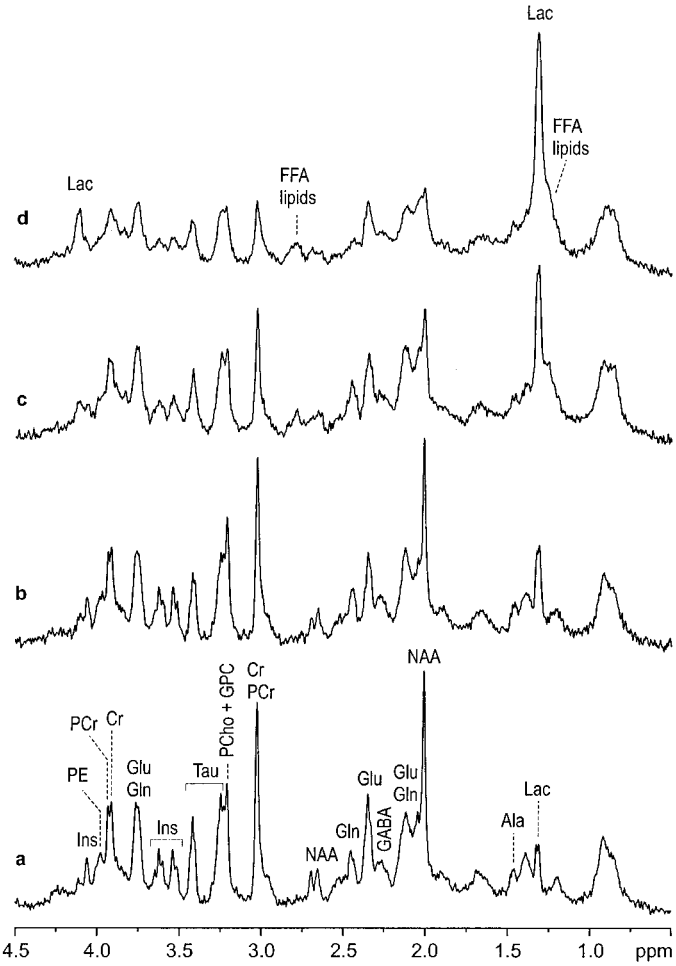

Fig. 4. Preliminary demonstration of the changes in the ^{1}H NMR spectra that can potentially be detected with increasing QA dose. From the striatum of an intact rat (**a**) compared to the spectra from the striatal QA lesion induced by increasing QA dose (**b**) 150 nmol, (**c**) 300 nmol, (**d**) 450 nmol. STEAM, TE = 2 msec, TM = 5 msec, TR = 4 sec, VOI = 22 μl, NT = 640, Gaussian line broadening of 1 Hz. For an assignment of signals see Figures 2 and 3.

J-modulation of coupled spin systems and signal losses. The high sensitivity of the method resulted in minimal partial volume effects as evidenced by the small volumes of 22 μl, which were considered adequate for the striatum for the present pilot study. Given that the changes we observed were on average half of those reported in the literature for glutamate, NAA, creatine, taurine and GSH, it is possible that residual partial volume effects may have reduced the apparent changes in metabolite concentrations. This effect can be further minimized by reducing the volume size by 50%, which can be achieved by reducing the localization dimensions by 20%. It remains to be seen, however, whether gender and or strain can affect the manifestation of this animal model of HD, as the majority of studies have used male Sprague–Dawley rats.

At 9.4 T, the spectral pattern was unique for each metabolite as a fingerprint, which resulted more robust data. The short T_1 background signals, attributed to macromolecules, were included in this analysis (LCModel) (Provencher, 1993; Pfeuffer et al., 1999a). Macromolecule signals were highly reproducible, provided excellent suppression of extra-cerebral lipid signals by outer volume suppression was achieved, which we consistently verified in our study (Tkáč, et al., 1999). Excellent stability of the baseline was attributed to the advanced water suppression methods used (Tkáč, et al., 1999), leading to an almost negligible water signal in the spectra. Taken together, these advances lead to the simultaneous quantification of more than fifteen metabolites non-invasively in the QA-lesioned and contralateral rat striatum, providing a paired comparison within each animal. The resulting quantification was robust as indicated by the very small inter-individual variations in measured metabolite concentrations. Total creatine was quantified with Cramér-Rao lower bounds of 2% and the coefficient of variation in non-lesioned striata was below 5%, which can be considered adequate for most neurochemical applications.

The sum of concentrations ([NAA] + [NAAG]) was reduced in the QA-lesioned striatum in agreement with previously published NMR data from lesions induced by QA (Strauss et al., 1997). The decreased NAA concentration in the QA lesion can by explained by the decreased NAA concentration in excitotoxically-impaired neurons as well as the selective loss of neurons in the lesion. The increased glutamine and decreased glutamate concentrations are in agreement with the results of chemical analysis of tissue extracts performed 12 hr after QA injection (Bordelon et al., 1997), although we did not observe such a substantial decrease in glutamate (Bordelon et al., 1997). The QA dose and the post-injection time, however, were not identical. It is also possible that a partial-volume effect, i.e., the lesion size was smaller than the measured volume of interest (22 μl), and a spatial heterogeneity of the lesion (Bordelon and Chesselet, 1999; Brickell et al., 1999) influenced our data.

Increased steady-state glutamine concentration can be interpreted as the result of a shifted equilibrium between Gln production in astrocytes (glutamine synthetase) and decreased uptake by damaged and dying neurons, which convert glutamine to glutamate via glutaminase (Greene and Greenamyre, 1996). The presence of elevated Gln in the lesion is evidence of a profound metabolic defect and is in agreement with biochemical extracts from transgenic mice (Jenkins et al., 2000). The observed increase in glutamine compared to the decrease in glutamate can be analyzed in terms of the metabolic trafficking between neurons and glia that occurs during glutamatergic action (Tsacopoulos and Magistretti, 1996; Daikhin and Yudkoff, 2000). The changes were highly significant for both Glu and Gln, however, the change in the sum of both was 0.3 ± 0.6 μmol/g, which was not significant ($P > 0.6$). Although the metabolite changes could be perceived as reflecting a shift in cellular composition of the tissue examined, the constancy of ([Glu] + [Gln]) may also imply a metabolic shift similar to what has been observed

in ischemia, namely an increased Gln concentration (in astrocytes) and a decreased (neuronal) glutamate concentration (Aas et al., 1993). Such a metabolic blockade at the level of the conversion of glutamine to glutamate (or glutamine transport out of the astrocyte) would imply that the action of QA on metabolism is indirect, by affecting the glutamine-glutamate interconversion, which has been linked to glutamatergic action (Yudkoff et al., 1993; Gruetter et al., 1998a; Sibson et al., 1998). Our data suggest that the conversion of glutamine to glutamate or transport of glutamine out of the glia and into the neurons are impaired. Because neurons lack the ability to synthesize TCA cycle intermediates de novo through anaplerosis, replenishment of the neurotransmitter glutamate pool from astrocytic glutamine appears to be inhibited at one of the many steps involved in converting glial glutamine to neuronal glutamate.

Interestingly, highly significant changes ($P < 0.002$) were detected in brain glutathione. Concentration of GSH can be measured by ^{1}H NMR spectroscopy (Pfeuffer et al., 1999a; Trabesinger et al., 1999; Trabesinger and Boesiger, 2001) and GSH metabolism can be followed after administration of [1-^{13}C]glucose (Choi and Gruetter, 2000). Glutathione is known as a free radical scavenger whose concentration has been postulated to reflect mitochondrial integrity. The decrease of glutathione ($-19 \pm 9\%$) in the excitotoxic lesion is in excellent agreement with a recent study measuring a $30 \pm 10\%$ decrease in GSH using biochemical extraction 1 week after 225 nmol QA injection (Cruz-Aguado et al., 2000). The somewhat smaller decrease in our study (Table I) probably reflects the smaller dose (150 nmol) used. These results strongly point to the involvement of oxidative stress possibly associated with impaired mitochondrial function in the pathogenesis of the toxin model of HD (Bordelon et al., 1997). Decreased GSH in QA lesions is also consistent with the observation that antioxidant treatment (Koroshetz et al., 1997; Matthews et al., 1998a) protected striatal neurons against excitotoxic insult, which supports the hypothesis that free radicals contribute to excitotoxic neuronal injury (Nakao et al., 1996).

We observed an 8% decrease in striatal GABA concentration between QA lesion (150 nmol dose) and contralateral striatum that was not significant due to the 25% coefficient of variation. Our inability to detect changes in GABA are not necessarily at odds with the well-established decrease of GABAergic neurons in the QA-lesioned striatum, when considering the experimental errors involved. First, only a 25% decrease was reported 2 weeks after administering 150 nmol of QA (Beal et al., 1986). Second, the scatter in the GABA measurements can be explained by the difficulties involved in detecting small changes in the GABA signal, that is comparatively weakly represented in the ^{1}H spectrum, even at 9.4 T.

The decreased concentration of Tau (-14 ± 6)% observed in our study is in agreement with the decrease in extracellular Tau concentration measured by microdialysis (Bockelmann et al., 1998) and with an 46% decrease in Tau concentration measured 7 days after 240 nmol QA (Ellison et al., 1987). Phosphocreatine was decreased and lactate was increased in the QA lesions relative to the contralateral striatum in five of seven rats, which supports the notion that QA induces impairments in energy metabolism (Bordelon et al., 1997).

Preliminary data indicated that progressively increasing the QA dose induced more pronounced changes in the ^{1}H NMR spectra (Fig. 4C,D), which is in agreement with dose-dependent changes in metabolite concentrations observed in QA lesions (Beal et al., 1986).

Our study thus illustrates the potential of high-field in vivo ^{1}H NMR spectroscopy to detect small neurochemical changes in animal models of HD. This emerging modality can be expected to be very useful in longitudinally assessing fetal graft viability as potential treatments of HD in animal models and human disease alike.

We conclude that high field in vivo ^{1}H NMR spectroscopy can detect changes in the neurochemical profile in the quinolinic acid-lesioned rat striatum, suitable to monitor the pathophysiology of the striatum after excitotoxic injury, and thus provide a better understanding of the neuropathological processes that underlie neurodegeneration. The observed increase in the concentration of glutamine and decrease in concentration of phosphocreatine/creatine, glutamate, glutathione, *N*-acetylaspartate, and taurine are consistent with neuronal cell loss, impaired energy metabolism and oxidative stress. Furthermore, the increased ratio of [Glu]/[Gln] in QA lesions indicates an imbalance between glutamine production in astrocytes and its uptake by damaged and dying neurons.

ACKNOWLEDGMENTS

Supported by NIH grant P41RR08079 from the Center for Research Resources, NIH grants R01NS38672 (RG), F30MH12157 (CDK), the Lyle French Fund (WL), and the Whitaker Foundation (RG).

REFERENCES

Aas JE, Berg-Johnsoen J, Hegstad E, Laake JH, Langmoen IA, Ottersen OP. 1993, Redistribution of glutamate and glutamine in slices of human neocortex exposed to combined hypoxia and glucose deprivation in vitro. J Cereb Blood Flow Metab 13:503–515.

Adriany G, Gruetter R. 1997. A half-volume coil for efficient proton decoupling in humans at 4 tesla. J Magn Reson 125:178–184.

Araujo DM, Cherry SR, Tatsukawa KJ, Toyokuni T, Kornblum HI. 2000. Deficits in striatal dopamine D_2 receptors and energy metabolism detected by in vivo microPET imaging in a rat model of Huntington disease. Exp Neurol 166:287–297.

Beal MF. 2000. Energetics in the pathogenesis of neurodegenerative diseases. Trends Neurosci 23:298–304.

Beal MF, Ferrante RJ, Swartz KJ, Kowall NW. 1991. Chronic quinolinic acid lesions in rats closely resemble Huntington disease. J Neurosci 11:1649–1659.

Beal MF, Kowall NW, Ellison DW, Mazurek MF, Swartz KJ, Martin JB. 1986. Replication of the neurochemical characteristics of Huntington disease by quinolinic acid. Nature 321:168–171.

Beal MF, Kowall NW, Swartz KJ, Ferrante RJ, Martin JB. 1988. Systemic approaches to modifying quinolinic acid striatal lesions in rats. J Neurosci 8:3901–3908.

Bockelmann R, Reiser M, Wolf G. 1998. Potassium-stimulated taurine release and nitric oxide synthase activity during quinolinic acid lesion of the rat striatum. Neurochem Res 23:469–475.

Bordelon YM, Chesselet MF. 1999. Early effects of intrastriatal injections of quinolinic acid on microtubule-associated protein-2 and neuropeptides in rat basal ganglia. Neuroscience 93:843–853.

Bordelon YM, Chesselet MF, Erecinska M, Silver IA. 1998. Effects of intrastriatal injection of quinolinic acid on electrical activity and extracellular ion concentrations in rat striatum in vivo. Neuroscience 83:459–469.

Bordelon YM, Chesselet MF, Nelson D, Welsh F, Erecinska M. 1997. Energetic dysfunction in quinolinic acid-lesioned rat striatum. J Neurochem 69:1629–1639.

Brickell KL, Nicholson LF, Waldvogel HJ, Faull RL. 1999. Chemical and anatomical changes in the striatum and substantia nigra following quinolinic acid lesions in the striatum of the rat: a detailed time course of the cellular and $GABA_{(A)}$ receptor changes. J Chem Neuroanat 17:75–97.

Ceresoli-Borroni G, Guidetti P, Schwarcz R. 1999. Acute and chronic changes in kynurenate formation following an intrastriatal quinolinate injection in rats. J Neural Transm 106:229–242.

Choi IY, Gruetter R. 2000. Dynamic detection of *N*-acetyl-aspartate and glutathione turnover using in vivo ^{13}C NMR. J Neurochem 74:S86A.

Clarke DD, Lajtha AL, Maher SD. 1989. Intermediary metabolism. In: Siegel GJ, editor. Basic neurochemistry. Molecular, cellular, and medical aspects. New York: Raven Press. p 541–564.

Cruz-Aguado R, Francis-Turner L, Diaz CM, Antunez I. 2000. Quinolinic acid lesion induces changes in rat striatal glutathione metabolism. Neurochem Int 37:53–60.

Daikhin Y, Yudkoff M. 2000. Compartmentation of brain glutamate metabolism in neurons and glia. J Nutr 130:1026S–1031S.

Ellison DW, Beal MF, Mazurek MF, Malloy JR, Bird ED, Martin JB. 1987. Amino acid neurotransmitter abnormalities in Huntington disease and the quinolinic acid animal model of Huntington disease. Brain 110:1657–1673.

Ferrante RJ, Andreassen OA, Jenkins BG, Dedeoglu A, Kuemmerle S, Kubilus JK, Kaddurah-Daouk R, Hersch SM, Beal MF. 2000. Neuroprotective effects of creatine in a transgenic mouse model of Huntington disease. J Neurosci 20:4389–4397.

Ferrer I, Martin F, Serrano T, Reiriz J, Perez-Navarro E, Alberch J, Macaya A, Planas AM. 1995. Both apoptosis and necrosis occur following intrastriatal administration of excitotoxins. Acta Neuropathol 90:504–510.

Greene JG, Greenamyre JT. 1996. Bioenergetics and glutamate excitotoxicity. Prog Neurobiol 48:613–634.

Gruetter R. 1993. Automatic, localized in vivo adjustment of all first- and second-order shim coils. Magn Reson Med 29:804–811.

Gruetter R, Seaquist ER, Kim S, Ugurbil K. 1998a. Localized in vivo ^{13}C-NMR of glutamate metabolism in the human brain: initial results at 4 tesla. Dev Neurosci 20:380–388.

Gruetter R, Tkáč, I. 2000. Field mapping without reference scan using asymmetric echo-planar techniques. Magn Reson Med 43:319–323.

Gruetter R, Weisdorf SA, Rajanayagan V, Terpstra M, Merkle H, Truwit CL, Garwood M, Nyberg SL, Ugurbil K. 1998b. Resolution improvements in vivo ^1H NMR spectra with increased magnetic field strength. J Magn Reson 135:260–264.

Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH. 1996. Mitochondrial defect in Huntington disease caudate nucleus. Ann Neurol 39:385–389.

Haque NS, Borghesani P, Isacson O. 1997. Therapeutic strategies for Huntington disease based on a molecular understanding of the disorder. Mol Med Today 3:175–183.

Jenkins BG, Brouillet E, Chen YC, Storey E, Schulz JB, Kirschner P, Beal MF, Rosen BR. 1996. Non-invasive neurochemical analysis of focal excitotoxic lesions in models of neurodegenerative illness using spectroscopic imaging. J Cereb Blood Flow Metab 16:450–461.

Jenkins BG, Klivenyi P, Kustermann E, Andreassen OA, Ferrante RJ, Rosen BR, Beal MF. 2000. Nonlinear decrease over time in *N*-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington disease mice. J Neurochem 74: 2108–2119.

Jenkins BG, Koroshetz WJ, Beal MF, Rosen BR. 1993. Evidence for impairment of energy metabolism in vivo in Huntington disease using localized 1H NMR spectroscopy. Neurology 43:2689–2695.

Jenkins BG, Rosas HD, Chen YC, Makabe T, Myers R, MacDonald M, Rosen BR, Beal MF, Koroshetz WJ. 1998. ^1H NMR spectroscopy studies of Huntington disease: correlations with CAG repeat numbers. Neurology 50:1357–1365.

Koroshetz WJ, Jenkins BG, Rosen BR, Beal MF. 1997. Energy metabolism defects in Huntington disease and effects of coenzyme Q10. Ann Neurol 41:160–165.

Lee WT, Lee CS, Pan YL, Chang C. 2000. Temporal changes of cerebral metabolites and striatal lesions in acute 3-nitropropionic acid intoxication in the rat. Magn Reson Med 44:29–34.

Matthews RT, Yang L, Browne S, Baik M, Beal MF. 1998a. Coenzyme Q_{10} administration increases brain mitochondrial concentrations and exerts neuroprotective effects. Proc Natl Acad Sci USA 95:8892–8897.

Matthews RT, Yang L, Jenkins BG, Ferrante RJ, Rosen BR, Kaddurah-Daouk R, Beal MF. 1998b. Neuroprotective effects of creatine and cyclocreatine in animal models of Huntington disease. J Neurosci 18: 156–163.

McIlwain H, Bachelard HS. 1985. Biochemistry and the central nervous system. New York: Churchill Livingstone. p 30–53, 154–201.

Mena FV, Baab PJ, Zielke CL, Zielke HR. 2000. In vivo glutamine hydrolysis in the formation of extracellular glutamate in the injured rat brain. J Neurosci Res 60:632–641.

Nakao N, Grasbon-Frodl EM, Widner H, Brundin P. 1996. Antioxidant treatment protects striatal neurons against excitotoxic insults. Neuroscience 73:185–200.

Perry TL. 1982. Handbook of neurochemistry. In: Lajtha A, editor. Cerebral amino acid pool. New York: Plenum Press. p 151–180.

Pfeuffer J, Tkáč, I, Gruetter R. 2000. Extracellular-intracellular distribution of glucose and lactate in the rat brain assessed noninvasively by diffusion-weighted ^1H nuclear magnetic resonance spectroscopy in vivo. J Cereb Blood Flow Metab 20:736–746.

Pfeuffer J, Tkáč, I, Provencher SW, Gruetter R. 1999a. Toward an in vivo neurochemical profile: quantification of 18 metabolites in short-echo-time ^1H NMR spectra of the rat brain. J Magn Res 141:104–120.

Pfeuffer J, Tkáč, I, Ugurbil K, Garwood M, Gruetter R. 1999b. Detection and assignment of phosphocreatine signal in vivo in ^1H NMR spectra at 9.4 T. 7th Annual Meeting Proc, ISMRM, Glasgow, UK, 22–28 May, 1999. p 1363.

Portera-Cailliau C, Hedreen JC, Price DL, Koliatsos VE. 1995. Evidence for apoptotic cell death in Huntington disease and excitotoxic animal models. J Neurosci 15:3775–3787.

Provencher SW. 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magn Res Med 30:672–679.

Reynolds NC Jr, Lin W, Meyer Cameron C, Roerig DL. 1997. Differential responses of extracellular GABA to intrastriatal perfusions of 3-nitropropionic acid and quinolinic acid in the rat. Brain Res 778:140–149.

Rodriguez-Martinez E, Camacho A, Maldonado PD, Pedraza-Chaverri J, Santamaria D, Galvan-Arzate S, Santamaria A. 2000. Effect of quinolinic acid on endogenous antioxidants in rat corpus striatum. Brain Res 858: 436–439.

Schwarcz R, Whetsell WO Jr, Mangano RM. 1983. Quinolinic acid: an endogenous metabolite that produces axon-sparing lesions in rat brain. Science 219:316–318.

Sharp AH, Ross CA. 1996. Neurobiology of Huntington disease. Neurobiol Dis 3:3–15.

Shear DA, Dong J, Gundy CD, Haik-Creguer KL, Dunbar GL. 1998. Comparison of intrastriatal injections of quinolinic acid and 3-nitropropionic acid for use in animal models of Huntington disease. Prog Neuropsychopharmacol Biol Psychiatry 22:1217–1240.

Sibson NR, Dhankhar A, Mason GF, Rothman DL, Behar KL, Shulman RG. 1998. Stoichiometric coupling of brain glucose metabolism and glutamatergic neuronal activity. Proc Natl Acad Sci USA 95:316–321.

Strauss I, Williamson JM, Bertram EH, Lothman EW, Fernandez EJ. 1997. Histological and ^{1}H magnetic resonance spectroscopic imaging analysis of quinolinic acid-induced damage to the rat striatum. Magn Reson Med 37:24–33.

Tabrizi SJ, Cleeter MW, Xuereb J, Taanman JW, Cooper JM, Schapira AH. 1999. Biochemical abnormalities and excitotoxicity in Huntington disease brain. Ann Neurol 45:25–32.

Terpstra M, Ugurbil K, Gruetter R. 2001. Direct in vivo measurement of cerebral GABA in humans using MEGA-editing at 7 T. 9th Annual Meeting Proc, ISMRM, Glasgow, UK, 21–27 April, 2001. p 961.

Tkáč, I, Kim J, Ugurbil K, Gruetter R. 2001. Quantification of 7 T ^{1}H NMR spectra of the human brain using LCModel and spin simulation. 9th Annual Meeting Proc, ISMRM, Glasgow, UK, 21–27 April, 2001. p 214.

Tkáč, I, Starcuk Z, Choi IY, Gruetter R. 1999. In vivo ^{1}H NMR spectroscopy of rat brain at 1 msec echo time. Magn Reson Med 41: 649–656.

Trabesinger AH, Boesiger P. 2001. Improved selectivity of double quantum coherence filtering for the detection of glutathione in the human brain in vivo. Magn Reson Med 45:708–710.

Trabesinger AH, Weber OM, Duc CO, Boesiger P. 1999. Detection of glutathione in the human brain in vivo by means of double quantum coherence filtering. Magn Reson Med 42:283–239.

Tsacopoulos M, Magistretti PJ. 1996. Metabolic coupling between glia and neurons. J Neurosci 16:877–885.

Yudkoff M, Nissim I, Daikhin Y, Lin ZP, Nelson D, Pleasure D, Erecinska M. 1993. Brain glutamate metabolism: neuronal-astroglial relationships. Dev Neurosci 15:343–350.