Identification of a High Concentration of *Scyllo*-Inositol in the Brain of a Healthy Human Subject Using ^1H - and ^{13}C -NMR

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The peak at 3.35 ppm in the ^1H -NMR spectrum characteristic for *scyllo*-inositol may be a marker for cerebral pathology, although it has a well-known constant concentration relative to *myo*-inositol. Such a peak was observed with an intensity at least 300% above normal in the brain of a healthy volunteer. The *scyllo*-inositol signal was assigned based on the detection of a corresponding peak at 74.5 ppm in the ^{13}C -NMR spectrum and on the demonstration of singlet characteristics of the proton signal. The presence of substantial brain concentrations of *scyllo*-inositol suggests that *scyllo*-inositol metabolism may be regulated independently from *myo*-inositol and that such concentrations are compatible with normal health.

Key words: *scyllo*-inositol, human brain, inositol phosphates, spectroscopy.

INTRODUCTION

Scyllo-inositol is one of five naturally occurring stereoisomers of inositol and it has been found to be the second isomer in abundance to *myo*-inositol in the brains of healthy humans and other mammalian species. This isomer has six equivalent CH protons that yield a singlet resonance at a chemical shift of 3.35 ppm. Based on quantification of the *scyllo*-inositol peak, healthy human brain tissue has been suggested to contain .4–.6 mM based on ^1H MRS at 2 Tesla (1). This concentration seems to be tightly coupled to the *myo*-inositol concentration at a 12:1 ratio in healthy brain, in agreement with previous biochemical assays in animal brain. The function of this isomer remains uncertain, but it is readily rearranged to form *myo*-inositol, the precursor to *myo*-inositol 1-phosphate (2), which is of key importance in signal transduction and protection of membrane integrity (3). Concentrations of this isomer have been found to be increased in patients with mitochondrial enzyme deficiency (1), human immunodeficiency virus (HIV) infection (4), and some brain tumors (5). Recently, we observed an unusu-

ally intense signal at 3.35 ppm in the brain of a healthy man, which suggests that *scyllo*-inositol concentration is independently regulated from that of *myo*-inositol and that elevated cerebral concentration can occur in asymptomatic people.

METHODS

^1H Spectroscopy

Experiments were performed on a 4.0-Tesla/125-cm Siemens magnet interfaced with a Varian console using a 15-cm-diameter quadrature semivolume coil and a 3-cm head gradient insert with a 250- μs rise time to 30 mT/m. Localization was based on STEAM ($TE = 20$ ms, $TM = 33$ ms, $TR = 3$ s, 27-ml nominal volume) (6). Shimming was performed with fast, automatic skimming techniques by mapping along projections (FASTMAP) (7). Water suppression was accomplished by using four 25-ms Gaussian pulses using 3,1-DRYSTEAM (6). Spectra were acquired in 3-minute blocks, zero-filled, and apodized using a 2-Hz Lorentz filter.

^{13}C -MRS experiments were performed on the same 4.0-Tesla magnet used for ^1H spectroscopy using a 7-cm ^{13}C coil with a 12-cm quadrature ^1H surface coil (8) and a whole-body gradient capable of switching 25 mT/m in 1.2 ms. Localization was based on fast low angle shot (FLASH) imaging. Shimming was performed by FASTMAP (7). The ^1H decoupler signal was passed through a ^1H bandpass filter and the ^{13}C coil port was filtered with a low-pass filter (0.1-dB insertion loss). Localization was performed using proton excitation for carbon-13 image selected localized in vivo spectroscopy (PRECISELY), as described previously (9): Briefly, image-selected in vivo spectroscopy (ISIS) was used for localization on the ^1H z magnetization, which was transferred to the ^{13}C nucleus using distortionless enhanced polarization transfer (DEPT). WALTZ-16 was applied at 30 W for 171 ms, $TR = 3$ s, $SAR < 3$ W/kg.

Data Analysis

Estimation of isomer concentration was accomplished by comparison to the creatine peak at 3.03 ppm. The area of this peak was assumed to represent a 10-mM concentration of creatine, the concentration previously reported in extracts of cortical tissue using high-field ^1H -MRS (10–12), and recently supported by *in vivo* measurements of human brain in which creatine and phosphocreatine, along with minor contributions from GABA, lysine, and glutathione, were found to contribute to the area of the peak at 3.03 ppm (13, 14). Data are reported as mean $\pm$ SEM, unless otherwise stated.

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CASE REPORT

The subject was a 43-year-old man who presented for participation in MR studies as a normal control. His medical history was significant for asthma and a previously diagnosed anxiety disorder that was currently inactive. His only medication was an albuterol inhaler, which he used on an as-needed basis. He did not smoke and he drank wine with dinner about four nights each week. His family history was significant for the diagnosis of Marfan syndrome in his father. He was married and employed as a custodian. A recent HIV titer performed at a blood donor center was negative. The subject was 180 cm tall and weighed 75 kg. His blood pressure was 115/72 and his heart rate was 72 beats per minute. His physical examination was unremarkable, including examination of his mental status, gait, cerebellar function, strength, and sensation. MR images of his brain were unremarkable.

RESULTS

^1H spectroscopy of the subject's occipital lobe revealed the presence of a clearly resolved peak at 3.35 ppm (Fig. 1) on three separate occasions during a 12-month period. A similar peak was not observed in the spectrum of a healthy control and an *in vivo* difference spectrum created by subtracting a control spectrum from the case subject's spectrum demonstrate that the 3.35-ppm peak is clearly larger in the case subject than in the control (Fig. 2). The excellent subtraction of all peaks but the peak at 3.35 ppm was also evident when subtracting spectra from other subjects. Additional spectra were acquired from the cerebellum (Fig. 1), and the periventricular white matter of the subject also displayed the presence of the peak at 3.35 ppm that has been identified as

FIG. 1. ^1H spectra acquired from the brain of the case subject. ^1H spectra were acquired from both the occipital cortex (bottom) and the cerebellum (top) of the subject. Peaks at 3.35 ppm were detected in data from both regions, suggesting that the cerebral tissue of this subject contains high concentrations of *scyllo*-inositol. (Abbreviations: Cho, choline groups; Cr, creatine; NAA, N-acetyl aspartate.)

FIG. 2. *In vivo* difference spectrum. ^1H spectra were acquired from the occipital cortex of the case subject (middle spectrum) and a healthy control (bottom spectrum). The peak at 3.35 ppm in the *in vivo* difference spectrum (top spectrum) demonstrates the degree of elevation of *scyllo*-inositol in the case subject.

scyllo-inositol. These peaks were absent in spectra acquired from the triceps surae (calf) muscle.

A series of echo times revealed positive intensity at 3.35 ppm compatible with apparent singlet characteristics. Fitting an exponential decay to the measured peak area at 3.35 ppm gave a T_2 of approximately 170 ms (Fig. 3). T_2 of the methyl peak of creatine at 3.03 ppm was 115 ms and that of choline at 3.22 ppm was determined at 106 ms. Inversion recovery nulled the signal at 3.35 ppm, along with the signal from the methyl peak of creatine (data not shown), suggesting a very similar T_1 of *scyllo*-inositol and the creatine methyl peak.

To further document that the subject had high brain and not muscle concentrations of *scyllo*-inositol, ^{13}C spectroscopy was performed. In unlocalized ^{13}C MR spectra of occipital cortex, the *scyllo*-inositol peak expected at 74.5 ppm (15) was readily identified (Fig. 4). Localized spectra further demonstrated the presence of this isomer signal in the occipital gray matter and comparison with previously reported ^{13}C spectra demonstrates that this signal is difficult to detect in the brains of other healthy volunteers (9, 16). ^{13}C spectroscopy of the triceps surae muscle failed to reveal a *scyllo*-inositol peak, adding further evidence that *scyllo*-inositol is not present in skeletal muscle tissue.

The concentration of *scyllo*-inositol in the occipital cortex of our subject was estimated to be 1.4 ± 0.1 mM ($n = 5$); a concentration substantially higher than the concentration of 0.43 ± 0.11 mM (mean $\pm$ SD, $n = 23$) previously reported for human gray matter in normal controls (1). Since the difference spectrum between our subject and a normal control failed to reveal the presence of a *myo*-inositol peak at 3.56 ppm relative to creatine (Fig. 2), we estimate that our subject has a normal concentration of *myo*-inositol. Using the previously determined *myo*-inositol concentration of 6–7 mM in gray matter (1, 13, 16), we calculated a *myo*-inositol to *scyllo*-

FIG. 3. Characterization of signal at 3.35 ppm in the ^1H -NMR spectrum. A series of echo times demonstrates the peak at 3.35 ppm (arrow) to have the expected singlet characteristics of *scyllo*-inositol and a T_2 of 170 ms.

inositol ratio of approximately 5:1 in the occipital cortex of our subject.

DISCUSSION

Scyllo-inositol is one of five naturally occurring isomers of inositol. Found in high concentrations in plant tissue, *scyllo*-inositol has been detected in the central nervous system, the male reproductive tract, and the spleen of several mammalian species (1, 2, 17, 18). The function of *scyllo*-inositol is unknown, but it is readily rearranged by inositol epimerase to form *myo*-inositol (2, 19). *Myo*-inositol can subsequently be phosphorylated to *myo*-inositol-1-phosphate, which is essential in the maintenance of membrane integrity and signal transduction (3). Intracerebral *scyllo*-inositol is derived from the blood and it enters the brain across the choroid plexus via a saturable transport system (20). Since *myo*-inositol has an equally high affinity for this transport system, *scyllo*-inositol levels may serve to regulate the entry of *myo*-inositol into the brain (20, 21).

Elevated signal from *scyllo*-inositol was identified in our proton spectra at 3.35 ppm with the aid of natural abundance ^{13}C NMR. Although this assignment is generally accepted, Preece and colleagues have questioned whether this resonance could be due to methanol contamination that may be present in extracts (22). Since our subject was not suffering from methanol poisoning and the ^{13}C chemical shift of methanol is different from *scyllo*-inositol, the peak observed in the subject at 3.35

ppm is mostly from *scyllo*-inositol. Our observations support those of Michaelis and colleagues (23), who assert that the resonance at 3.35 ppm is due to *scyllo*-inositol, but the *myo*-inositol H2 triplet may contribute to the peak as well (24).

High concentrations of cerebral *scyllo*-inositol have been very recently identified by NMR in humans with mitochondrial enzyme deficiency (1), some brain tumors (5), and long-standing HIV infection (4). Our observation of increased intracerebral *scyllo*-inositol in a healthy subject suggests that such a finding is compatible with a normal neurological status. In addition, although most studies have suggested that the ratio of *myo*-inositol to *scyllo*-inositol is regulated at approximately 12:1 in healthy brain (1), our subject was found to have a ratio of 5:1. These observations demonstrate that elevated *scyllo*-inositol concentrations can be found in the nervous tissues of healthy humans even when *myo*-inositol concentrations are normal, suggesting that metabolism of *scyllo*-inositol may be independently regulated from that of *myo*-inositol.

It is, however, possible that our subject had an underlying, not yet detected or clinically not relevant disease that caused increased *scyllo*-inositol concentration. The persistence of these findings over 12 months while our subject enjoyed excellent health makes this unlikely, as do the normal MR images of his brain. Our subject had previously been diagnosed with an anxiety disorder, and we can speculate that this psychiatric disease was responsible for the biochemical alteration observed. However, since this problem had been inactive for more than a decade, we do not believe it is responsible for our findings. Subjects previously found to have high signal at 3.35 ppm had serious medical diseases consisting of long-standing HIV infection complicated by substance abuse (4). Neither of these clinical situations are applicable to our subject. Therefore, we do not believe he has an underlying disease that could increase the *scyllo*-inositol concentrations in his brain and we conclude that such concentrations can be found in the absence of serious cerebral pathology.

FIG. 4. Unlocalized ^{13}C spectra from the occipital cortex. A peak was resolved at the 74.5-ppm location at which *scyllo*-inositol is found in the ^{13}C -NMR spectrum, demonstrating that the occipital cortex of this subject contains a high concentration of *scyllo*-inositol. (Abbreviations: Glu, glutamate; Gln, glutamine; Cr, creatine; Cho, choline groups; NAA, *N*-acetyl aspartate.)

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