

# NEUROLOGY

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*Neurology* 2004;62;1016-1018

**This information is current as of May 27, 2008**

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# Proton MR spectroscopy in succinic semialdehyde dehydrogenase deficiency

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**Abstract**—Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare hereditary disorder of the CNS catabolism of  $\gamma$ -aminobutyric acid (GABA), leading to accumulation of the metabolite 4-hydroxybutyrate (GHB). Here the authors report on 1.5 and 3.0 T proton MR spectroscopy in a patient with SSADH deficiency. A characteristic pattern with clearly elevated GABA levels and traces of GHB was found in both the white and the gray matter of the brain. In vivo spectroscopy may be useful for diagnosis and monitoring SSADH deficiency.

NEUROLOGY 2004;62:1016–1018

Succinic semialdehyde dehydrogenase (SSADH) deficiency is a rare autosomal recessive disorder affecting the catabolism of the neurotransmitter  $\gamma$ -aminobutyric acid (GABA).<sup>1</sup> In the absence of SSADH, transamination of GABA to succinic semialdehyde is followed by its reduction to 4-hydroxybutyrate (GHB).<sup>1</sup> As detection of GHB is commonly missed by routine gas chromatography mass spectrometry (GC-MS) methods, diagnosis of SSADH deficiency is established by selective ion-monitoring GC-MS or assessment of SSADH activity in lymphocytes.<sup>2</sup> The clinical syndrome is defined by mental retardation, seizures, and impaired motor function.<sup>1,2</sup> We studied whether the specific pattern of biochemical abnormalities of GABA metabolism in SSADH deficiency is detectable by proton MR spectroscopy (<sup>1</sup>H-MRS).

**Methods.** *Case report.* We studied a 20-year-old patient with delayed speech since early childhood and deficits in motor functions. He was untreated for the last 5 years. Examination showed severely impaired language, mild deficits in fine motor functions, and severe anxiety/mutism. Metabolic screening revealed elevated GHB in urine (188 mmol/mol of creatinine) and plasma (276  $\mu$ mol/L). Diagnosis of SSADH deficiency was confirmed by absent SSADH activity in lymphocytes. Vigabatrin (25 to 75 mg/kg/day) was discontinued owing to a lack of amelioration and recurrence of seizures (one to two per year).

*<sup>1</sup>H-MRS.* The patient and four healthy volunteers (ages 20 to 29) were examined on 1.5/3.0 T MR systems (Magnetom Symphony and Trio, Siemens, Germany). Three orthogonal sets of T2-weighted images were acquired for positioning of volumes of interest (VOI). Single-voxel spectroscopy in a  $2 \times 2 \times 2$ -cm<sup>3</sup> VOI was performed in frontal (interhemispheric) and occipital gray matter (GM) as well as frontal and parietal white matter (WM). A point-resolved spectroscopy (PRESS) sequence (echo time [TE] = 30 milliseconds, repetition time [TR] = 2,000 milliseconds, no. of excitations [NEX] = 128) was used at both scanners. Reference spectra were obtained for each VOI without water suppression with a long TR of 10 seconds using two excitations. This spectrum was used for eddy current correction and for absolute quantification using the fully relaxed water signal as internal reference.

*Spectra analysis.* The LCModel software<sup>3</sup> was used to estimate the absolute concentrations of N-acetyl compounds, total creatine, choline-containing substances, myo-inositol, glutamate and glutamine (Glx), and GABA. The LCModel basis sets for 1.5 and 3.0 T were previously obtained with the same sequences at both scanners and consisted of fully relaxed model spectra (TR = 10,000 milliseconds, TE = 30 milliseconds, NEX = 128) of 15 metabolite solutions measured at room temperature and pH 7.2. To allow for possible GHB contributions, in vitro spectra of GHB were acquired and included into the fitting procedure (figure 1). All in vivo spectra were evaluated using the standard LCModel basis set and the basis set containing the spectrum of GHB. No correction for metabolite T1 and T2 relaxation effects was performed.

*Image segmentation.* A T1-weighted three-dimensional image data set was acquired with a spatial resolution of 1 mm in each direction. Segmentation of the three-dimensional image data set was performed using SPM99 software (Mathworks, Newton, MA) to determine the fractional content of GM and WM as well as CSF within the VOI ( $p$ [GM],  $p$ [WM], or  $p$ [CSF]). The metabolite concentrations obtained by LCModel were divided by the fractional content of brain tissue ( $p$ [GM] +  $p$ [WM]) to correct for CSF contribution.

**Results.** Figure 2A shows the position of the VOI in the frontal GM of the patient. <sup>1</sup>H-MRS spectra obtained in the frontal GM of the patient and an age-matched volunteer at 1.5 and 3.0 T are shown in figure 2B. At 1.5 T, a higher <sup>1</sup>H-MRS signal was found in the spectra of the patient as compared with the healthy control in the spectral range between 2.20 and 2.40 ppm. As shown by the LCModel fits for GABA, glutamate, and glutamine at 1.5 T (see figure 2C), the resonances of these metabolites overlap in this spectral region. At 3.0 T, the higher spectral resolution allowed a better discrimination of these resonances and revealed that the elevation is mostly attributable to the peaks at about 2.30 and 2.35 ppm. The LCModel fits show that these two peaks are mainly contributions from GABA and glutamate, which can be well separated owing to the additional triplet of glutamate centered at 3.75 ppm (see figure 2C).

The table summarizes tissue compositions of the VOI

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Supported by Deutsche Forschungsgemeinschaft (grants NA 395/1-1 and TH 812/1-1).

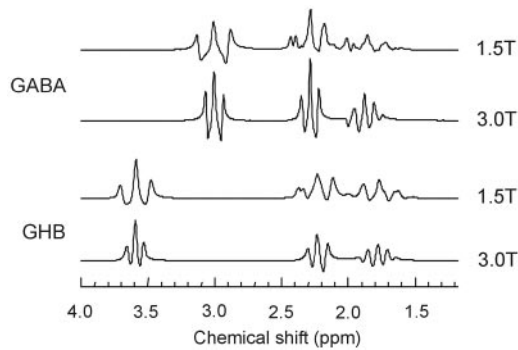
Received August 14, 2003. Accepted in final form November 10, 2003.

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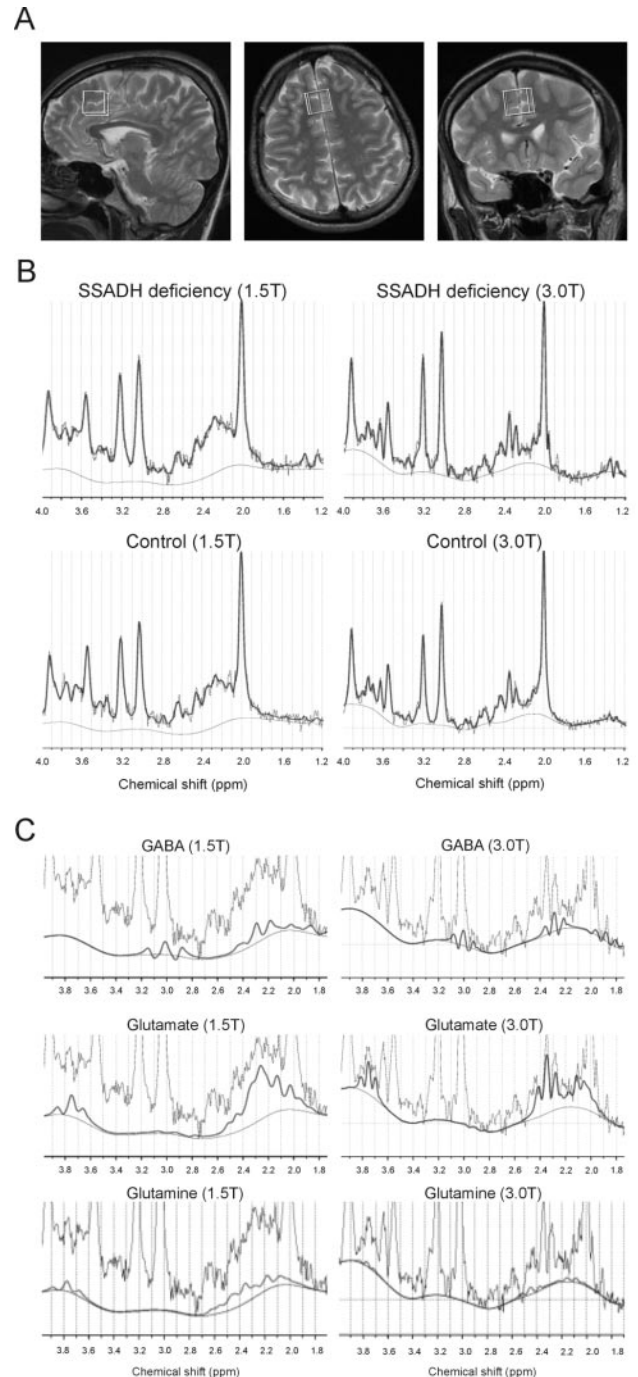


**Figure 1.** *In vitro* proton MR spectroscopy spectra of  $\gamma$ -aminobutyric acid (GABA) and  $\gamma$ -hydroxybutyrate (GHB) at 1.5 and 3.0 T.

and absolute metabolite concentrations of Glx, GABA, and GHB using the standard LCModel basis set (LCM) and the basis set including that of GHB (LCM + GHB) at 3.0 T. The most obvious result is an elevation of GABA concentrations, which were significantly higher in all examined brain regions at 3.0 T. Elevated GABA levels were also detectable at 1.5 T (data not shown), but accuracy of the measurements was reduced. Thus, significantly higher concentrations were found only in frontal GM. The inclusion of the spectral information of GHB into the LCModel did not result in any major changes in the determination of GABA concentrations (see the table), and only traces of GHB were detected. The levels of *N*-acetyl compounds, creatine, choline-containing substances, and myo-inositol did not reveal abnormalities (data not shown).

**Discussion.**  $^1\text{H}$ -MRS demonstrates intracerebral elevation of GABA and traces of GHB in SSADH deficiency. This metabolic pattern reflects the defect in metabolizing GABA into succinate, leading to accumulation of GABA and GHB through alternative pathways.<sup>1,2</sup> Monitoring the metabolic abnormalities *in vivo* is crucial to test and validate new therapeutic approaches. Therefore,  $^1\text{H}$ -MRS might be the appropriate tool to establish innovative treatments in SSADH deficiency.

Determination of brain GABA levels by  $^1\text{H}$ -MRS is challenging because of its low concentration. The disadvantages of the established methods are limited availability and long acquisition times.<sup>4</sup> We showed that conventional  $^1\text{H}$ -MRS at 3.0 T in combination with LCModel is useful for rapid detection (acquisition time <5 minutes) of elevated brain GABA levels. GABA was found to be significantly elevated in all brain regions of our SSADH deficiency patient (see the table). In cerebral GM, there was a threefold increase, consistent with a recent publication<sup>5</sup> and predictions from the murine knockout model.<sup>6</sup> As accumulation of GHB occurs in SSADH deficiency, contributions of GHB to these resonances need to be excluded by analyzing resonances of GABA at 3.01 ppm and GHB at 3.58 ppm (see figure 1). Thus, we extended the standard basis set by the resonance spectra of GHB, as LCModel can identify only compounds included into its basis set. This evaluation



**Figure 2.** (A) Position of the volume of interest in frontal gray matter of the succinate semialdehyde dehydrogenase (SSADH) patient on T2-weighted images. (B) *In vivo* proton MR spectroscopy ( $^1\text{H}$ -MRS) spectra of frontal gray matter. In the upper row are the spectra of SSADH patient at 1.5 T (left) and 3.0 T (right); in the lower row are control spectra at 1.5 T (left) and 3.0 T (right). (C) *In vivo* LCModel fit of  $^1\text{H}$ -MRS spectra of  $\gamma$ -aminobutyric acid (GABA; upper row), glutamate (middle row), and glutamine (lower row) at 1.5 T (left) and at 3.0 T (right).

yielded similar values for GABA, indicating that the observed elevations of GABA are reliable. However, elevation of brain GABA might not be specific for SSADH deficiency, because, for example, GABA

**Table** Metabolite concentrations of Glx, GABA, and GHB as determined by LCMoel at 3.0 T

Concentrations	White matter, frontal lobe		White matter, parietal lobe		Gray matter, frontal lobe		Gray matter, occipital lobe	
	Patient	Control	Patient	Control	Patient	Control	Patient	Control
<i>Tissue composition of VOI, %</i>								
Gray matter	39.63	31.67 ± 14.15	26.91	9.99 ± 4.21	60.69	65.88 ± 3.52	58.23	70.26 ± 0.16
White matter	57.56	66.74 ± 14.84	70.96	89.19 ± 4.65	16.32	17.71 ± 5.27	20.11	20.11 ± 1.11
CSF	2.96	1.74 ± 0.81	2.37	0.97 ± 0.64	23.22	16.78 ± 2.90	10.28	10.28 ± 1.09
<i>Metabolites, mM</i>								
Glx	6.78	6.25 ± 0.78	6.23	5.06 ± 1.72	7.93*	10.20 ± 0.58	8.47	8.44 ± 0.41
GABA (LCM)	0.47*	ND	0.41*	ND	1.49*	0.42 ± 0.16	1.46*	0.28 ± 0.25
GABA (LCM + GHB)	0.64*	0.02 ± 0.04	0.50*	ND	1.47*	0.44 ± 0.15	1.48*	0.29 ± 0.25
GHB (LCM + GHB)	0.09*	0.02 ± 0.02	0.15*	0.02 ± 0.02	0.03	0.01 ± 0.01	0.07*	ND

Concentrations of  $\gamma$ -aminobutyric acid (GABA) and 4-hydroxybutyrate (GHB) were determined by the standard LCM basis set (LCM) and the basis set including GHB (LCM + GHB). No correction for metabolite  $T_1$  and  $T_2$  relaxation effects was performed. Control values are means  $\pm$  SD, n = 4.

\* >3 SD from control.

Glx = glutamate + glutamine; VOI = volume of interest.

transaminase deficiency can also result in an accumulation of intracerebral GABA.<sup>7</sup>

Additionally, higher concentrations of GHB were found in the patient. GHB levels in normal mammalian and rodent brain are similar.<sup>6,8</sup> If the 40- to 60-fold increase in GHB levels observed in the mouse model<sup>6</sup> also occurs in human SSADH deficiency, the concentrations of GHB were in the expected range. However, GHB could not be reliably quantified. The concentrations of GHB were much smaller than those of GABA, which contrasts previous findings in CSF, where GHB concentrations were about 25 times higher than those of GABA.<sup>1,2</sup> This favors the proposed rapid efflux of GHB from brain tissue into CSF.<sup>9</sup> Our findings would support the notion that, in contrast to the traditional hypothesis,<sup>1</sup> the pathophysiologic mechanisms leading to the clinical syndrome in SSADH deficiency might be related to elevated intraparenchymal GABA rather than GHB levels. Pathologic levels of other compounds of the GABA metabolism might contribute to clinical features. However, intracerebral elevation of glutamate/glutamine (Glx in <sup>1</sup>H-MRS) has not been shown in SSADH-deficient mice or in our investigation.<sup>6,10</sup> Most likely, the clinical picture is not caused by one single metabolic abnormality, but by a concert of neurochemical alterations.

## Acknowledgment

The authors thank Sonja Fuchs for technical assistance in the MRS examinations.

## References

- Gibson KM, Hoffmann GF, Hodson AK, Bottiglieri T, Jakobs C. 4-Hydroxybutyric acid and the clinical phenotype of succinic semialdehyde dehydrogenase deficiency, an inborn error of GABA metabolism. *Neuropediatrics* 1998;29:14–22.
- Pearl PL, Gibson KM, Acosta MT, et al. Clinical spectrum of succinic semialdehyde dehydrogenase deficiency. *Neurology* 2003;60:1413–1417.
- Provencer SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magn Res Med* 1993;30:672–679.
- Rothman DL, Petroff OA, Behar KL, Mattson RH. Localized 1H-NMR measurements of gamma-aminobutyric acid in human brain in vivo. *Proc Natl Acad Sci USA* 1993;90:5662–5666.
- Novotny EJ, Fulbright RK, Pearl PL, Gibson KM, Rothman DL. Magnetic resonance spectroscopy of neurotransmitters in human brain. *Ann Neurol* 2003;54(suppl 6):25–31.
- Hogema BM, Gupta M, Senephansiri H, et al. Pharmacologic rescue of lethal seizures in mice deficient in succinate semialdehyde dehydrogenase. *Nat Genet* 2001;29:212–216.
- Medina-Kauwe LK, Nyhan WL, Gibson KM, Tobin AJ. Identification of a familial mutation associated with GABA-transaminase deficiency disease. *Neurobiol Dis* 1998;5:89–96.
- Dotherty JD, Hattox SE, Snead OC, Roth RH. Identification of endogenous  $\gamma$ -hydroxybutyrate in human and bovine brain and its regional distribution in human, guinea pig, and rhesus monkey brain. *J Pharmacol Exp Ther* 1978;207:130–139.
- Shumate JS, Snead OC. Plasma and central nervous system kinetics of gamma-hydroxybutyrate. *Res Commun Chem Pathol Pharmacol* 1979;25:241–256.
- Gibson KM, Schor DSM, Gupta M, et al. Focal neurometabolic alterations in mice deficient for succinate semialdehyde dehydrogenase. *J Neurochem* 2002;81:71–79.



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