Eur Radiol (2002) 12:858–861
DOI 10.1007/s003300101073

PEDIATRIC

T. A. G. M. Huisman
T. Thiel
B. Steinmann
G. Zeilinger
E. Martin

Proton magnetic resonance spectroscopy of the brain of a neonate with nonketotic hyperglycinemia: in vivo–in vitro (ex vivo) correlation

Received: 31 August 2000
Revised: 9 April 2001
Accepted: 10 July 2001
Published online: 28 August 2001
© Springer-Verlag 2001

T. A. G. M. Huisman (✉) · T. Thiel ·
E. Martin
Department of Neuroradiology and Magnetic Resonance,
University Children's Hospital Zurich,
Zurich, Switzerland
E-mail: thierry@nmr.mgh.harvard.edu
Phone: +1-617-7242223
Fax: +1-617-7267422

B. Steinmann
Department of Metabolism
and Molecular Pediatrics,
University Children's Hospital Zurich,
Zurich, Switzerland

G. Zeilinger
Division of Neonatology, Regional
Hospital Aarau, Aarau, Switzerland

Present address: T. A. G. M. Huisman, Neuroradiology Section, MGH-NMR Center, Massachusetts General Hospital and Harvard Medical School, Mailcode CNY 149–2301, Boston, MA 02119, USA

Abstract Nonketotic hyperglycinemia (NKH) is an inborn error of amino acid metabolism caused by a defect in the glycine cleavage multienzyme complex resulting in high concentrations of glycine within the brain and spinal cord. Quantitative magnetic resonance spectroscopy (^{1}H-MRS) allows measurement of absolute glycine concentrations within different parts of the brain in vivo. In addition, ^{1}H-MRS may be useful in monitoring treatment of NKH and to differentiate this disease from other disorders of glycine metabolism.

Keywords Non-ketotic hyperglycinemia · MR spectroscopy · Brain · Neonate

Introduction

Nonketotic hyperglycinemia (NKH) is an autosomal recessively inherited disease characterised by elevated concentrations of glycine in plasma, brain, cerebrospinal fluid (CSF) and urine due to deficiency of the glycine cleavage enzyme system [1, 2]. In the majority of children a devastating and often lethal symptomatology develops with onset in early postnatal life. After a short period of well being, muscular hypotonia, lethargy, poor feeding with hiccups and intractable seizures ensue. Progression to respiratory insufficiency, apnea, coma and death usually follows. Those children who survive have severe mental retardation, refractory seizures and hypotonia that can progress to spasticity [2, 3].

This article shows that quantitative ^{1}H-MRS allows reliable measurements of glycine within different parts of the brain in vivo.

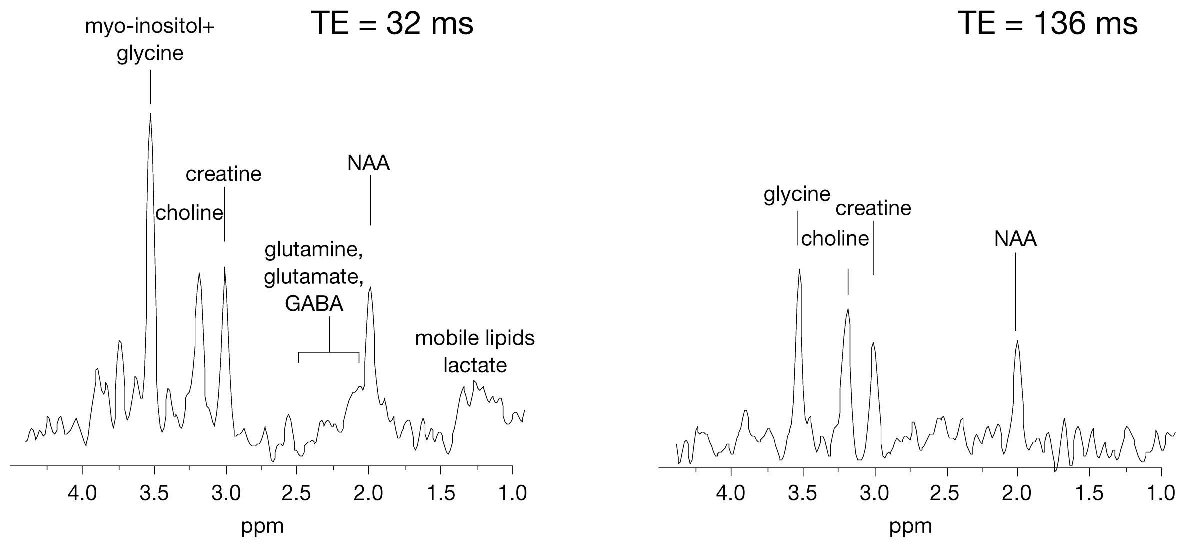

Fig. 1 Short- and long-echo-time water-suppressed proton (^{1}H) MRS spectra from parieto-occipital white matter. Both spectra demonstrate the high intensity of glycine in the brain at 3.55 ppm

Methods and results

We report on a newborn boy who was delivered at term after an uneventful pregnancy. The child developed tonic–clonic seizures and respiratory insufficiency on the third day of life. Since birth a generalised muscular hypotonia as well as poor feeding with failure to suck and high-pitch cry was observed. In addition, intractable hiccups were noticed, which the mother had already noticed during pregnancy. The EEG on the first day of life showed a burst suppression pattern suggestive for NKH [2]. Glycine levels were elevated in plasma (3590 μmol/l, normal value 243 ± 20 μmol/l), CSF (646 μmol/l, normal value < 13 μmol/l) and urine (12354 μmol/mol creatine). The ratio of CSF to plasma glycine concentration was elevated (0.18; normal value ~0.02) and diagnostic for NKH. Ketoacidosis and organic acidaemia were excluded. On day 7 the child was referred for a neuroradiological work-up under artificial ventilation. Magnetic resonance imaging revealed a hypoplasia of the corpus callosum. ^{1}H-MRS showed increased concentrations of glycine within the frontal and parieto-occipital white matter (Fig. 1), the basal ganglia, the cerebellar white matter and the CSF (Table 1). Further treatment was stopped and the boy died at day 9. Post-mortem glycine levels in brain tissue samples were measured by ion-exchange chromatography (Table 1). The diagnosis of NHK was further confirmed enzymatically in the liver (courtesy of Dr. M. Roland, Lyon). The parents gave informed consent for the study.

Quantitative, water-suppressed proton (^{1}H) -MRS (PRESS) was performed on a 2 Tesla whole-body MR system (Tomikon S200A, Bruker Medical Systems) using a standard quadrature head coil. Two- to 3.5-ml volume voxels were positioned in different brain areas, mentioned previously. Long echo time spectra at a TE of 136 ms were acquired using repetition times of 2000 ms. In addition, short-echo-time spectra were obtained at echo times of TE = 32 ms. Two hundred fifty-six scans were averaged at a bandwidth of 2 kHz. Chemical shifts (δ) were set using the signal of N-acetylaspartate (δ = 2.02 ppm) as internal reference. Calibration of cerebral metabolite concentrations was achieved utilising an external phantom as standard consisting of a solution of 50 mmol/l creatine. The coil load for the calibration measurement was adjusted to match that of the brain examination.

The peak area of the methylene group of glycine at 3.5 ppm and the methyl group of creatine at 3 ppm in the long-echo-time spectra were fitted by time-domain data processing using the home-built SD Software. This software is based on the Levenberg-Marquardt algorithm to minimise the sum of squared differences between the data and the model function. The cramer rao lower bounds of the fit are listed in Table 1 as standard deviations. For glycine they were in the region of 6–9 %. At short echo times a consistent time-domain analysis failed due to the strong signal overlap of glycine with resonances of myo-inositol (MI).

The glycine concentrations (Table 1) were calculated according to the following equation:

$$[Gly] = [Phan]\ (A_{Gly}/A_{Phan}) \times (n_{Phan}/n_{Gly}),$$

where A_{Gly} is area of glycine peak, A_{Phan} is area of peak of phantom constituent, and n is number of protons of corresponding methylene and methyl groups.

Our calculations are based on the assumption of comparable T2 relaxation times of creatine and glycine. Due to the impossible quantification of glycine at short echo times, no correction for the T2 relaxation was performed. In order to reduce the systematic error of the quantification, no T2 correction for the phantom constituent was applied. The concentrations of the other metabolites

Table 1 Concentration of glycine in different regions of the brain

Brain region	Quantitative ^{1}H-MRS[a] (mmol/l)	Chemical quantification[b] (mmol/l)
CSF	< 1	0.46
Occipito-parietal white matter	4.0 ± 0.3	4.24
Frontal white matter	5.0 ± 0.5	4.86
Basal ganglia	5.5 ± 0.5	6.61
Cerebellar white matter	8.0 ± 0.7	Not available

[a] Study done on postnatal day 7
[b] Study done on postnatal day 9, 12 h post mortem, standard error 3–5 %

including choline, creatine, NAA and lactate are within normal limits for neonates.

The brain was obtained at autopsy 12 h post mortem and stored at –20 °C. Quantitative chemical analysis of amino acids from the corresponding parts of the brain which were measured by ^{1}H-MRS was done. The samples were homogenised, extracted with 19 volumes of 4 % sulfosalicylic acid containing 1 mmol/l sarcosine as an internal standard and centrifuged. Glycine was determined in the supernatants by ion-exchange chromatography. The measured concentrations of glycine are summarised in Table 1. The standard errors were in the region of 3–5 %.

Discussion

The elevated levels of glycine within the brain and spinal cord differentiate NKH from ketotic and other forms of hyperglycinemia. In NKH, glycine accumulates in the central nervous system to reach very high levels, which is reflected in the CSF. In the other forms of hyperglycinemia, despite high plasma glycine levels, brain and CSF-glycine is normal. Since these children usually have no neurological symptoms, the high glycine levels within the brain are supposed to be responsible for the neurological symptoms in NKH [1, 2]. It was stated that high concentrations of glycine in the brain could overstimulate the N-methyl-D-aspartate (NMDA) receptor system [2, 4]. The excitatory properties of glycine at the glutaminergic NMDA receptor or at a site closely associated with NMDA receptor plays an important role in glutamate-induced neurotoxicity. Glycine increases the frequency of NMDA receptor channel opening by accelerating the recovery of the receptor following glutamate-induced desensitisation. In this way, glycine potentiates the excitotoxic action of glutamate, which is believed to be a result of an excessive elevation of intracellular free Ca^{2+} [5]. Apart from this excitatory property, glycine is a major inhibitory neurotransmitter at the postsynaptic, strychnine-sensitive receptors that are mainly located in the basal ganglia, brainstem, cerebellar cortex and spinal cord [6]. Neurophysiological studies indicate that excessive activation of excitatory amino acid receptors, especially NMDA receptor, has been implicated in the pathogenesis of neuronal injury in numerous neurological disorders including hypoxia-ischaemia, hypoglycaemia and brain trauma. McDonald and Johnston have shown that especially the developing brain shows increased susceptibility to NMDA-mediated brain injury and high levels of glycine may be particularly devastating to the central nervous system of the neonate [7]. The neuropathology of NKH consists of diffuse alterations in the postnatal myelination. All myelinated areas have a striking spongy appearance due to the vacuolation of the myelin. In accordance with the neonatal stage of myelination, the spinal cord, brain stem, cerebellar white matter, posterior limb of the internal capsula and optical tracts are mainly involved in NKH [8, 9].

Previous reports demonstrated that the measured glycine concentration varied within different brain regions, with higher levels in the spinal cord, the cerebellar cortex and basal ganglia than in the cerebral cortex [1, 10]. Diagnosis of NKH is established by measuring glycine levels in plasma and CSF. Glycine in plasma and CSF is usually elevated 2–8 and 10–30 times of normal, respectively [11]. The elevated ratio of CSF to plasma glycine differentiates NKH from other disorders of glycine metabolism.

Magnetic resonance spectroscopy is the only method to quantify glycine non-invasively in the brain in vivo. Heindel has already shown the value of MRS, although his quantification of glycine was based on an assumed creatine concentration of 5 mmol/l as internal standard [12]; however, the concentration of creatine not only varies with early brain development, but more so between different regions of the brain, and is not known to remain normal in NKH. ^{1}H-MRS with use of an external phantom/reference allowed a reliable quantification of glycine with results comparable to the post-mortem biochemical quantification of glycine (Table 1). It is important to compare short-echo with long-echo proton spectra to distinguish resonances of short relaxing MI from glycine. If there were only glycine, the ratio of the glycine signal to the other singulett signals would be approximately the same at long and short TE, if a similar T2-relaxation time for all singuletts is assumed. Therefore, the double size of the signal at 3.55 ppm as compared with creatine is explained by the presence of approximately equal proportions of protons from glycine and MI at short TE. Our results also confirm previous findings of regionally different glycine concentrations measured biochemically in vitro [1, 10], the biological significance of which remains elusive [2].

In conclusion, multi-voxel ^{1}H-MRS allows non-invasive measurement of glycine concentrations within the brain in vivo at multiple sites. Thus, ^{1}H-MRS can be a useful tool in monitoring the effects of therapeutic interventions. In addition, ^{1}H-MRS can differentiate NKH from other metabolic disorders with increased plasma glycine levels.

Acknowledgement This study was supported by grants from the Swiss National Science Foundation no. 32–52647–97 to E. M and 32–59445.99 to B. S.

References

1. Perry TL, Urquhart N, MacLean J, Evans ME, Hansen S, Davidson GF (1975) Non ketotic hyperglycinemia. Glycine accumulation due to absence of glycine cleavage in brain. N Engl J Med 292: 1269–1273
2. Hamosh A, Johnston MV (2001) Nonketotic hyperglycinemia. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8th edn. McGraw-Hill, New York, pp 2065–2078
3. Steiner RD, Sweetser DA, Rohrbaugh JR, Dowton SB, Toone JR, Applegarth DA (1996) Nonketotic hyperglycinemia: atypical clinical and biochemical manifestations. J Pediatr 128: 243–246
4. Tada K, Kure S, Takayanagi M, Kume A, Narisawa K (1992) Non-ketotic hyperglycinemia: a life-threatening disorder in the neonate. Early Hum Dev 29: 75–81
5. Choi DW (1991) Excitotoxicity. In: Meldrum BS (ed) Excitatory amino acid antagonists. Blackwell, Oxford, pp 216–236
6. Naas E, Zilles K, Gnahn H, Betz H, Becker CM (1991) Glycine receptor immunoreactivity in rat and human cerebral cortex. Brain Res 561: 139–146
7. McDonald JW, Johnston MV (1990) Physiological and pathophysiological roles of excitatory amino acids during central nervous development. Brain Res Rev 15: 41–70
8. Shuman RM, Leech RW, Scott CR (1978) The neuropathology of the nonketotic and ketotic hyperglycinemias: three cases. Neurology 28: 139–146
9. Brun A, Borjeson M, Hultberg B, Sjoblad S, Akesson H, Litwin E (1979) Nonketotic hyperglycinemia: a clinical, biochemical and neuropathologic study including electronic microscopy findings. Neuropediatrie 10: 195–205
10. Bachmann C, Mihatsch MJ, Baumgartner RE, Brechbühler T, Bühler UK, Olafsson A, Ohnacker H, Wick H (1971) Nicht-ketotische Hyperglyzinämie: perakuter Verlauf im Neugeborenenalter. Helv Paed Acta 26: 228–243
11. Tada K, Hayasaka K (1987) Non-ketotic hyperglycinemia: clinical and biochemical aspects. Eur J Pediatr 146: 221–227
12. Heindel W, Kugel H, Roth B (1993) Noninvasive detection of increased glycine content by proton MR spectroscopy in the brains of two infants with nonketotic hyperglycinemia. AJNR 14: 629–635