^{1}H MRS of cortical and cerebellar brain extracts of ataxic migraine mice

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Introduction

Familial hemiplegic migraine type-1 (FHM1) is an autosomal dominant subtype of migraine with aura caused by mutations in the *CACNA1A* gene. This gene encodes the pore forming α1-subunit of $Ca_v2.1$ (P/Q-type) calcium channels. FHM1 mutation S218L produces a particularly severe phenotype of ataxia, hemiplegic migraine triggered by mild head trauma, associated with cerebral edema and fatal coma that is sometimes preceded by a generalized seizure [1]. We generated a transgenic S218L FHM1 knockin (KI) mouse model using homologous recombination. We isolated cortical and cerebellar tissue from these mice and analyzed metabolite concentrations in brain extracts using NMR. The aim was to provide a metabolic profile for the migraine mice, as a corroboration of *in vivo* MRS data that will be obtained for the same brain regions.

Methods

Female 4-month-old wild-type, heterozygous and homozygous S218l FHM1 KI mice (littermates) were euthanized by cervical dislocation. Brains were rapidly isolated and cerebellum and cortex were snap-frozen in liquid N_2. Brain tissue was extracted by methanol-chloroform-water extraction, essentially as described previously [2]. Methanol and water (4 °C, 1:1 v/v) were added to the frozen tissue. After homogenation, methanol, chloroform and water (4 °C, 2:3:2 v/v/v) was added and the tissue–solvent mixture was thoroughly vortexed and sonicated. After approximately 30 min in contact with the first solvents, the samples were centrifuged at 3,000 rpm for 20 min at 4 °C. The upper phase was separated from the lower phase using a glass pipette. The protein pellets from the first tissue extraction were re-extracted and pooled with the original tissue extract. The extracts were lyophilized O/N. Lyophilized extracts were redissolved in 1 ml KH_2PO_4 buffered D_2O (pH 7.0) and stored at -80 °C until NMR spectroscopy.

^{1}H NMR spectra were recorded at room temperature on a Bruker DMX 400 spectrometer, using 256 accumulations, repetition time 4.7 s, spectral width 6009.6 Hz, data size 16 K and water presaturation (**Figure 1**). Chemical shifts were referenced to (trimethylsilyl) propionic-2,2,3,3,d4-acid (TSP) at 0.00 ppm. Concentrations (μmol/g wet weight) of taurine (Tau), creatine (Cr), glutamine (Gln), glutamate (Glu), γ-aminobutyric acid (GABA) and n-acetyl-aspartate (NAA) were determined from fully relaxed ^{1}H NMR spectra of extracts using TSP as external standard. Fourier transformation was performed with 0.5 Hz line broadening. Localized baseline correction was performed and peak areas were integrated using XWinNMR software (Bruker, Ettlingen, Germany). All values are expressed as means ± SEM.

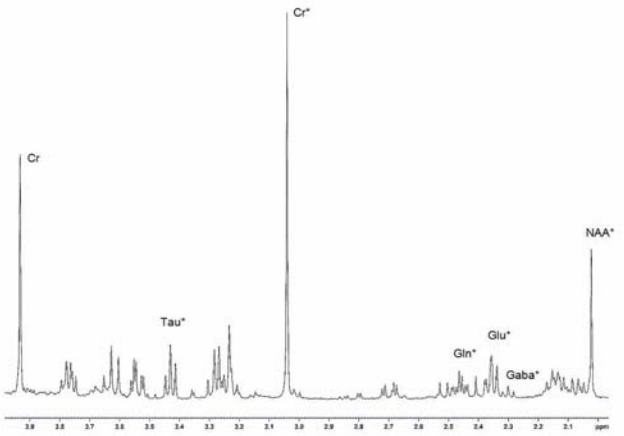

Figure 1 Representative aliphatic portion of a proton NMR spectrum depicting cytosolic metabolites from adult mouse brain following CM extraction. Quantitation of each metabolite was made from the resonance indicated by an asterisk and listed as follows: taurine (Tau, SCH2); creatine (Cr, CH3); glutamine (Gln, β CH2) glutamate (Glu, γ CH2); GABA (α CH2); NAA (CH3). Chemical shifts are expressed in ppm referenced to TSP at 0.00 ppm

Results

Solution state ^{1}H NMR spectra from cerebellar and cortical extracts were examined for specific metabolites: Tau, Cr, Glu, GABA and NAA levels (**Table 1**). Glu, Gln and NAA levels were decreased by ~15% in homozygous S218L KI mice. Glu, Gln and NAA levels in heterozygous mice did not significantly differ from wild-type. Tau, Cr and GABA levels were not altered between genotypes. Cerebellar wet weight of homozygous S218L KI mice significantly differed by ~12% from that of wild-type and heterozygous mice. Principal component analysis was used to search for possible differences between groups.

Table 1 ^{1}H NMR metabolite profile of cerebellum and cortex

	Tau	Cr	Gln	Glu	GABA	NAA	Wet weight
Cerebellum							
Wild-type (n = 5)	9.09 ± 0.67	22.59 ± 1.16	6.36 ± 0.27	9.88 ± 0.50	1.73 ± 0.07	9.68 ± 0.46	56.8 ± 1.07
S218L/wt (n = 5)	8.84 ± 0.16	21.49 ± 0.53	5.86 ± 0.16	9.18 ± 0.31	1.81 ± 0.11	9.18 ± 0.30	60.2 ± 1.46
S218L/S218L (n = 8)	8.95 ± 0.18	20.53 ± 0.52	5.49 ± 0.14**	8.43 ± 0.19**	1.66 ± 0.07	8.38 ± 0.23*	50.3 ± 0.73***
Cortex							
wt/wt *(n = 6)*	11.46 ± 0.37	16.24 ± 0.43	5.93 ± 0.11	12.29 ± 0.42	3.04 ± 0.13	12.63 ± 0.38	218.2 ± 4.22
S218L/wt *(n = 5)*	12.05 ± 0.40	16.74 ± 0.65	6.05 ± 0.25	12.50 ± 0.44	3.18 ± 0.17	12.87 ± 0.53	225.4 ± 8.10
S218L/S218L *(n = 7)*	11.70 ± 0.30	16.04 ± 0.42	5.50 ± 0.18	11.90 ± 0.24	2.93 ± 0.10	12.10 ± 0.29	202.5 ± 4.26*

Means of metabolites are expressed in μmoles/g wet weight ± s.e.m. Means of wet weight are expressed in mg ± s.e.m. Two-tailed Student's t-test compared to wild-type; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Discussion

Decreased levels of Glu, Gln and NAA were found in the cerebellum of homozygous S218L KI mice. This is in close agreement to what has been previously reported for FHM patients [3]. In these patients, also cerebellar atrophy was reported. Cortical weight was decreased by 9%, whereas cerebellar weight decreased by ~12%. This indicates that in these mice, also cerebellar atrophy may be present, although histological analyses did not reveal gross anatomical cerebellar abnormalities indicating atrophy, such as cell loss or shrinkage. However, abnormal Purkinje cell dendritic morphology is present in homozygous S218l FHM1 KI mice. Reduced levels of glutamate and glutamine may indicate reduced parallel or climbing fiber function, which may ultimately result in disturbed Purkinje cell development [4, 5]. Further studies are necessary to indicate if glutamate release and/or uptake levels are altered.

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