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SHORT REPORT

Visual evoked potentials in succinate semialdehyde dehydrogenase (SSADH) deficiency

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Summary In mammals, increased GABA in the central nervous system has been associated with abnormalities of visual evoked potentials (VEPs), predominantly manifested as increased latency of the major positive component P100. Accordingly, we hypothesized that patients with a defect in GABA metabolism, succinate semialdehyde dehydrogenase (SSADH) deficiency (in whom supraphysiological levels of GABA accumulate), would manifest VEP anomalies. We evaluated VEPs on two patients with confirmed SSADH deficiency. Whereas the P100 latencies and amplitudes for binocular VEP analyses were within normal ranges for both patients, the P100 latencies were markedly delayed for left eye (OS) (and right eye (OD), patient 1) and monocular OS (patient 2): 134–147 ms; normal <118 ms. We hypothesize that elevated GABA in ocular tissue of SSADH patients leads to a use-dependent downregulation of the major GABAergic receptor in eye, $GABA_C$, and that the VEP recordings' abnormalities, as evidenced by P100 latency and/or amplitude measurements, may be reflective of abnormalities within visual systems. This is a preliminary finding that may suggest the utility of performing VEP analysis in a larger sample of SSADH-deficient patients, and encourage a neurophysiological assessment of $GABA_C$ receptor function in Aldh5a1$^{-/-}$ mice to reveal new pathophysiological mechanisms of this rare disorder of GABA degradation.

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Abbreviations

GABA	4-aminobutyrate/γ-aminobutyrate
$GABA_CR$	$GABA_C$ receptor
OD	right eye
OS	left eye
SSADH	succinate semialdehyde dehydrogenase
VEP	visual evoked potentials

Introduction

Succinic semialdehyde dehydrogenase (SSADH; aldehyde dehydrogenase 5a1 (Aldh5a1); OMIM 271980),

deficiency (OMIM 610045) is a rare defect of GABA degradation featuring supraphysiological accumulations of both γ-aminobutyric acid (GABA) and the GABA-analogue, γ-hydroxybutyric acid (GHB). In the corresponding murine knockout model (termed Aldh5a1$^{-/-}$ mice), developed by standard gene targeting, significant alterations of $GABA_A$ receptor ($GABA_AR$) and $GABA_BR$ structure and function have been demonstrated (Buzzi et al. 2006; Wu et al. 2006). We have hypothesized that elevated GABA, levels potentially compounded by increased GHB, have led to these alterations in the mouse model. These GABAergic abnormalities detected in the murine model have recently provided important insight into pathophysiology in humans. For SSADH-deficient patients, imaging studies have confirmed alterations of $GABA_AR$ (employing [^{11}C]flumazenil binding) and $GABA_BR$ (characterized using transcranial magnetic stimulation) binding (Pearl et al. 2007; Reis et al. 2007); the latter have been proposed as outcome measures for future clinical trials. Nonetheless, despite these GABAergic alterations documented in both SSADH-deficient patients and mice, no studies of $GABA_CR$ function have been undertaken, perhaps owing to the ocular localization of this receptor.

Long-term treatment with vigabatrin (γ-vinyl-GABA; Sabril), an antiepileptic drug that elevates intracellular GABA concentrations by irreversible inhibition of GABA-transaminase, was shown to induce VEP anomalies (Harding et al. 2000; Schroeder et al. 1992). The P100 wave is the major positive component of VEP tracings resulting from transient changes of brain activity after intermittent visual stimulation. P100 delayed latency and decreased amplitude can indicate abnormalities of visual neurotransmission in several neurological disorders (Celesia and Peachey 1999). Since SSADH-deficient patients and those receiving vigabatrin manifest GABA increases in physiological fluids, it is tempting to hypothesize that a use-dependent downregulation of the $GABA_CR$ might occur in the presence of elevated ligand GABA. To begin to evaluate this hypothesis, we report VEPs analyses in two patients with SSADH deficiency.

Patients and methods

Clinical features of patient 1

The patient is a 4-year-old boy born at term from a normal pregnancy. At birth, he presented asphyxia and cyanosis, and poor cry and sucking. At follow-up, the child presented delayed psychomotor milestones, hypotonia and absent speech. He had poor interest in environmental stimuli and absent active grasp. At 10 months, he presented focal seizures with eye rolling and mouth deviation. Carbamazepine treatment was started with significant seizure reduction. The ictal EEG showed high-voltage posterior slow waves. Nerve conduction, electromyography and funduscopy were normal. MRI revealed T2-hyperintensity in the subcortical white matter and mild frontal cortical atrophy. Urine organic acid analysis by gas chromatography–mass spectrometry revealed the presence of GHB (161–225 mmol/mol creatinine; normal <10), *threo*- and *erythro*-4,5-dihydroxyhexanoic acid (DHHA; 8–37 and 7–50 mmol/mol creatinine; undetectable in normal) and the corresponding internal lactones of DHHA (50 and 45 mmol/mol creatinine; undetectable in normal). DHHA may well represent a pathognomonic marker for SSADH deficiency (Brown et al. 1987).

Clinical features of patient 2

Patient 2 was reported at 9 years of age and has recently been briefly presented after a 20-year follow-up (Crutchfield et al. 2008; Gibson et al. 1988). In brief, at age 9 years she manifested psychomotor delay, hypotonia, microcephaly and hyperkinesis. Urine organic acid analysis confirmed the presence of GHB and enzymatic and molecular confirmation of the diagnosis was previously achieved in white cells (Akaboshi et al. 2003; Gibson et al. 1988). After 20 years, she remains delayed but the clinical course has remained essentially static (Crutchfield et al. 2008).

VEP methodology

VEPs were recorded at 10 months of age (patient 1) and age 9 years (patient 2). During the examination patient 1 was lying in his mother's lap with his head held to look straight ahead during the recording. The experimental room was semi-dark and quiet. Four gold-cup electrodes were placed on the subject's scalp, previously treated with an abrading paste in order to reduce and maintain the scalp impedance at 5 kohm (3 kohm, patient 2). An electrolyte conducting paste was used to attach the electrodes. The recording electrodes were placed on O_1 (left occiput) and O_2 (right occiput), each referenced to a third electrode placed at C_z. The fourth earth electrode was placed at Fp1. The stimulating lamp was placed at a 30 cm (20 cm, patient 2) distance from the subject and directed

toward his eyes. Each electrode lead was connected to an amplifier which, in turn, was connected to a two-channel Galileo-Syrius (for patient 2, a two channel Cadwell Quantum 84 Microprocessor was employed). The Galileo-Syrius (Cadwell, patient 2) averaged each O_1 and O_2 channels, as elicited and averaged by each flash stimulus presented.

The stimulus was a diffuse flash, 0.2 J intensity, at a rate of 1.7 Hz. The flash intensity for patient 2 was a diffuse flash, Grass Photostimulator, intensity 8. Band-pass filters were set at 0.5–500 Hz (1–30 Hz, patient 2); the VEP sweep time was set at 590 ms (500 ms for patient 2). A 50 Hz (60 Hz, patient 2) notch filter was used to avoid alternating current disturbance. In patient 1, both monocular and binocular VEPs averaged 100 flash stimuli, and each was replicated 6 times to assure reliable P_{100} latencies and peak-to-peak amplitudes. The frequency of stimulus delivery was unchanged in the replications. Each VEP recording was performed without an inter-trial interval (ITI), whereas in patient 2 there was a 2-minute ITI between each VEP recording. Monocular and binocular VEPs were recorded simultaneously and replicated for patient 1; for patient 2, OS, OD and binocular VEPs were recorded separately and replicated. Responses with excessive artefacts were automatically discarded. All averaged responses were saved and stored electronically. The P_{100} latencies and amplitudes were calculated by computer in microvolts (μV) and milliseconds, respectively. All VEPs were replicated at least once to assure reliable P_{100} latencies and amplitudes. VEP latencies and amplitudes were compared with normative values for each laboratory matched by age and sex. Electroretinography could not be performed because of poor cooperation of the patients.

Additional components of VEP methodology for patient 2

The subject was seated and instructed not to talk, to relax (especially the neck, face and jaw), not to blink, to remain as still as possible, and to look straight ahead during all VEP recordings. The recording room was maintained at the same luminance throughout the recording session. Binocular VEPs were initially averaged after 100 flash stimuli were presented (1 flash per second). OS and OD VEPs averaged 6 flash stimuli at a frequency of one flash per second, followed (after a 2-minute ITI) by 35 averaged flash stimuli, also presented at 1 flash per second. An opaque eye patch was placed over the subject's non-stimulated eye during the monocular VEP recordings (to ensure that light would not enter the non-stimulated eye).

Results

Confirmation of the gene defect in patient 1

Genomic DNA of the proband was amplified by using specific primer pairs (Blasi et al. 2002). Sequence analysis of the PCR products revealed a homozygous state for a novel missense mutation (c.667T>C; p.Cys223Arg) in exon 4 of the SSADH coding sequence (GenBank Y11192). This mutation affects the same amino acid as c.668G>A, which was found to be associated with a dramatic reduction of enzyme activity (Akaboshi et al. 2003), strongly supporting the pathogenic role of this novel nucleotide alteration. The homozygous state for the missense mutation was confirmed by sequencing genomic DNA from his parents, who were T/C heterozygotes at position c.667. In addition, both parents turned out to be G/A heterozygotes for SNP rs2744583, providing further evidence that they carried two copies of the SSADH gene not deleted in exon 4. The proband inherited two copies of the rs2744583(A) allele, thus confirming the homozygous state at exon 4.

VEP findings for patient 1

Binocular flash VEPs elicited synchronous P_{100} latencies for both the O_1 (left occiput) and the O_2 (right occiput) channels at 134 ms. The same 134 ms positive components' latencies were present for the replication VEPs. Monocular flash VEPs were averaged after 100 stimuli and replicated 6 times. The P_{100} component latencies were 144 ms for O_2-C_z (OD) and 150 ms for O_1-C_z (OS). These latencies values were increased compared to normal ranges for the patient's age (110–118 ms). The P_{100} peak-to-peak amplitudes were 1.56 μV in OS, 3.13 μV in OD and 9.6 μV with binocularly elicited responses. These values were within the normal range for our laboratory (5.6±3.7 μV).

VEP findings for patient 2

Binocular flash VEPs elicited synchronous P_{100} components, with latencies for both the O_1 and the O_2 channels at 97.9 ms. The same 97.9 ms P100 latencies were present for the replication VEPs. These latencies were normal for age. Monocular OD flash VEPs were averaged from 6 flash stimuli, and then replicated with 35 averaged flash stimuli. Although a slight amplitude decrement was observed, with an increase in flash stimuli presented, the P_{100} components for O_1 and O_2 were synchronous and well within the normal range at

104 ms. Monocular OS flash VEPs were initially averaged with 6 flash stimuli presented, and were replicated with an increase to 35 flash stimuli presented. The initial VEP P_{100} components' peak-to-peak amplitudes were 70 μV and 66 μV, respectively, with synchronous latencies; the latencies were markedly delayed at 147 ms for both the O_1 and O_2 channels. With an increase in averaged flash stimuli (from 6 flash stimuli averaged, with a 2-minute ITI, to 35 flash stimuli presented), replication VEPs elicited a pronounced peak-to-peak amplitude decrement for the P_{100} positive component. The P_{100} latencies remained abnormally delayed.

Discussion

As the major inhibitory neurotransmitter in mammals, GABA acts on three receptors in the nervous system, $GABA_A$, $GABA_B$ and $GABA_C$ (Hinton et al. 2008). Of these, two facilitate rapid inhibition via ligand-gated ion channels, while the $GABA_B$ receptor drives slow inhibition via the adenylate cyclase/G-protein system. Whereas the $GABA_A$ and $GABA_B$ receptors have widespread neural localizations (and differential subunit structure for both receptor classes), only the $GABA_C$ receptor is predominantly localized to ocular tissue. Compared to the $GABA_A$ receptor, the $GABA_C$ receptor mainly mediates synaptic responses (both excitatory and inhibitory) in mammalian bipolar rod cells (Eggers and Lukasiewicz 2006; Lukasiewicz and Shields 1998). Accordingly, it is not unreasonable to hypothesize that elevated GABA in ocular tissue would have an adverse effect on $GABA_C$ receptors. In dogs receiving high-dose vigabatrin there was an increase in central latencies of the VEPs, which subsequently normalized upon cessation of vigabatrin intervention (Schroeder et al. 1992). Similarly, VEP analyses revealed delayed oscillatory potentials in 8 patients receiving vigabatrin intervention (Harding et al. 2000). GABA is significantly elevated in cerebrospinal fluid of SSADH-deficient patients (Gibson et al. 2003), an observation supported by *in vivo* studies using magnetic resonance spectroscopy (Novotny et al. 2003), yet the ocular concentration in SSADH-deficient patients (or Aldh5a1$^{-/-}$ mice) has not been determined. Nonetheless, it is tempting to hypothesize that increased GABA levels in our patients were causally related to the underlying VEPs abnormalities we observed.

Whereas VEP measurements have been performed in other genetic disorders (e.g. ocular albinism, Hurler disease, Wilson disease) (Bradfield et al. 2007; Das et al. 2007; Hoffmann et al. 2007; Husain 2006), to our knowledge this is the first report of VEP analysis in heritable SSADH deficiency. In patient 2, the lack of early wave components in the monocular OS flash VEPs was indicative of fibre dysfunction in the left optic radiations; the delayed latencies suggested a diffuse disturbance of cerebral function ipsilateral to the stimulated eye. The fibres are predominantly ipsilateral, retrochiasmally, but some are ipsilateral within the visual system. The monocular OS flash stimulation revealed abnormal VEPs for the P_{100} components of both the O_1 and O_2 channels, as well as unusually large peak-to-peak amplitude. An increase in averaged flash stimuli for the replication VEPs elicited a rapid decrement in the amplitude of the P_{100} values. These features were unlikely to have been the result of thioridazine intervention (due to the patient's extreme hyperkinesis). In a double-blind analysis of 62 hyperkinetic children receiving either placebo, thioridazine or *d*-amphetamine, patients receiving thioridazine manifested increased latencies and decreased amplitudes on VEP analysis (Saletu et al. 1975), although a clear understanding of the processes involved remains to be determined. The increased latencies in the P_{100} components in both patients (patient 1, 144–150 ms; patient 2, 147 ms) suggest that altered latencies represent an associated marker of pathophysiology in SSADH deficiency.

In childhood, the occurrence of large peak-to-peak amplitudes of the P_{100} components elicited by monocular VEP recordings is often indicative of neurological compromise (Chu 1986; Nevsímalová et al. 1990). Neurochemical or neurotransmitter dysfunction, including alterations in receptor function and/or abnormal transmitter metabolism, could explain these findings (Crutchfield et al. 1986; Hrbek et al. 1982; Jones 1986). This hypothesis is supported by the well-documented visual field defects associated with vigabatrin intervention (Hammoudi et al. 2005; Lawden et al. 1999). In conclusion, this preliminary study provides the necessary groundwork to support a larger evaluation of SSADH-deficient patients for both VEPs and ERGs. Moreover, the role as well as the concentration of GABA (and GHB) in ocular tissues, not yet evaluated in either humans or Aldh5a1$^{-/-}$ mice, should be investigated. These studies are currently in progress.

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