Spectroscopic imaging of frontal neuronal dysfunction in hyperekplexia

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Summary
We used proton magnetic resonance spectroscopic imaging (MRSI) to assess in vivo cortical neuronal involvement in hyperekplexia. Cerebral neuronal function was measured using proton MRSI in four unrelated patients with hyperekplexia and 20 healthy controls. All patients had the major form of hyperekplexia, with additional atypical clinical features in two of them. Family history was positive in three patients and absent in one. The neuronal marker N-acetylaspartate (NAA), choline-containing compounds (Cho) and creatine (Cr) were measured in frontal, central and parietal areas. The MRSI showed a reduction of the relative resonance intensity of NAA/(Cr + Cho) in frontal and central regions in three patients, and in the right frontal region of the fourth. In one patient a second MRSI showed normal relative NAA resonance intensities over both temporal lobes as well as in the brainstem. In two subjects the topography of EEG abnormalities in the frontal lobes coincided with the MRSI findings. This proton MRSI study indicates the presence of frontal neuronal dysfunction in hyperekplexia. Whether this represents cortical dysfunction or an epiphenomenon of diencephalic or brainstem abnormalities remains open. However, the observation of normal proton MRSI in the temporal regions and brainstem in one of the patients seems to concur with the hypothesis of a facilitatory role of cortical dysfunction within areas of sensorimotor representation in the generation of the pathological startle reaction in hyperekplexia.

Keywords: startle reaction; hyperekplexia; proton magnetic resonance spectroscopy

Abbreviations: Cho = choline-containing compounds; Cr = creatine; MRSI = proton magnetic resonance spectroscopic imaging; NAA = N-acetylaspartate

Introduction
Hyperekplexia (MIM: 149400), or startle disease, is a rare disorder (Suhren et al., 1966; Andermann et al., 1980; Ryan et al., 1992; Shiang et al., 1993; Bernasconi et al., 1996) characterized by an exaggerated and persistent startle reaction to unexpected auditory, somatosensory and visual stimuli. It may occur in a minor form, in which the response is quantitatively different from normal (i.e. the startle response is more violent), or a major form (Suhren et al., 1966), in which patients present with generalized muscular rigidity during the neonatal period. The muscle stiffness usually returns towards normal during the first years of life, but during childhood, affected individuals develop falling attacks caused by brief generalized tonic spasms without impairment of consciousness. Some adult patients report episodes of a sensation of muscle stiffness or slowness of movement (Dooley and Andermann, 1989), and they often have spontaneous bouts of (usually nocturnal) clonus (Suhren et al., 1966; Andermann et al., 1980; Bernasconi et al., 1996). In some patients, there are features of more diffuse cerebral involvement with developmental delay (Andermann et al., 1980). The symptoms usually respond dramatically to clonazepam (Andermann et al., 1980; Ryan et al., 1992; Bernasconi et al., 1996), and the clinical evolution is usually favourable. However, the excessive backward jerking of the head elicited by tapping the forehead or nose (Shahar et al., 1991) generally persists. Exceptionally, symptoms arise later in life (Suhren et al., 1966; Andermann et al., 1980; Dooley and Andermann, 1989; Brown et al., 1991a; Hochman et al., 1994) or improve with valproic acid (Dooley and Andermann, 1989), vigabatrin (Stephenson, 1992), 5-hydroxytryptophan or piracetam (Saenz-Lope et al., 1984).

In some families with autosomal dominant inherited hyperekplexia, the genetic defect has been identified as a point mutation in the α1-subunit of the inhibitory glycine receptor on chromosome 5q33-q35 (Shiang et al., 1993, 1995; Tijssen et al., 1995b). However, a different mutation
in the same exon of this subunit has also been reported in patients with autosomal recessive inheritance (Rees et al., 1994), and there are hyperekplexia families in which a mutation in the glycine receptor α1-subunit was not identified (Turecki et al., 1996).

The pathophysiological basis of hyperekplexia remains unclear. The excessive startle reflex has been ascribed to a normal but exaggerated startle response (Andermann et al., 1980; Brown et al., 1991a) or to increased neuronal cortical excitability (Markand et al., 1984). Hyperekplexia has also been considered to be an independent or sui generis phenomenon within the stimulus-sensitive myoclonic disorders with mixed pontospinal origin (Matsumoto et al., 1992) or to represent a combination of reticular and cortical reflex myoclonus (Wilkins et al., 1986).

Physiological studies have focused on brainstem and spinal mechanisms in startle disease. However, there is good evidence that the startle reflex activates frontal abnormalities, and that both reticular and cortical mechanisms contribute to the clinical picture (Chauvel et al., 1992). In order to understand the underlying cerebral dysfunction in hyperekplexia, we performed proton magnetic resonance spectroscopic imaging (MRSI) studies in four patients with the major form of this condition.

### Methods

#### Subjects

Demographic and clinical characteristics of the patients are summarized in Table 1. We studied three unrelated patients with familial hyperekplexia and one (Patient 2) with an apparently sporadic form. The transmission of hyperekplexia in these families was consistent with autosomal dominant inheritance, but members of other generations had the minor form of the disorder. Clinical features of Patients 1–3 have been described earlier (Andermann et al., 1980; Dooley and Andermann, 1989). All except Patient 4 presented with a variable history of muscle stiffness, which persisted through adulthood in Patient 3. All but Patient 4 had bouts of spontaneous generalized clonus and presented with exaggerated backward jerking of the head on tapping the tip of the nose. Patients 2 and 3 could consistently provoke a stereotyped motor response with blinking, facial grimacing and backward jerking of the head by tapping themselves on the tip of the nose. Generalized hyper-reflexia with flexor plantar responses and variable degrees of uncertain gait were present in all. After walking a short distance, Patient 3 presented progressive stiffness of his legs and stumbled frequently. He also had difficulty extending the right arm quickly, with limitation of passive movements. All patients were treated with clonazepam (2–6 mg/day).

None of the patients had the described dominant (G1192A, G1192T) (Shiang et al., 1993) or recessive (T1112A) (Rees et al., 1994) inherited point mutations in the gene encoding for the α1-subunit of the glycine receptor (E. A. Shoubridge, unpublished data).

All patients underwent serial EEG recordings using the International 10–20 system for electrode placement. The EEG in the resting state showed mild generalized intermittent disturbance of cerebral activity (Patient 1), intermittent slow wave activity in the theta frequency range from both frontocentrotemporal regions (Patient 2) and some sharp waves in frontocentral regions with left sided predominance (Patient 3). There was no epileptic activity.

Informed consent was obtained from all patients and healthy volunteers after the nature of the procedure had been fully explained. The protocol was approved by the Research Ethics Committee of the Montreal Neurological Institute.

#### Neuroimaging

MRI scans were obtained on a 1.5-T Philips Gyroscan (Philips Medical Systems, Best, The Netherlands) using spin echo sequences (field of view 250 mm; 256 × 256 matrix and 4–6 mm slice thickness depending on the plane). Sagittal T1-weighted (TR 550 ms, TE 19 ms) images were followed by transverse proton density (TR 2000, TE 20 ms) and T2-weighted (TR 2100 ms, TE 20 or 78 ms) as well as coronal T1-weighted images. In addition, a second MRI study was performed using a T1-weighted gradient-echo volume acquisition (TR = 18 ms; TE = 10 ms; 30° angle; 160 contiguous slices, 1 mm thick) for multiplanar reconstruction. All four patients had normal MRI examinations.

A proton MRSI was obtained using the same scanner.

### Table 1 Demographic and clinical characteristics of the four patients

<table>
<thead>
<tr>
<th>Patient/ Sex/Age</th>
<th>Rigidity</th>
<th>Age at onset of excessive startle</th>
<th>Abdominal hernia</th>
<th>Motor development</th>
<th>Spontaneous clonus</th>
<th>Abnormal response to nose tapping</th>
<th>Insecure gait</th>
<th>Generalized hyper-reflexia</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/38</td>
<td>At birth, transitory</td>
<td>13 months</td>
<td>No</td>
<td>Delayed</td>
<td>Nocturnal</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Clonazepam</td>
</tr>
<tr>
<td>2/M/32</td>
<td>At birth, transitory</td>
<td>18 months</td>
<td>No</td>
<td>Delayed</td>
<td>Nocturnal</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Clonazepam</td>
</tr>
<tr>
<td>3/M/24</td>
<td>13 years, persistent None</td>
<td>13 years</td>
<td>No</td>
<td>Delayed</td>
<td>Diurnal</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Clonazepam, valproic acid</td>
</tr>
<tr>
<td>4/F/15</td>
<td>None</td>
<td>10 years</td>
<td>No</td>
<td>Delayed</td>
<td>None</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Clonazepam, valproic acid</td>
</tr>
</tbody>
</table>
After preliminary images in axial and sagittal planes, a multislice spin echo MRI (TR 2000, TE 30) was obtained parallel to the intercommissural line. A large volume of interest was defined, including frontal, central and anterior parietal areas, and excluding bone (Fig. 1). A water-suppressed proton MRSI was obtained from that volume of interest (TR 2000 ms, TE 272 ms, 250 × 250 mm field of view, 32 × 32 phase-encoding steps), followed by a proton MRSI without water suppression (TR 850 ms, TE 272 ms, 250 × 250 mm field of view, 16 × 16 phase-encoding steps). The post-processing included zero-filling the water-suppressed MRSI to obtain 32 × 32 profiles, dividing the water-suppressed MRSI by the water-unsuppressed MRSI, and application of a mild Gaussian K-space filter and an inverse 2D Fourier (Cendes et al., 1994). Residual water signal was removed by applying the linear HSVD fitting method (de Beer et al., 1992). The nominal voxel size in plane was ~8 × 8 mm, and 12 × 12 mm after K-space filtering.

Resonance intensities in individual spectra were determined by integration of peak areas using locally developed software (Cendes et al., 1994). The values for N-acetylaspartate (NAA), choline-containing compounds (Cho), creatine (Cr), and NAA/(Cr + Cho) were determined by averaging values from spectra in the frontal and central/post-central regions, respectively for each hemisphere (Fig. 1). Values for each region were compared with those obtained from a group of 20 age-matched normal control subjects. Patient 3 underwent a second proton MRSI obtained from temporal lobes following a protocol published previously (Cendes et al., 1994). The NAA/(Cr + Cho) values were determined for the medial and posterior region of each temporal lobe as well as for the brainstem and compared with those from another group of 20 normal control subjects.

**Statistical analysis**

We performed analysis of variance (ANOVA) to compare the resonance intensities of NAA/(Cho + Cr) in the frontal and central/post-central regions of patients and controls, followed by post hoc comparisons of all possible pairs of groups, using Tukey’s test which performs post hoc pairwise comparisons and corrects appropriately for multiple comparisons (Sokal and Rohlf, 1981).

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**Fig. 1** (A) Axial T₁-weighted MRI slice through the frontocentral regions. The volume of interest for the spectroscopic image (thick grey line) and the original phase-encoding grid are shown superimposed on the image. The metabolite values were determined by averaging spectra in the frontal and central/post-central regions as outlined by the white rectangle over the volume of interest on the right hemisphere. (B) Typical average spectra from the right and (C) from the left frontal region of a normal individual (left side of figure) and Patient 2. Note the reduced NAA peak from the patient compared with that from the normal subject.
Proton MRSI findings

<table>
<thead>
<tr>
<th>NAA/(Cho + Cr)</th>
<th>Frontal</th>
<th>Central/post-central</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.18*</td>
<td>1.12*</td>
</tr>
<tr>
<td>2</td>
<td>1.02*</td>
<td>1.00*</td>
</tr>
<tr>
<td>3</td>
<td>1.09*</td>
<td>1.00*</td>
</tr>
<tr>
<td>4</td>
<td>1.22*</td>
<td>1.18</td>
</tr>
<tr>
<td>Controls (n = 20)</td>
<td>1.41</td>
<td>1.28</td>
</tr>
<tr>
<td>Mean</td>
<td>1.28</td>
<td>1.13</td>
</tr>
<tr>
<td>SD</td>
<td>0.069</td>
<td>0.073</td>
</tr>
</tbody>
</table>

*Value < 2 SD.

Results

Group comparisons

NAA/(Cho + Cr) values for frontal and central/post-central regions were both significantly different in patients and controls (P < 0.0001). Pair-wise post hoc comparisons showed that NAA/(Cho + Cr) values in patients with hyperekplexia were (i) significantly different from controls in both left (P = 0.02) and right (P < 0.001) frontal regions, and for the right central/post-central region (P = 0.02); and (ii) not different from controls in the left central/post-central region (P > 0.5).

Individual patient comparisons

NAA/(Cho + Cr) values were significantly decreased (>2 SD below the mean of controls) in both frontal and central regions in three subjects (Patients 1, 2 and 3), and in the right frontal region in one (Patient 4) (see Table 2). Patient 3, who underwent a second proton MRSI of temporal regions, had normal NAA/(Cr + Cho) values over both temporal lobes as well as from the brainstem.

We have analysed the Cho : Cr ratios as well as the left : right ratios of Cho and Cr metabolites (data not shown here), and found no significant abnormalities. The only consistent abnormalities were found in the NAA/(Cho + Cr) values. This indicates that the abnormal ratios found here largely, if not entirely, reflect a decrease in NAA resonance intensity, indicating neuronal loss or damage. For simplicity, we choose to present here only data concerning NAA/(Cho + Cr) values.

Discussion

All patients had a variable degree of reduction of the relative NAA resonance intensity in both frontal and central regions. NAA is present only in neurons and their axons (Simmons et al., 1991), and NAA reduction is a marker for neuronal loss or dysfunction. In addition, proton MRSI can detect subtle widespread tissue abnormality in the absence of focal structural lesions in high resolution MRI (Cendes et al., 1994; Garcia et al., 1995; Petroff et al., 1995). The inter-hemispheric difference between patients and control subjects, in the central/post-central region, could be explained by a lack of sensitivity of the MRSI or by an asymmetry in the disease process that needs to be investigated further.

The variability of the partial volume effect which arises due to differences in metabolite levels between grey and white matter could be compensated for by introducing an image segmentation analysis procedure (Michaelis et al., 1993), but it was not performed in the present study. Therefore, we could not determine whether the abnormality in the relative NAA resonance intensities is more pronounced in the grey matter, white matter or both.

In two subjects, the topography of the non-epileptic EEG abnormalities in the frontal lobes coincided with the MRSI findings. The clinical characteristics and the severity of the disease did not correlate with the extent of MRSI anomalies. However, the patient without hypertonia, spontaneous clonus or abnormal response to nose tapping (Patient 4) showed the least NAA reduction.

The motor response in the normal auditory startle reflex in man is organized in the medial reticular formation, which may be activated by subcortical or cortically relayed afferent inputs. The specific motor response may result from a caudally and rostrally spreading single volley (Brown et al., 1991b) or from a polysynaptically generated muscle activation organized by a reticular generator capable of spatiotemporal sequencing (Chokroverty et al., 1992). Testing spinal inhibitory pathways in five patients with hereditary hyperekplexia, Floeter et al. (1996) found definite abnormalities in only one of the two forms of inhibition mediated by glycinergic interneurons. Therefore, spasticity, hyper-reflexia and muscle stiffness in patients with hyperekplexia are probably not related to spinal hyperexcitability. On the other hand, the clonus (Suhren et al., 1966; Andermann et al., 1980; Dooley and Andermann, 1989), the EEG abnormalities (Suhren et al., 1966; Andermann et al., 1980; Dooley and Andermann, 1989), the shortened latency of the blink reflex (Brown et al., 1991a) and the large somatosensory evoked potentials (Fariello et al., 1983; Markand et al., 1984; Brown et al., 1991a) may indicate that the excessive startle reflex is related to increased cortical (Markand et al., 1984) or reticular neuronal excitability (Matsumoto et al., 1992). Studying saccadic eye movements, Tijssen et al (1995a) found no evidence of a lack of cortical inhibition in hyperekplexia. On the other hand, some observations support the hypothesis of a lack of inhibition by higher cortical centres (Suhren et al., 1966). Fariello et al. (1983) reported a patient in whom an infarction involving the subthalamic nucleus and the dentatorubro-ventrolateral thalamic pathway led to the reappearance of pre-existing hyperekplexia. He postulated that the interruption of thalamic pathways can eliminate the descending inhibition of the startle reflex. Hochman et al. (1994) reported a sporadic case of hyperekplexia with decreased cerebral blood flow in
the frontal lobe using SPECT (single photon emission computed tomography), and assumed that this abnormality could represent a ‘functional cortical lesion of a descending pathway that normally inhibits the startle reflex’. Thus, considering previous neurophysiological studies, the neuronal dysfunction indicated by the reduced NAA in frontocentral regions seems to support the hypothesis that the pathological startle reflex in these patients may be associated with a lack of cortical inhibition. The finding of MRSI abnormalities in the presence of completely normal high resolution MRI studies indicates the presence of neuronal metabolic dysfunction or neuronal loss. Considering the possible involvement of GABA in this condition (Ryan et al., 1994; Shiang et al., 1993), an in vivo analysis of GABA distribution in the brain by proton MRSI (Rothman et al., 1993) could lead to further clarification.

The peculiar sensibility to sensory stimuli of the central face area in some of our patients may also be related to these cortical abnormalities. This is in keeping with the fact that patients with startle-provoked epileptic seizures show a preponderance of EEG and structural abnormalities within the frontal and central regions (Aguglia et al., 1984; Chauvel et al., 1992; Manford et al., 1996). Therefore, our observations support the concept of a common pathway in the pathophysiology of epileptic and non-epileptic startle disorders.

The hypertonia and the generalized hyper-reflexia, as well as the cautious and apractic gait are clinical signs of dysfunction in the pyramidal corticospinal and cortico-bulbo-cerebellar pathways. Spontaneous clonic jerks occur frequently in hyperekplexia (Suhren et al., 1966; Andermann et al., 1980; Saenz-Lope et al., 1984; Dooley and Andermann, 1989). De Groen and Kamphuisen (1978) attributed this form of clonus to reticular hyperexcitability. Previous studies have demonstrated that cortical myoclonus may represent an enhancement of sensory inputs to the motor cortex or abnormal relays within the sensorimotor cortex (Obeso et al., 1985; Reutens et al., 1993). Magnetoencephalography studies in patients with cortical myoclonus showed that an abnormal sensorimotor cortex can contribute to the generation of the cortical myoclonus (Uesaka et al., 1996) and that anomalies of the motor cortical inhibition facilitate the spread of the myoclonic activity responsible for generalized jerks (Brown et al., 1996). Therefore, in our patients with hyperekplexia and spontaneous clonus, the cortical abnormalities could also contribute to the generation of the spontaneous clonus.

None of our patients with familial and sporadic hyperekplexia, or their examined relatives, had the previously described point mutations of the α1-subunit of the glycine receptor (Shiang et al., 1993, 1995; Rees et al., 1994; Bernasconi et al., 1996). It is therefore evident that the clinical phenotypes of hyperekplexia, both the major and the minor form, as well as familial and sporadic cases, can result from more than one genetic abnormality, indicating genetic heterogeneity. In addition, the two strains of mutant mouse that resemble the startle disease phenotype have a genetic defect involving two different genes, coding for the α-subunit (Ryan et al., 1994) and the β-subunit (Kingsmore et al., 1994) of the glycine receptor, respectively. These glycine receptor subunits in the mammalian CNS were found in brainstem, spinal cord and brain (Matzenbach et al., 1994). Therefore, it is possible that other mutations can be found in genes coding for the different subunits of the glycine receptor in human hyperekplexia syndromes (Shiang et al., 1995).

The results of this study with proton MRSI show that patients with hyperekplexia have a frontal neuronal dysfunction and are consistent with previous observations. Whether this represents cortical dysfunction or an epiphenomenon of diencephalic or brainstem abnormalities remains open. However, the observation of normal proton MRSI in the temporal lobe and brainstem of one of the patients seems to concur with the hypothesis of a facilitatory role of cortical dysfunction in sensorimotor pathways leading to generation of the pathological startle reaction and the spontaneous generalized clonus in hyperekplexia.

References


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