Short-latency somatosensory-evoked potentials demonstrate cortical dysfunction in patients with Angelman syndrome

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ABSTRACT

Background: Angelman syndrome (AS) is a neurodevelopmental disorder, causal gene of which is maternally expressed UBE3A. A majority of patients results from the large deletion of relevant chromosome which includes GABA A receptor subunit genes (GABARs) as well as UBE3A (AS Del). We previously reported aberrantly desynchronized primary somatosensory response in AS Del by using magnetoencephalography. The purpose of this study is to estimate cortical and subcortical involvement in the deficit of primary somatosensory processing in AS.

Methods: We analyzed short-latency somatosensory-evoked potentials (SSEPs) in 8 patients with AS Del. SSEPs were recorded on a 4-channel system comprising of two cortical electrodes which were placed on the frontal and centro-parietal areas. The peak and onset latency of each component were measured to compare latency and interval times.

Results: The first-cortical peak latency (N20, P20), and N13-N20 peak interval times were significantly prolonged in AS Del compared to healthy controls. In contrast, there was no difference in latencies between subcortical components up to N20 onset or for N11-N20 onset interval times.

Conclusion: Highly desynchronized first-cortical SSEP components and normal latencies of subcortical components indicated cortical dysfunction rather than impairment of afferent pathways in AS Del patients, which might be attributed to GABAergic dysfunction due to loss of UBE3A function and heterozygosity of GABARs.

1. Introduction

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe developmental delay, speech impairment, ataxic gaits, and characteristic behaviors such as paroxysmal laughter [1,2]. The imprinted UBE3A gene in chromosome region 15q11-q13 is the causal gene for AS; it encodes a ubiquitin protein ligase whose function is yet to be determined [3,4]. In recent murine studies, loss of Ube3a function induced neurobehavioral deficits due to abnormal neural plasticity [5,6] or disruption of excitatory/inhibitory balance [7–9]. The majority of AS patients show a 4-Mb deletion of maternal origin involving three non-imprinted GABA A receptor-subunit genes (GABRB3, GABRA5, and GABRG3, which encode the receptor subunit proteins β3, α5, and γ3, respectively), in addition to UBE3A. The β3 subunit is expressed throughout the brain, especially during development [10] and hemizygosity for this gene may affect the clinical severity of AS through GABAergic dysfunction [11–13].

We previously showed aberrant prolongation of the first somatosensory-evoked fields (SEFs) component in AS patients with a deletion in 15q11-q13 (AS Del) using magnetoencephalography [14]. The desynchronized responses were not observed in AS patients without the deletion. We speculated that such responses arose from functional deficits in the somatosensory cortex and largely attributed to the hemizygosity of GABA A receptor-subunit genes. These results suggested the clinical potential of analyzing primary somatosensory responses to evaluate the cortical dysfunctions relevant to GABAergic systems.

Magnetoencephalography is superior to electroencephalography for estimating primary somatosensory cortex activities. However, since

Abbreviations: AS, Angelman syndrome; GABARs, GABA A receptor subunit genes; SEFs, Somatosensory-evoked fields; AS Del, AS patients with a deletion in 15q11-q13; SSEPs, Short-latency somatosensory-evoked potentials; VPA, Valproic acid; CZP, Clonazepam; CCT, Central conduction time.

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magnetoencephalography cannot detect subcortical activities [15], our earlier work could not exclude the possibility that the delayed cortical somatosensory responses reflected impairment of the afferent pathway due to events such as demyelination of the white matter. Although previous pathological and MRI studies suggested that most AS patients have no organic impairment that may affect afferent pathway conductivity [16,17], more recent studies discussed the possibility that AS patients in young age could have delayed myelination of the white matter [18,19]. It has been shown that the subcortical conductivity could be evaluated precisely by short-latency somatosensory-evoked potentials (SSEPs) following median nerve stimulation using onset-to-onset and peak-to-peak methods [20,21]. The current study therefore measured SSEPs in patients with AS Del to evaluate subcortical as well as cortical somatosensory activities.

2. Subjects and methods

2.1. Subjects

Eight patients with genetically confirmed AS Del (four female and four male patients aged 5–28 years, mean ± SEM: 11.6 ± 2.5) and 11 age-matched healthy volunteers (four female and seven male normal controls aged 5–26 years, mean ± SEM: 10.5 ± 1.7) participated in the study. The AS Del patient profiles are shown in Table 1. Three patients took valproic acid (VPA), and five patients took VPA and clonazepam (CZP) as antiepileptic medication. All subjects or their parents provided written informed consent. This study was approved by the Internal Review Board, Hokkaido University Graduate School of Medicine.

2.2. Recording methods and evaluation of SSEPs

The subjects were assessed while supine on a bed in a quiet, electrically shielded room. AS patients were sedated by intravenous administration of thiopental sodium (approximately 3–4 mg/kg), and control subjects were encouraged to relax or sleep naturally. The right median nerve was stimulated by square-wave impulses (duration: 0.2 ms, frequency: 3 Hz) with sufficient intensity to produce a definitive thumb twist. SSEPs were recorded on a 4-channel electromyographic device (Keypoint™, Dantec Dynamics, Bristol, UK) with the following montages: (1) right Erb’s point (Erb) to noncephalic (NC) electrode placed on the left shoulder, (2) the spinous process of C6 (cv6) to Fz in the International 10–20 system, (3) C3’ (2 cm posterior to C3) to A2, and (4) F3 to A2. Electrode impedance was maintained below 5 kΩ. Amplified evoked potentials were filtered between 10 and 3000 Hz. Five hundred responses were averaged with an analysis time of 100 ms, and at least two averaged recordings were obtained for reproducibility.

The peaks were labeled according to their polarity and modal peak latency (Fig. 1). The highest peak was measured when multiple peaks were present. For the N9, N11, P13/14, and N20 peaks, we also measured onset latencies based on previous reports [20,22]. The onset of N20 was identified as the fork at which the frontal and centroparietal waves diverged from superimposing traces with subcortical components [20,21]. The suffix ’p’ and ’o’ in the labels indicated peak and onset of each component, respectively. We also analyzed subcortical interval parameters (from N9o to N11o, from N11o to N20o, and from P13/14o to N20o). In addition, interval times between N13p and N20p (N13p-N20p) and total N20 duration (between N20o and the latency for the frontal and the centroparietal waves crossing again [23]) were also evaluated. Unpaired Student’s t-test was used to evaluate the difference between AS Del patients and control subjects in each latency interval and parameter. The difference in N13p-N20p interval times between AS Del patients who had and who had not taken CZP was also analyzed using the Mann Whitney U test. Statistical significance was set at P < 0.05.

3. Results

Fig. 1A and B show representative SSEPs findings for healthy controls and the AS Del patients, respectively. For AS Del patients, the cortical N20 component in the centroparietal scalp comprised of dull double (6 patients) or triple (2 patients) peaks. Six of the eight showed a prolonged frontal P20 peak (Fig. 1B) and 2 patients had double peaks. All components were identified in both AS Del and control subjects. The latencies and interval times are listed in Table 2. Cortical N20, P20 peak latencies, and N20 duration were significantly prolonged in AS Del patients. In contrast, the AS subcortical latencies up to N20o and subcortical interval parameters were not different from those in control subjects. N13p-N20p times were also prolonged in AS Del patients, whereas N11o-N20o times were not prolonged. There was no difference in N13p-N20p times between AS Del patients who had taken and not taken CZP (11.7 ± 2.7 ms versus 15.9 ± 2.6 ms, p = 0.39).

4. Discussion

The present study evaluated SSEPs in AS Del patients. It revealed prolonged N13p-N20p times as well as dispersed cortical N20 and P20 components. The N13p-N20p times are conventionally regarded as the central conduction time (CCT), and a similarly prolonged ’CCT’ was reported in an earlier study of AS patients [24]. However, other detailed analyses including normal subjects revealed that conventional CCT was inappropriate for estimating central afferent conductivity. This was because the times varied among individuals due to the high intracortical-pathway variability (from N20o, indicating the cortical arrival time, to N20p segments in the N13p-N20p times [20,22]). The intervals between N11o and N20o were proposed as a better parameter by which to evaluate the central (subcortical) afferent pathway conductivities. A previous study documented the central conduction abnormality in diabetes mellitus by prolonged N11o-N20o interval times [25]. In contrast, the N11o-N20o interval times in AS Del patients were not different from those in control subjects. Accordingly, our results showed that impairments in the subcortical afferent pathway did not prolong somatosensory reactions in AS Del patients. Instead, the dispersed cortical response was caused by intracortical-function deficits.

The current findings for SSEPs were in line with that for SEPs in the preceding study [14], and support our proposal of a correlation between desynthesized sensory cortex activities and GABAergic dysfunction. Recent murine studies revealed that loss of Ubε3α function induced neurobehavorial deficits due to abnormal synaptic formation and neural plasticity [5,6]. Furthermore, lack of the GABA_A receptor β3 subunit gene also caused disruption of the neuronal networks [26]. Neuronal plasticity could be highly disturbed in AS Del patients due to both the loss of Ubε3α function and GABA_A receptor-subunit gene hemizygosity, which together may lead to desynthesized somatosensory cortical responses reflected by the prolonged cortical N20, P20 components in SSEPs. Our recent positron emission topographic study evaluating

| Table 1 | Clinical profiles of the AS Del patients. |
|---------|----------------------------------------|
| Patient No. | Age/sex | Anticonvulsant | Walking impairment | Language ability |
| 1 | 5/M | VPA | mild | simple words |
| 2 | 6/M | VPA, CZP | severe | no words |
| 3 | 8/F | VPA | severe | no words |
| 4 | 10/F | VPA | severe | no words |
| 5 | 10/M | VPA, CZP | moderate | simple words |
| 6 | 12/F | VPA, CZP | severe | simple words |
| 7 | 14/F | VPA, CZP | severe | no words |
| 8 | 28/M | VPA, CZP | severe | no words |

VPA: valproic acid, CZP: clonazepam, Classification of walking impairment: severe = impossible to walk without help, moderate = possible to walk without help, but is using a wheelchair, mild = possible to walk without help, and is not using a wheelchair.
GABA<sub>A</sub> receptor expression suggested a disturbed GABAergic network in AS patients [27]. Following studies illustrating disruption of inhibitory neuron firings [7] or decrement of tonic form of inhibition [8] in Ube3a deficit mice support our results. Previous studies have revealed that synchronous feed-forward and feed-back inhibition using GABAergic transmission shapes neuronal responses by allowing a brief window of excitability [28,29]. In the mammalian somatosensory cortex, neural excitation increases, with increased duration, when GABAergic inhibition is blocked [30,31]. Our findings are in accordance with these studies. Thus, GABAergic dysfunction may be the principal pathophysiology for shaping the aberrant cortical responses of SSEPs in AS Del patients. Further investigation using animal models would be required to clarify the details of GABAergic dysfunction in AS for a seeking novel therapeutic strategy.

The projection from the primary somatosensory cortex plays an important role in learning and coordinating motor function [32,33]. Therefore, the desynchronized primary somatosensory activity in AS Del, which is represented by prolonged N20 duration in the current study, can be associated with motor dysfunction. If the correlation between N20 duration and the severity of motor dysfunction is proven in a
further study with a greater number of subjects, N20 duration can be utilized as a biomarker for evaluating brain dysfunctions in AS and therapeutic effects of clinical trials.

In the current study, only AS patients and not control subjects were sedated and given antiepileptic medications. A role for drug effects in subcortical dysfunctions in patients with neurodevelopmental disorders.

Declaration of competing interest

None.

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