SHORT REPORT

Presynaptic inhibition of cerebellar GABAergic transmission by glutamate decarboxylase autoantibodies in progressive cerebellar ataxia

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Abstract

Autoantibodies against glutamic acid decarboxylase (GAD) have been found in stiff-man syndrome, insulin dependent diabetes mellitus, and progressive cerebellar ataxia. A patient with progressive cerebellar ataxia is described who was positive for GAD autoantibodies, and had Sjögren's syndrome. Immunohistochemical studies using CSF and serum samples from the patient showed immunoreactivities in axon terminals of cerebellar GABAergic neurons. A whole cell patch clamp technique recording from rat cerebellar slices showed that the CSF, presumably through GAD autoantibodies, presynaptically inhibited GABAergic transmission. Intravenous administration of immunoglobulin failed to improve clinical symptoms and immunoreactivities examined after therapy. The findings suggest that GAD autoantibodies play a pathogenic part in reducing GABA release in in vitro slices.

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Glutamic acid decarboxylase (GAD) catalyzes the conversion of glutamic acid to γ-aminobutyric acid (GABA) and is concentrated in CNS inhibitory neurons and pancreatic β-cells. GAD autoantibodies have been found in patients with stiff-man syndrome, insulin dependent diabetes mellitus, and polyendocrine autoimmune syndrome.[1] A few reports have also identified patients with cerebellar ataxia associated with GAD autoantibodies.[2–5]

The GAD autoantibodies are considered to have a pathogenic role in various neurological disorders.[6 7] For example, previous studies have shown that these autoantibodies in CSF obtained from an ataxic patient reduce GABA release from nerve terminals, thereby suppressing cerebellar inhibitory transmission.[5 8] However, it remains to be elucidated whether inhibitory transmission is actually depressed by CSF immunoglobulins (IgGs) of other ataxic patients with GAD autoantibodies

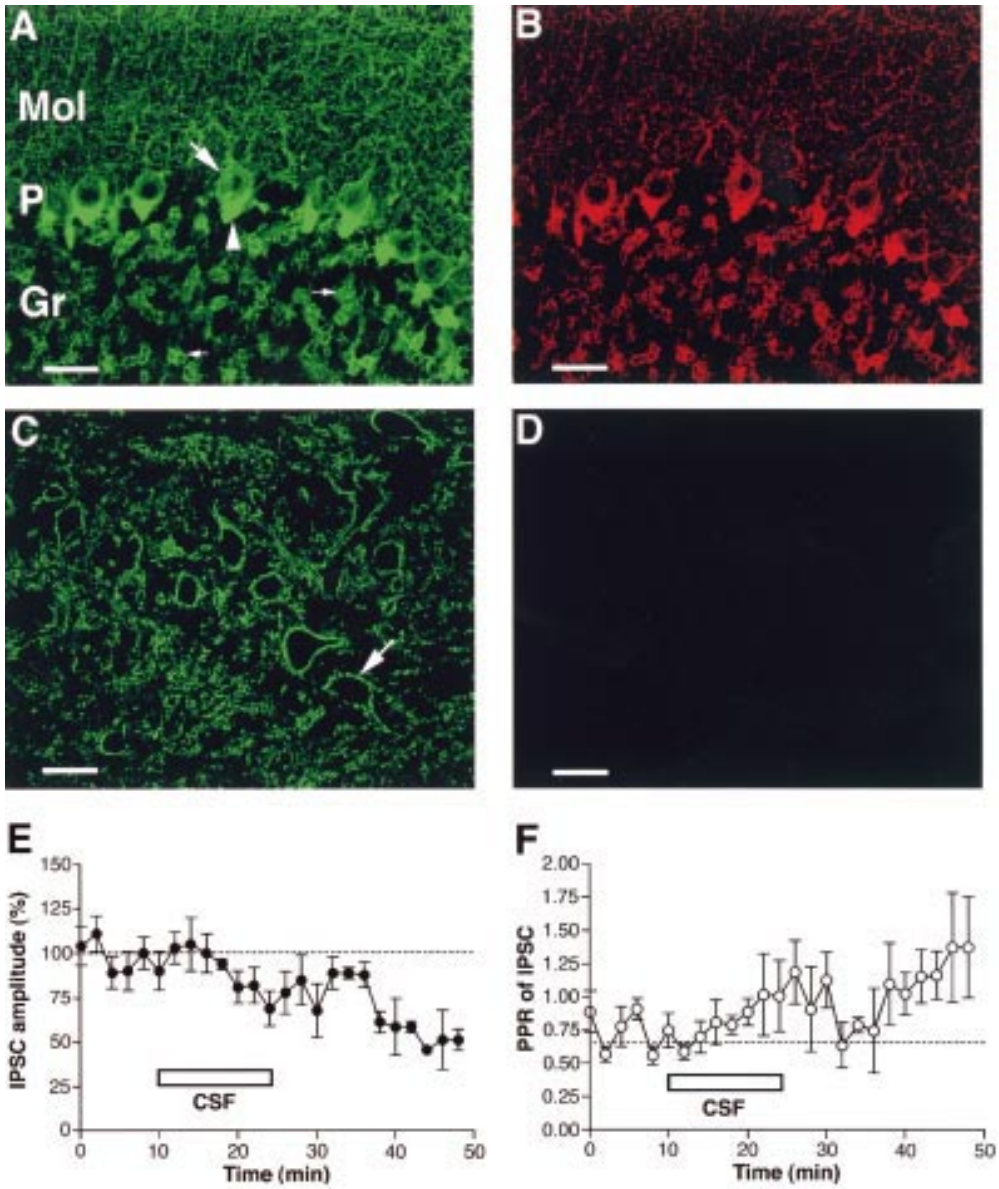
We report on a woman with progressive cerebellar ataxia who was positive for GAD autoantibodies. She also had Sjögren's syndrome. We analyzed the pathophysiological actions of CSF from this patient on cerebellar inhibitory synaptic transmissions using whole cell recording in rat cerebellar slices.

Patient and methods

CASE REPORT

A 72 year old woman noticed gait instability and dysarthria in February 1997. The family history was negative for cerebellar ataxia. She had had Sjögren's syndrome since the age of 68 and diabetes mellitus since the age of 69. She was referred to our clinic in December 1998 (at the age of 74) because of vertigo and oscillopsia. On admission (March 1999), she showed ataxic dysarthria, gaze evoked down beat nystagmus, truncal ataxia, and left side predominant limb ataxia. Tendon reflexes were moderately hyperactive and pathological reflexes were positive in both lower limbs. The visual suppression of the vestibulo-ocular reflex was impaired, indicating cerebellar oculomotor disturbances. There were no signs of stiff-man syndrome and no history of seizures. No muscle weakness, sensory disturbance, urinary disturbance, involuntary movements, or dementia were recognised.

Serological tests showed positive antinuclear antibodies, anti SS-A/ Ro antibodies, and anti-RNP antibodies, which were associated with Sjögren's syndrome. The following other organ specific autoantibodies were also detected: gastric parietal cell, pancreatic islet cell, intrinsic factor, thyroglobulin, and thyroid microsomal antibodies. Analysis of CSF was normal except for the presence of GAD autoantibodies. Oligoclonal IgG bands were negative. There was no evidence of any malignancy on radiological studies or paraneoplastic cerebellar degeneration including anti-Hu, anti-Yo, and anti-Ri antibodies. No tumour markers were higher than the normal cut off concentrations. Genetic analysis did not show CAG repeat expansion in the CACNL1A4 gene causing spinocerebellar ataxia type 6. Titres of GAD autoantibodies measured with an assay kit (Cosmic Co, Tokyo, Japan) were 95 500



(A-D) Double immunostaining of the postnatal 56 day old rat cerebellar cortex by (A) 1:50 diluted CSF from the patient and (B) 1:500 diluted anti-GAD antibody. Distribution of immunoreactivity by the same CSF in (C) the cerebellar nucleus and (D) by 1:50 diluted CSF from a patient with olivopontocerebellar atrophy without GAD autoantibodies, as a negative control. Large arrows in A and C indicate punctuate immunoreactivity in the rim of the soma of Purkinje cells and neurons of the cerebellar nucleus, respectively. Large arrowhead in A indicates intense immunoreactivity in the initial segment of the axons of Purkinje cells. Small arrows in A indicate dense immunoreactivity in the glomerulus. Mol=molecular layer; P=Purkinje cell layer; Gr=granule cell layer. Bars in A–D indicate 40 μm. (E and F) Effects of the diluted 1:100 CSF on inhibitory postsynaptic current (IPSC) and paired pulse ratio (PPR). The PPR was defined as the ratio of the amplitude of the first IPSC over that of the second IPSC to paired pulse nerve stimuli. Each data point represents the mean (SD) of six successive synaptic responses evoked at 0.05 Hz in a single experiment. CSF suppressed the IPSC amplitude, which was associated with an increase in PPR. CSF was applied by perfusion during the period indicated by the horizontal bar.

U/ml in serum and 2500 U/ml in CSF, with a serum/CSF GAD autoantibody ratio of 38.2.

Brain MRI showed no abnormal findings including cerebellar atrophy. However, single photon emission computed tomography (SPECT) and ^{18}F-deoxyglucose positron emission tomography (FDG-PET) indicated reduced blood flow and glucose metabolism mainly in the left cerebellar hemisphere. These asymmetric findings were considered to reflect the laterality of functional cerebellar abnormalities. Echography of the thyroid showed a multinodular goitre.

The patient was treated with intravenous immunoglobulin (IVIg; 0.4 g/kg/day for 5 consecutive days). However, this treatment did not result in improvement of cerebellar symptoms. Furthermore, a rise in serum GAD autoantibody concentration to 140 000 U/ml was noted 3 months after therapy.

IMMUNOHISTOCHEMISTRY

Blood and CSF samples were obtained with informed consent from this patient and other patients with cerebellar ataxia without GAD autoantibodies.

Immunohistochemistry was conducted using cerebellar cryosections of 56 day old rats.[5 8] Sections were incubated with CSF and serum from the patient (1:50–1:500 dilutions of CSF and 1:2000–1:8000 dilutions of serum in 0.1M of phosphate buffered saline with 0.2% Triton X) overnight, and specific labelling was detected by biotinylated goat antihuman IgG (Chemicon, Temecula, CA, USA) and avidin-FITC conjugate (ICN, Costa Mesa, CA, USA). For double immunostaining, sections were incubated with mixtures of the patient's CSF and a rabbit anti-GAD antibody (Affinity Research Products, Manhead, UK) as previously reported.[5] GAD immunoreactivity was visualised by TRITC-conjugated goat antirabbit IgG (Cappel, Durham, NC, USA). Sections were examined by a confocal laser scanning microscopy (BioRad, Hemel Hempstead, UK).

ELECTROPHYSIOLOGY

Whole cell voltage clamp recording was made from visually identified Purkinje cells in rat cerebellar slices obtained from 14 day old rats as previously reported.[5 8] Sagittal cerebellar slices (250 μm thick) were cut using a microtome. The slices were continuously perfused with artificial CSF. Purkinje cells were identified under Nomarski optics (Olympus, Tokyo, Japan). Membrane currents were recorded from Purkinje cells through an amplifier (HEKA, Lambrecht, Germany) using the whole cell configuration with patch clamp electrodes. Synaptic currents were evoked by stimulation (single pulse of 10–30 V and 20–200 μs) via glass microelectrodes within the molecular layer.

Results

IMMUNOHISTOCHEMISTRY AND ELECTROPHYSIOLOGY

Immunohistochemical studies using 1:50 diluted CSF before IVIg showed nerve terminals with punctuate immunoreactivity surrounding the soma and axon hillock of Purkinje cells (figure A). Positive immunoreactivity was diffusely distributed in the molecular layer and densely in the glomerulus of the granular layer (figure A). The distribution of immunoreactivities on double immunostaining using anti-GAD antibody was similar to that seen using the patient's CSF (figure B), suggesting that autoantibodies in CSF recognise GAD in GABAergic nerve terminals. We also confirmed the lack of cross reactivity between the patient's CSF and goat antirabbit IgG, and also between rabbit anti-GAD antibody and goat anti-human IgG (data not shown). In cerebellar nuclei, punctuate immunoreactivity surrounding the soma of large neurons was noted (figure C). These immunoreactivities also colocalised with those identified by anti-GAD antibody (data not shown), indicating that the stained structures were GAD positive axon terminals of Purkinje cells. We obtained the same findings using 1:200 diluted CSF after IVIg and 1:2000 and 1:8000 diluted serum before and after IVIg (data not shown). These results suggest that the same or increased concentrations of GAD autoantibodies were still present in CSF and serum after IVIg. We found no particular immunoreactivities by using serum samples or CSFs from seven patients with cerebellar ataxia without GAD autoantibodies (figure D), or by using the same immunostaining procedure except for omitting the CSF or serum of the patient (data not shown).

The functional significance of this humoral autoimmune response at nerve terminals of GABAergic neurons was then analyzed using whole cell recording in the cerebellar slices. Focal stimulation within the molecular layer in the cerebellar cortex produces inhibitory postsynaptic currents (IPSCs) in Purkinje cells, which are mediated by GABA released from basket cells.[5 8] The diluted CSF samples (1:100) gradually decreased the amplitude of IPSCs (mean 35 (SD 6)% of the control, n=3) (figure E). The degree of specificity was ascribed to the tested CSF, because control CSF obtained from ataxic patients without GAD autoantibodies (n=7) had no effect on IPSCs. The ratio of the magnitude of the second IPSC to that of the first IPSC to paired pulse stimuli was defined as the paired pulse ratio (PPR). During the inhibitory period, PPR increased to 179 (SD 6)% of the control (n=3) (figure F). Because increased PPR represents the suppression of the transmitter release from nerve terminals,[8] these results indicated that CSF containing GAD autoantibodies presynaptically suppressed GABAergic transmissions from basket cells to Purkinje cells.

Discussion

GAD autoantibodies are found in patients with stiff-man syndrome, insulin dependent diabetes mellitus, and polyendocrine autoimmune syndrome.[1] Our ataxic patient with GAD autoantibodies was complicated by insulin dependent diabetes mellitus and Sjögren's syndrome. Sjögren's syndrome is often associated with involvement of the CNS, and the pathogenic mechanism of neural complication is thought to be mainly due to angitis.[9 10] However, our patient showed neither MRI abnormalities nor CSF findings that reflected angitis. On the other hand, it is possible that GAD autoantibodies in our patient were produced by non-specific activation of B cells related to associated Sjögren's syndrome, but this is also unlikely because the combined presence of GAD autoantibodies and Sjögren's syndrome is very rare. Thus, it is likely that in our patient, development of ataxia and high concentration of the antibodies are not directly linked to Sjögren's syndrome.

Recently, a few patients with progressive cerebellar ataxia who were positive for GAD autoantibodies have been reported.[2–5] These patients were all women and the onset of ataxia was in the 40s to 60s age range. Their ataxia was more prominant in the trunk than in the limbs. These clinical profiles matched those of our patient. Furthermore, the concentration of GAD autoantibodies were markedly high and were compatible with autoimmune cerebellar ataxia as defined by Saiz *et al*.[3] Immunohistochemical studies using CSF and serum

samples in our study showed positive immunoreactivities in the cerebellar cortex and cerebellar nuclei, which coincided with those of GAD in the nerve terminals of GABAergic interneurons. These findings indicated that GAD autoantibodies in CSF and serum are linked to the cerebellar ataxia in our patient.

Previous reports indicated that IVIg was effective in some patients with stiff-man syndrome and cerebellar ataxia associated with GAD autoantibodies.[4 11 12] In our patient, IVIg did not have a therapeutic effect on the neurological symptoms or GAD autoantibody titres. Although the mechanisms of action of IVIg have not been completely elucidated, it is considered to have multiple functions as an immunomodulating substance on both humoral and cell mediated immune systems.[13] In our patient, the gravity of autoimmunity against GAD might be too strong or the cerebellar GABAergic neurons had already been irreversibly damaged.

Electrophysiological studies using CSF samples showed suppression of the amplitude of IPSCs and increased PPR. Increased PPR is the hallmark of depressed release of transmitters[8] and the tested CSF suppressed cerebellar inhibitory transmission presynaptically. Such presynaptic suppression seems to be mediated by GAD autoantibodies in CSF, as the actions of CSF mimicked those of polyclonal GAD autoantibodies described in our previous report.[5] These results suggest that GAD autoantibodies in CSF obtained from ataxic patients acted on the nerve terminals of GABAergic neurons to reduce the release of GABA.[5 8] In this regard, Dinkel *et al*[14] recently showed that GAD autoantibodies from patients with stiff-man syndrome decreased GABA synthesis. Therefore, one of the possible mechanisms of autoantibody induced presynaptic suppression is GAD autoantibody induced down regulation of GABA synthesis, which results in a reduction of GABA release from nerve terminals.

Together with our previous reports,[5 8] the present study indicated that GAD autoantibodies obtained from a group of patients with cerebellar ataxia elicit a functional impairment by reducing GABA release in in vitro slices. These results reconcile with the notion that GAD autoantibodies are directly involved in the pathogenesis of cerebellar ataxia and stiff-man syndrome.[7 14] However, if such autoantibodies suppress inhibitory transmission in stiff-man syndrome as well as in cerebellar ataxia, then there is a need to identify the factors that determine the aetiological differences between the two diseases. In addition, it is important to determine the mechanisms that limit the actions of GAD autoantibodies to selective regions of the CNS compared with the widespread distribution of GAD. Thus, further studies are necessary to determine the mechanisms by which the pathogenic synaptic suppression by GAD autoantibodies in vitro leads to specific dysfunction of the cerebellum or spinal cord in vivo.

The first two authors contributed equally to the study.

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1 Solimenta M, Folli F, Aparisi R, *et al*. Autoantibodies to GABA-ergic neurons and pancreatic beta cells in stiff-man syndrome. *N Engl J Med* 1990;**322**:1555–60.
2 Giometto B, Miotto D, Faresin F, *et al*. Anti-gabaergic neuron autoantibodies in a patient with stiff-man syndrome and ataxia. *J Neurol Sci* 1996;**143**:57–9.
3 Saiz A, Arpa J, Sagasta A, *et al*. Autoantibodies to glutamic acid decarboxylase in three patients with cerebellar ataxia, late-onset insulin-dependent diabetes mellitus, and polyendocrine autoimmunity. *Neurology* 1997;**49**:1026–30.
4 Abele M, Weller M, Mescheriakov S, *et al*. Cerebellar ataxia with glutamic acid decarboxylase autoantibodies. *Neurology* 1999;**52**:857–9.
5 Ishida K, Mitoma H, Song SY, *et al*. Selective suppression of cerebellar GABAergic transmission by an autoantibody to glutamic acid decarboxylase. *Ann Neurol* 1999;**46**:263–7.
6 Solimena M, DeCamilli P. Autoimmunity to glutamic acid decarboxylase (GAD) in stiff-man syndrome and insulin-dependent diabetes mellitus. *Trends Neurosci* 1991;**14**:452–7.
7 Ellis TM, Atkinson MA. The clinical significance of an autoimmune response against glutamic acid decarboxylase. *Nat Med* 1996;**2**:148–53.
8 Mitoma H, Song SY, Ishida K, *et al*. Presynaptic impairment of cerebellar inhibitory synapses by an autoantibody to glutamate decarboxylase. *J Neurol Sci* 2000;**175**:40–4.
9 Alexander GE, Provost TT, Stevens MB, *et al*. Sjögren syndrome: central nervous system manifestations. *Neurology* 1981;**31**:1391–6.
10 Kaplan JG, Rosenberg R, Reinitz E, *et al*. Invited review: peripheral neuropathy in Sjögren syndrome. *Muscle Nerve* 1990;**13**:570–9.
11 Amato AA, Cornman EW, Kissel JT. Treatment of stiff-man syndrome with intravenous immunoglobulin. *Neurology* 1994;**44**:1652–4.
12 Barker RA, Marsden CD. Successful treatment of stiff man syndrome with intravenous immunoglobulin. *J Neurol Neurosurg Psychiatry* 1997;**62**:426–7.
13 Dalakas MC. Mechanism of action of intravenous immunoglobulin and therapeutic considerations in the treatment of autoimmune neurologic diseases. *Neurology* 1998; **51**(suppl 5):S2–8.
14 Dinkel K, Meinck HM, Jury KM, *et al*. Inhibition of α-aminobutyric acid synthesis by glutamic acid decarboxylase autoantibodies in stiff-man syndrome. *Ann Neurol* 1998;**44**:194–201.