J Neurol (2010) 257:281–285
DOI 10.1007/s00415-009-5331-z

SHORT COMMENTARY

Effects of immunotherapy on motor cortex excitability in Stiff Person Syndrome

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Received: 14 January 2009 / Revised: 8 September 2009 / Accepted: 16 September 2009 / Published online: 10 October 2009
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Abstract A number of cortical and spinal excitability variables have been tested in a patient with Stiff Person Syndrome (SPS), before and after immunotherapy with mycophenolate mofetil, intravenous immunoglobulin and corticosteroids, which normalized plasma levels of anti-GAD antibodies and dramatically improved the clinical picture. The overlapping time-course of neurophysiological, clinical and bio-umoral findings suggests that immunotherapy might have changed GABA/Glutamate balance at cortical level, favoring the former, as reflected by normalization of the startle reflex, lengthening of the cortical silent period and clear-cut reduction of intracortical facilitation to paired-pulse transcranial magnetic stimulation. This represents the first report investigating effects of immunotherapy on cortical excitability in SPS.

Keywords Stiff Person Syndrome · TMS · Mycofenolate mofetil · Anti-GAD antibodies · IViG

Introduction

Stiff Person Syndrome (SPS) is a rare acquired, progressive, immunomediated disease characterized by the presence of intrathecal and circulating anti-glutamic acid decarboxilase (GAD) antibodies (Ab), frequent association with other immunomediated disorders and auto-Ab, and variable response to immunotherapy [1]. Clinical features are stiffness and episodic axial, oro-pharyngeal and limb muscular spasms, due to co-contraction of agonist and antagonist muscles, with continuous motor unit firing at rest: these are thought to reflect spontaneous depolarization of motor neurons [2], probably as consequence of a dysfunction of GABAergic inhibitory pathways mostly in the motor and premotor areas, as suggested by recent positron emission tomography (PET) investigations [3] and magnetic resonance (MR) spectroscopy in vivo [4] .

Evidence that such motor cortex hyperexcitability could be causal for SPS symptoms comes from transcranial magnetic stimulation (TMS) investigations: indeed, TMS can test several variables of cortical excitability, each one reflecting the net cortical state of different physiological mechanisms regulating the excitability of corticospinal neurons at a pre-synaptic level [5, 6]. In SPS, an increased intracortical facilitation (ICF) to paired-pulse TMS correlates positively with anti-GAD circulating auto-Ab levels [7]. Increased ICF is likely the result of a relative hyperglutamatergic tone [8], since it presumably reflects the activity of excitatory cortical interneuronal pools under a powerful GABAergic inhibitory control [8, 9], which is overtly dysfunctional in SPS [1]. While GABA activity can be assessed by neuroimaging techniques [3], novel and reliable PET tracers for Glutamate are under investigations but not yet available for routine clinical applications [10].

Immunotherapy has a central role in SPS therapy and is known to reduce anti-GAD Ab levels in most cases [11]. However, studies investigating the effects of immunotherapy on relationships between cortical excitability variables, clinical features and bio-umoral markers are still lacking.

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Case report

A 39 year-old woman suffered for 2 years from spasm attacks, firstly in the left upper limb and then spreading to the axial district and to all limbs. Treatment with diazepam (20 mg/day) and then with clonazepam (6 mg/day) and baclofen (100 mg/day), had poor symptomatic effect. At the time of symptoms' appearance, serum anti-GAD65 Ab levels were slightly increased (13 IU/ml, upper normal limits 5 IU/ml) (Fig. 1). She was also taking warfarin for an aortic valvular implant. She had no diabetes, ataxia, history of epilepsy, and EEG did not show interictal epileptic activity.

She came to our observation for a severe worsening in frequency of attacks that extended to oro-pharyngeal muscles, with dysphagia, dysphonia and dyspnea. Serum anti-GAD65 Ab were dramatically increased (Fig. 1). The episodic muscular spasms, lasting 10 to 60 min, were spontaneous or reflex to sudden acoustic or tactile stimuli. These also induced exaggerated startle reflex involving limb and trunk muscles. Neuropsychological testing revealed state and trait anxiety with mild depression. Brain and spinal MR images were normal and work-up for malignancies negative. She also had thymic hyperplasia (without clinical signs, electrophysiological and bio-umoral markers suggestive for myasthenia), and increased titres of anti-thyroid Ab without overt clinical signs. Tendon reflexes were symmetrically hyperexcitable.

Due to anticoagulant therapy, diagnostic lumbar puncture and therapeutic plasma exchange were not carried out. A first cycle of intravenous immunoglobulin (IViG) (2 gr/Kg) was administered with transient (2 weeks) improvement of the clinical features and reduction of the anti-GAD Ab value (Fig. 1). Since successive cycles of IViG were clinically ineffective, mycophenolate mofetil (MMF) (2,000 mg/day) and prednisone (25 mg/day) were introduced, with normalization of anti-GAD Ab (Fig. 1) and quite complete disappearance of SPS symptoms. Clinical improvement and normal anti-GAD Ab titres persist after 2 years.

Neurophysiological testing and results

Many neurophysiological testing (Table 1) were carried out before and after immunotherapy (Fig. 1), keeping unchanged symptomatic therapy (clonazepam 6 mg/day, baclofen 100 mg/day). TMS variables were recorded, during monitoring of the silence of the EMG activity in the right arm (in the intervals between SPS attacks), by placing the figure-of-eight coil on the left primary motor cortex and recording motor evoked potentials (MEPs) from the right abductor pollicis brevis (ABP) muscle, according to international guidelines [12].

Resting motor threshold (RMT) (i.e., the percentage of the maximal stimulator output (monophasic Magstim Bi-Stim) eliciting MEPs of at least 50 μV with 50%

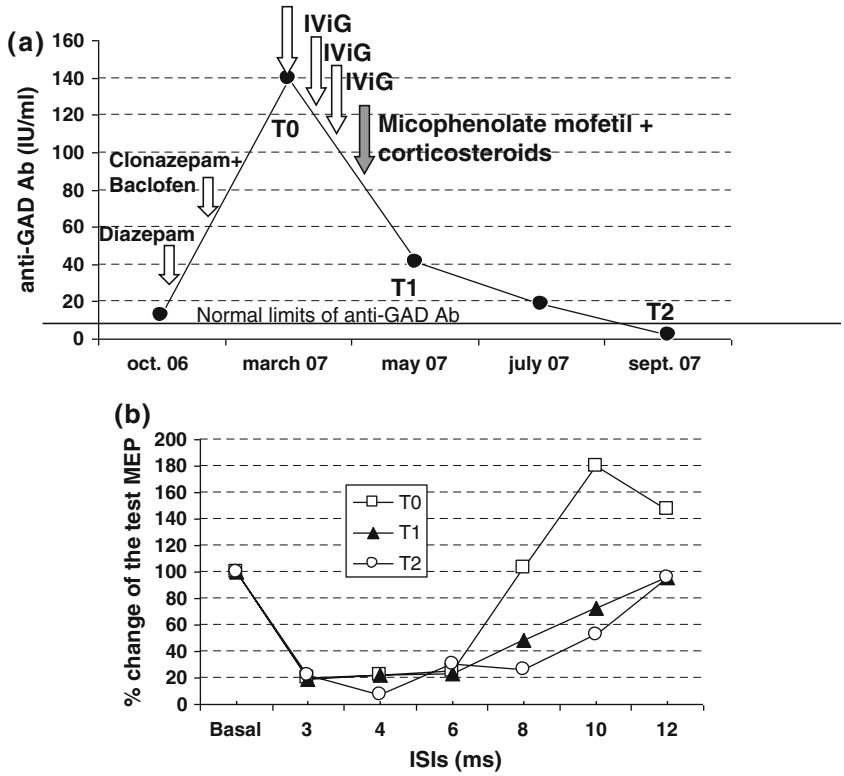

Fig. 1 **a** Timing of neurophysiological recordings in relation to anti-GAD Ab levels, symptomatic therapy and immunotherapy. **b** Paired-pulse TMS curve at T0, T1 and T2. Short-term intracortical inhibition (SICI) at ISIs of 2, 4 and 6 ms is unchanged. Intracortical facilitation (ICF) is dramatically reduced after immunotherapy, at T1 and T2

Table 1 Neurophysiological variables tested at baseline (T0) and at different times following immunotherapy (T1 and T2) according to Fig. 1

Tested variable	T0	T1	T2
SICI-ICF	See Fig. 1 (SICI: 44.6 ± 26.2%; ICF: 168.8 ± 68.8%)	See Fig. 1	See Fig. 1
RMT (% of the stimulator output)	61% (59.9 ± 10.3)	59%	60%
CSP (ms)	74.1 ± 5.8 (83.6 ± 25.3)	98 ± 11.6	91.4 ± 7.7
SAI (% reduction vs. basal MEP)	66% (42.3 ± 21)	65.3%	67.7%
Startle response	Exaggerated	Not done	Normal
F wave (minimal latency, frequency, chronodispersion)	Normal	Normal	Normal
H reflex	Absent	Absent	Absent

Normal values of our lab are reported in parenthesis. They come from a sample of 80 healthy subjects (for RMT), 40 subjects (for CSP), 16 subjects (for SICI-ICF and SAI)

SICI short-term intracortical inhibition, *ICF* intracortical facilitation, *RMT* resting motor threshold, *CSP* cortical silent period, *SAI* short-latency afferent inhibition, *MEP* motor evoked potential

probability [12]) reflecting ion channel function [13], was normal and did not change in time.

For short-latency afferent inhibition (SAI), the conditioning stimulus was an electric pulse delivered on the median nerve at wrist, at an intensity producing a non-painful thumb twitch. The test stimulus was a supra-threshold single TMS pulse evoking, when unconditioned, a stable MEP in the ABP muscle of 800–1000 μV [14]. The interstimulus interval (ISI) between the two stimuli was 21 ms (i.e., 1 ms after the arrival of the peripheral afferent volley to the contralateral sensorimotor cortex). The peak-to-peak amplitudes of five conditioned MEPs were averaged and expressed as a percentage of the average of five test MEPs' amplitude. SAI, which reflects cholinergic mechanisms [14, 15] or their pre-synaptic gabaergic modulation [16] did not change with immunotherapy.

For cortical silent period (CSP), ten single supra-threshold (130% of the RMT) TMS pulses (ISI > 7-s) were delivered on the contralateral motor cortex. These induced a suppression of the EMG activity related to a voluntary maximal contraction. The length of the CSP was determined on the rectified trace from the MEP peak to the reappearance of the EMG activity. The CSP, reflecting long-lasting GABAb-mediated inhibition in its later part [17, 18], was prolonged by immunotherapy.

Short-latency intracortical inhibition (SICI) and intracortical facilitation (ICF) were obtained by paired-pulse TMS with randomized ISIs of 2, 4, 6 (SICI) and 8, 10, 12 (ICF) ms (conditioning stimulus 80% of RMT, test stimulus as for SAI). The peak-to-peak amplitudes of five conditioned MEPs for each ISI were averaged and expressed as a percentage of the average of the five test MEPs amplitude. SICI, which mainly reflects intracortical GABAa-mediated inhibition [9, 13, 19], was unchanged by immunotherapy. ICF, which mainly reflects glutamatergic mechanisms [20, 21], was dramatically reduced after immunotherapy, in parallel with the normalization of anti-GAD Ab.

Classical indices of spinal excitability, obtained with conventional procedures in right median nerve, did not vary: H-reflex was always absent and F-wave parameters (frequency, latency, chronodispersion) were unremarkable.

The startle response, obtained by sudden acoustic stimulation [see 23] and surface EMG recordings from neck, trunk and limb muscles, showed clear reflex responses in all muscle groups at T0, and a normal pattern, involving only neck muscles, at T2.

Discussion

RMT was within normal limits, in keeping with previous studies [7, 22] and not modified by immunotherapy, suggesting that anti-GAD Ab did not influence the membrane excitability of cortical neurons.

The time course of SAI, not yet tested in SPS patients, was similar, therefore suggesting that reduction of anti-GAD Ab levels was uneffective in this sense. The lack of changes might indicate that SAI reflects exclusively cholinergic mechanisms [14, 15], hence not causal for SPS in which cholinergic systems are spared. Otherwise, the concomitant treatment with benzodiazepines and baclofen made GABAergic interneural pools acting on SAI at a presynaptic level [16] more resistant to anti-GAD Ab actions. Indeed, these auto-antibodies ultimately reduce GABA synthesis, therefore simultaneously shifting GABA/Glutamate balance in favor of the latter. Such a relative increase of Glutamate likely leads to dishinibition at cortical level, as suggested by exaggerated startle reflex, shortened CSP, and increased ICF at T0 in comparison to T2, despite the GABAergic chronic treatment. Increased

acoustic startle reflex [23, 24], shortened CSP [7, 22] and increased ICF [7, 22] in untreated SPS patients have been already reported. Moreover, there is a positive correlation between increased ICF and levels of circulating anti-GAD Ab [7]. These data suggest that a relative hyperglutamatergic state, besides the exclusive impairment of GABAergic circuitries, may contribute to the genesis of the clinical picture of SPS.

Counterintuitively, spinal reflexes were normal in this patient, but in keeping with previous larger series of SPS patients [25]. Similarly, it might be argued that, due to the central GABAergic dysfunction in SPS [3, 4], a reduction of ICI levels at T0 (and, possibly, SAI impairment) could be expected. This was not the case, as already found in a previous larger study investigating SICI in SPS [22]: probably, the chronic therapy (5 months, see Fig. 1) with high dosage of GABAmimetic benzodiazepines and baclofen made the ICI and the other GABA-mediated mechanisms tested less vulnerable to the GABAergic dysfunction due to anti-GAD Ab. Withdrawal of these drugs was not attempted before T0 because potentially dangerous [26].

Because of such a pharmacological unavoidable bias, it is difficult to interpret current findings in terms of absolute normality or abnormality. Nevertheless, they can still be informative as to how anti-GAD Ab further modulate cortical excitability mechanisms in the context of a stable pharmacologically induced GABAergic hypertone. The CSP, despite still in the lower limit of normality at T0, was considerably lengthened at T1 and T2 (Table 1). The extremely high amount of SICI at T0 and T1 (i.e., conditioned MEP size about 20% of the basal MEP), was further increased at T2. The ICF, which was lacking at T0 and T1, reappeared at T2. These changes occurred in parallel with the progressive normalization of anti-GAD Ab levels, despite the persistence of the GABAergic hypertone.

MMF, an inosine monophosphate dehydrogenase inhibitor, is an immunosuppressive agent increasingly investigated in controlled studies for some T- and B-cell mediated neurological diseases, such as myasthenia gravis, multifocal motor neuropathy and chronic inflammatory demyelinating polyneuropathy. MMF has been unsuccessfully employed in the treatment of a single SPS patient which later responded to rituximab [27]. In the current case, MMF and corticosteroids were more effective than standard immunomodulating therapy with repeated IViG cycles [11] in reducing anti-GAD Ab levels (see Fig. 1) and in improving the clinical picture. Therefore, MMF could be considered among the potentially useful drugs for treating SPS [28] .

While the decrement of anti-GAD Ab levels following immunotherapy is well established [11], the effects of immunomodulating and immunosuppressive drugs on cortical excitability measures are still lacking. Immunotherapy, by normalizing anti-GAD Ab levels, might have changed GABA/Glutamate balance at cortical level, favoring the former also due to the concomitant GABAergic therapy, as reflected by normalization of the startle reflex, lengthening of the CSP and disappearance of ICF (Table 1).

The evaluation of several indexes of cortical and spinal excitability allowed to infer neurophysiological mechanisms by which immunotherapy may improve symptoms in SPS. Further studies on patients, possibly free from GABAergic drugs, are required to substantiate this single but original observation.

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