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Axonal dysfunction with voltage gated potassium channel complex antibodies

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ABSTRACT

Objective: Although autoantibodies targeted against voltage-gated potassium channel (VGKC)-associated proteins have been identified in limbic encephalitis (LE) and acquired neuromyotonia (aNMT), the role of these antibodies in disease pathophysiology has not been elucidated. The present study investigated axonal function across the spectrum of VGKC–complex antibody associated disorders.

Methods: Peripheral axonal excitability studies were undertaken in a cohort of patients with LE (N = 6) and aNMT (N = 11), compared to healthy controls (HC; N = 20).

Results: Patients with LE demonstrated prominent abnormalities in peripheral axonal excitability during the acute phase, with reduced threshold change in threshold electrotonus (depolarizing 10–20 LE: 58.5 ± 3.1%; HC: 67.4 ± 0.9%; P < .005; S2 accommodation LE: 17.2 ± 1.4%; HC: 22.2 ± 0.6%; P ≤ .005) and in recovery cycle parameters (superexcitability LE: −16.0 ± 0.9%; HC: −23.4 ± 1.1%; P < .01; subexcitability LE: 8.5 ± 1.2%; HC: 13.8 ± 0.7%; P ≤ .005). The pattern of change in LE patients was dissimilar to the effects of antiepileptic medications, suggesting that these factors did not underlie excitability changes in LE. Normalization of excitability parameters was associated with recovery (TEd peak correlation coefficient = .868; P = .002), suggesting that peripheral excitability studies may provide a marker associated with clinical improvement. In contrast, patients with aNMT demonstrated no significant changes at the site of stimulation.

Conclusions: The lack of prominent excitability abnormalities in patients with aNMT likely reflects a distal origin of hyperexcitability, expected to be at the motor nerve terminal, while the prominent changes observed in patients with LE likely represent a complex disturbance at the level of the axonal membrane, combined with electrolyte imbalance and adaptive change.

Introduction

Voltage-gated potassium channels (VGKCs) are critical in modulating neuronal excitability through stabilizing membrane potential, repolarizing the nodal membrane and limiting repetitive discharges (Kiernan et al., 2001; Miller et al., 1995; Vucic et al., 2010). A number of autoimmune conditions have been linked to the production of antibodies targeting VGKC-complex components, including neuromyotonia, Morvan's syndrome, limbic encephalitis and forms of epilepsy (Hart et al., 1997; Newsom-Davis and Mills, 1993; Vincent et al., 2004). However, these disorders have a broad clinical spectrum, with varying degrees of central nervous system (CNS) and peripheral nervous system (PNS) involvement.

Limbic encephalitis (LE) is characterized by CNS-predominant symptoms including memory disturbance, hallucinations, and seizures, coupled with evidence of inflammation in the mesial temporal lobes or hippocampi (Vincent et al., 2004; Zuliani et al., 2012). By contrast, acquired autoimmune neuromyotonia (aNMT) is characterized by predominantly PNS involvement, including spontaneous muscle twitching, cramps and fatigue (Hart et al., 1997; Isaacs, 1961; Newsom-Davis and Mills, 1993), although many patients also exhibit some CNS disturbance (Hart et al., 2002).

Some of this clinical heterogeneity may be explained by recent findings identifying specific antigenic targets in patients with VGKC-associated autoantibodies. Specifically, antibodies against leucine-rich

Abbreviations: aNMT, acquired autoimmune neuromyotonia; CASPR2, contactin associated protein 2; CMAP, compound muscle action potential; EMG, electromyography; HC, healthy controls; LE, limbic encephalitis; LGI1, leucine rich inactivated glioma 1; TE, threshold electrotonus; VGKC, voltage gated potassium channel.

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glioma inactivated 1 (LGI1) have been identified in LE patients (Irani et al., 2010, 2011; Lai et al., 2010) and contactin-associated protein-2 (CASPR2) antibodies in some patients with aNMT and Morvan's syndrome (Irani et al., 2010, 2012; Vincent and Irani, 2010). Both LGI1 and CASPR2 are VGKC-complex associated proteins rather than components of the channel itself (Irani et al., 2010; Poliak et al., 1999). Accordingly, the role of direct VGKC dysfunction in the pathophysiology of these disorders remains unclear, although IgG preparations obtained from clinically affected patients have been reported to interfere with VGKC function and suppress fast K^+ currents in vitro (Lalic et al., 2011; Shillito et al., 1995). The aim of the present study was to investigate axonal membrane ion channel function across the spectrum of VGKC-complex antibody associated disorders in vivo.

Methods

Patient selection

Patients with LE were identified following their acute admission for seizures, short term memory loss and behavioral changes. The study was approved by South Eastern Sydney Area Health Service (Eastern Section) Human Research Ethics Committee and University of New South Wales Human Research Ethics Committee. Participants or family members provided written informed consent in accordance with the declaration of Helsinki.

Patients were diagnosed with LE determined by positive VGKC-complex antibody assays, MRI evidence of signal abnormalities or PET evidence of increased glucose metabolism in the medial temporal lobe. At the time of nerve excitability testing, all acute phase patients were receiving antiepileptic drugs — all patients were receiving sodium valproate (600 mg–1500 mg), and in addition two patients were receiving levetiracetam (1.5–2 g) and one patient carbamazepine (400 mg). During the 26 month follow-up period, LE patient 1 continued to be treated with sodium valproate and levetiracetam on all nerve excitability testing occasions. LE patients 5 and 6 were not receiving antiepileptic drugs at the time of excitability testing.

Results from LE patients were contrasted to studies in patients with a diagnosis of acquired autoimmune neuromyotonia (aNMT) according to previously established clinical criteria, with all patients displaying symptoms of muscle twitching or cramps in two or more skeletal muscle regions (Hart et al., 2002). In addition, EMG criteria were applied, including the presence of doublet, triplet or multiplet neuromyotonic discharges or fasciculations (Maddison et al., 1999). At the time of testing, five patients were receiving immunomodulatory treatment with IVIg, plasmapheresis, azathioprine or prednisone, four were receiving symptomatic relief with amitryptiline, two with gabapentin, two with carbamazepine and one with pregabalin.

Neurophysiological studies

Patients underwent nerve conduction studies using a Medelec synergy system (Oxford Instruments, Oxfordshire, UK). Nerve excitability studies were performed on the median nerve, stimulating at the wrist and recording compound motor action potentials (CMAPs) from the abductor pollicis brevis. Standard nerve excitability protocols and equipment included the computerized operational system Qtrac (©Institute of Neurology, London UK), with stimulation provided by the DS5 isolated linear bipolar constant current stimulator (Digitimer, Welwyn Garden City, UK).

Multiple excitability measures were recorded using threshold tracking techniques (Bostock et al., 1998), following a standardized protocol (strength–duration time constant, threshold electrotonus (TE), and recovery cycle) as described in Kiernan et al. (2000). Patient excitability results were compared to a healthy control group (N = 20; 53.4 ± 3.5 years), matched by age and temperature. A complete excitability profile was recorded for each participant, although only significant findings are discussed in the results. Threshold electrotonus parameters are described in terms of the direction: depolarising (TEd) or hyperpolarizing (TEh), and the timing after onset of the current pulse (i.e. 10–20 ms, or 40–60 ms). In addition TEd peak referred to the maximal threshold change (%) after onset of the depolarizing current pulse and S2 accommodation referred to the change in threshold between TEd peak and the TEd 90–100 ms. TEh overshoot and TEd undershoot were the peak threshold changes after the cessation of the polarizing current in both the hyperpolarizing and depolarizing directions. Superexcitability was the greatest threshold decrease in the recovery cycle of excitability, while subexcitability was the greatest threshold increase after an interstimulus interval of 10 ms. Strength duration time constant was measured as the relationship between stimulus width and stimulus strength, and calculated offline according to Weiss' law (Kiernan et al., 2000).

Mathematical model of motor axonal excitability

Excitability simulations using a mathematical model of human motor axonal excitability were undertaken. The two-compartment model, composed of a single node and internode, was implemented by MEMFIT software (Bostock, 2006) (described by Howells et al., 2012). Briefly, iterative alterations to reduce the discrepancy between the model and experimental data were undertaken using a least squares method (Howells et al., 2012).

Immunological studies

Serum samples from all patients were assessed by a standardized immunoprecipitation assay with ^{125}I-α-dendrotoxin-labeled rabbit brain extract designed to detect antibodies to $K_v1.1$, $K_v1.2$ and $K_v1.6$ channels, or proteins complexed with these channels, as reported previously (Irani et al., 2010). VGKC antibody titres were considered positive if greater than 100 pmol/L. Some of the patient cohort (2 of 6 LE patients; 9 of 11 aNMT patients) also underwent testing for specialized antigenic targets (LGI1, CASPR2, or contactin-2 antibodies Irani et al., 2011).

Statistical analysis

Data are presented as mean ± SEM. Independent T-tests were used to compare patient and healthy control results. Correlations were undertaken using Pearson product-moment correlation coefficients. Linear regression analysis was used to examine relationships between VGKC-complex antibody titres, serum electrolytes and excitability parameters and results are presented as coefficients of determination. In light of the multiple comparisons, a P value of <0.01 was considered significant (Kiernan et al., 2001). QTrac and SPSS (version 19, IBM, New York, NY) software packages were used for analysis.

Results

Clinical features and antigenic specificity

Patients with LE all demonstrated confusion, seizures and evidence of hyponatremia (due to syndrome of inappropriate antidiuretic hormone secretion; Table 1). A positive VGKC-complex antibody titre (>100 pM) was identified in each of the LE patients. Two LE patients who underwent testing for LGI1 antibodies (Irani et al., 2011) were found to be positive (patients 1 and 3). The other LE patients were unable to be tested for specialized antigenic targets due to unavailability of the testing at the time of presentation. Five of the LE patients received three days of pulse intravenous methylprednisolone, which produced a reduction in seizures, associated with improvements in memory and normalization of hyponatremia. Treatment was associated with