doi:10.1093/brain/awl084 Brain (2006), **129**, 1570–1584

Neuromyotonia and limbic encephalitis sera target mature *Shaker*-type K^+ channels: subunit specificity correlates with clinical manifestations

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Autoantibodies to *Shaker*-type (Kv1) K^+ channels are now known to be associated with three syndromes. Peripheral nerve hyperexcitability is the chief manifestation of acquired neuromyotonia; the combination of neuromyotonia with autonomic and CNS involvement is called Morvan's syndrome (MoS); and CNS manifestations without peripheral involvement is called limbic encephalitis (LE). To determine the cellular basis of these clinical manifestations, we immunostained mouse neural tissues with sera from patients with neuromyotonia (n = 10), MoS (n = 2) or LE (n = 5), comparing with specific antibodies to relevant K^+ channel subunits. Fourteen of 17 patients' sera were positive for Kv1.1, Kv1.2 or Kv1.6 antibodies by immunoprecipitation of ^{125}I-α-dendrotoxin-labelled rabbit brain K^+ channels. Most sera (11 out of 17) labelled juxtaparanodes of peripheral myelinated axons, co-localizing with Kv1.1 and Kv1.2. In the CNS, all sera tested (n = 12) co-localized with one or more areas of high Kv1.1, Kv1.2 or Kv1.6 channel expression: 10 out of 12 sera co-localized with Kv1.1 and Kv1.2 at spinal cord juxtaparanodes or cerebellar layers, while 3 out of 12 sera co-localized additionally (n = 2) or exclusively (n = 1) with Kv1.6 subunits in Purkinje cells, motor and hippocampal neurons. However, only sera from LE patients labelled the hippocampal areas that are enriched in excitatory, Kv1.1-positive axon terminals. All sera (17 out of 17) labelled one or more of these Kv1 subunits when expressed at the cell membrane of transfected HeLa cells, but not when they were retained in the endoplasmic reticulum. Again, LE sera labelled Kv1.1 subunits more prominently than did MoS or neuromyotonia sera, suggesting an association between higher Kv1.1 specificity and limbic manifestations. In contrast, neuromyotonia sera bound more strongly to Kv1.2 subunits than to Kv1.1 or Kv1.6. These studies support the hypothesis that antibodies to mature surface membrane-expressed *Shaker*-type K^+ channels cause acquired neuromyotonia, MoS and LE, and suggest that future assays based on immunofluorescence of cells expressing individual Kv1 subunits will prove more sensitive than the immunoprecipitation assay. Although more than one type of antibody is often detectable in individual sera, higher affinity for certain subunits or subunit combinations may determine the range of clinical manifestations.

Keywords: ion channels; juxtaparanodes; myokymia; Morvan's syndrome; hippocampus

Abbreviations: DAPI = 4′,6′-diamidino-2-phenylindole; DG = dentate gyrus; ER = endoplasmic reticulum; LE = limbic encephalitis; MoS = Morvan's syndrome

Received December 5, 2005. Revised March 8, 2006. Accepted March 13, 2006. Advance Access publication April 13, 2006

Introduction

Neuromyotonia is a syndrome of continuous muscle fibre activity and is usually an acquired disorder characterized by variable onset ranging from childhood to adult life, fasciculations, exercise-induced myokymia, pain, stiffness and muscle cramps (Newsom-Davis and Mills, 1993; Auger, 1994; Newsom-Davis, 1997; Vincent, 2000). Some patients

may develop autonomic or sensory manifestations, such as excessive sweating, laryngeal spasms, paresthesias and numbness. The combination of neuromyotonia and CNS manifestations including confusion, anxiety, agitation, delirium or insomnia is called Morvan's syndrome (MoS) (Lee *et al.*, 1998; Liguori *et al.*, 2001). Antibodies to *Shaker*-type K^+ channels (the Kv1 family) are strongly associated with acquired neuromyotonia and MoS (Shillito *et al.*, 1995; Newsom-Davis, 1997; Newsom-Davis *et al.*, 2003). They are detectable in ~50% of sera from neuromyotonia patients using the ^{125}I-α-dendrotoxin immunoprecipitation assay, which detects Kv1.1, Kv1.2 and Kv1.6, and in >85% using molecular/immunohistochemical assays (Hart *et al.*, 1997). Interestingly, the same antibodies are also detectable in patients with a newly defined form of limbic encephalitis (LE), who usually lack the manifestations of neuromyotonia (Buckley *et al.*, 2001; Vincent *et al.*, 2004; Thieben *et al.*, 2004).

Three distinct voltage-gated K^+ conductances have been recorded by patch clamping of myelinated axons in the mammalian PNS. The most prominent is a slowly activating current, K_s (Roper and Schwarz, 1989; Safronov *et al.*, 1993), ascribed to KCNQ2, which is localized to nodes of Ranvier (Devaux *et al.*, 2004). Kv3.1b may generate the fast nodal current, K_{f2} (Corrette *et al.*, 1991; Devaux *et al.*, 2003). Kv1.1 and Kv1.2, which are co-expressed at juxtaparanodes (Wang *et al.*, 1993; Arroyo *et al.*, 1999), account for the K_i (Safronov *et al.*, 1993; Reid *et al.*, 1999). All may contribute to the regulation of axonal excitability. Likewise, K^+ channels play a major role in regulating the excitability of hippocampal neurons. They modulate neurotransmitter release, post-synaptic responses to excitatory inputs, neuronal spike properties and firing frequency (Johnston *et al.*, 2000). At least six different *Shaker*-type K^+ channels (Kv1.1–Kv1.6) are expressed in the hippocampus (Sheng *et al.*, 1994; Wang *et al.*, 1994; Rhodes *et al.*, 1997; Monaghan *et al.*, 2001), and subunit composition varies across subfields (Southan and Owen, 1997; Monaghan *et al.*, 2001).

In neuromyotonia, the repetitive electrical muscle activity can be abolished by neuromuscular blocking agents, and more variably by nerve block, indicating that hyperexcitability originates along the peripheral nerve (Isaacs, 1961; Newsom-Davis and Mills, 1993), probably distally (Arimura *et al.*, 2005). The antibodies do not appear to affect the function of *Shaker*-type K^+ channels directly but, when applied for several hours, they reduce K^+ channel current amplitudes in cultured neuronal cell lines (Sonoda *et al.*, 1996; Nagado *et al.*, 1999), probably by increasing channel turnover (Tomimitsu *et al.*, 2004). The antibodies appear to be heterogeneous, binding to Kv1.1, Kv1.2 and Kv1.6 (Hart *et al.*, 1997, 2002; Newsom-Davis, 1997); this may be the molecular basis for the clinical diversity of the syndrome (Vincent, 2000). However, no correlation has been established between the clinical manifestations and the subunit specificity of the antibodies. To determine the targets of antibodies in the PNS and CNS and their correlation with clinical manifestations, we examined the binding of sera from 17 patients with neuromyotonia, MoS or LE to myelinated PNS axons and in clinically relevant regions of the CNS. Subunit specificity was also determined by binding to transfected HeLa cells.

Patients and methods

Patients

The clinical characteristics of the patients with acquired neuromyotonia, MoS or autoimmune LE, some of whom have been previously reported, are summarized in Table 1. Antibody titers were measured by immunoprecipitation of ^{125}I–α-dendrotoxin-labelled *Shaker*-type K^+ channels as described previously (Shillito *et al.*, 1995; Hart *et al.*, 1997).

Immunohistochemistry

Adult mice were deeply anaesthetized according to institutionally approved protocols with intraperitoneal injection of Avertin (Aldrich, Milwankee, WI, USA), and then transcardially perfused with 0.9% NaCl followed by fresh 4% paraformaldehyde in 0.1 M phosphate buffer. The sciatic nerves, lumbar spinal cord and brains were removed and placed in the same fixative for 30 min. Teased fibres were prepared from one nerve; the remaining tissues were cryoprotected by infiltration in 20% sucrose overnight, embedded in optimum cutting temperature (OCT) and immediately frozen in a dry ice–acetone bath. Unfixed frozen sections or teased fibres were prepared by immediately embedding tissues in OCT or by teasing the fibres. Ten-micron thick cryostat sections were thaw-mounted on SuperFrost Plus glass slides (Merzel, Braunschweig, Germany) and stored at −20°C. Teased fibres and sections were permeabilized by immersion in −20°C acetone for 10 min, blocked at room temperature for 1 h in 5% bovine serum albumin (BSA) containing 0.5% Triton X-100 in phosphate-buffered saline (PBS) and incubated overnight at 4°C with each of the sera from neuromyotonia/MoS/LE /LE patients or normal controls (diluted 1 : 350) and rabbit antisera against Kv1.1, Kv1.2, Kv1.4 or Kv1.6 (Alomone Labs, Jerusalem, Israel). Slides were washed and incubated with the appropriate fluorescein- and rhodamine-conjugated donkey cross-affinity purified secondary antibodies (diluted 1 : 100; Jackson ImmunoResearch, West Grove, PA, USA) for 1 h. Cell nuclei were visualized with 4′,6′-diamidino-2-phenylindole (DAPI). Slides were covered with mounting medium (Dako, Glostrup, Denmark) and images were photographed under a Zeiss fluorescence microscope with a digital camera using the Zeiss Axiovision software.

Cell transfections

Communication-incompetent HeLa cells were grown in low-glucose Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and antibiotics (100 μg/ml penicillin/streptomycin) in a humidified atmosphere containing 5% CO_2 at 37°C to achieve 80% confluency by the day of transfection. For transient transfection, FuGENE 6 Transfection Reagent (Roche, Basel, Switzerland) and DNA containing the *KCNA1/Kv1.1*, *KCNA2/Kv1.2* or *KCNA6/Kv1.6* open reading frame subcloned in the pcDNA3 vector (gifts from Prof. Olav Pongs) were incubated in Optimem (Gibco, Paisley, UK) according to manufacturer's instructions (ratio 6 : 1). In further experiments, in order to promote surface expression, Kv1.1 and Kv1.2 were co-transfected with a rat *Kcna4/Kv1.4* construct, and

Table 1 Clinical data of the patients with LE, MoS or neuromyotonia

Patient	Age/sex	Diagnosis	Kv1 antibody titers[a] (pM)	Outcome/response to therapy	Previous report
1	44M	LE	1958	Improvement	Vincent *et al.* (2004)
2	69F	LE	7082	NK	
3	76M	LE	4700	Improved cognitively but died of cardiac problems	Vincent *et al.* (2004)
4	66M	LE	1645	Some improvement with immunosuppression	
5	56M	LE	1635	Good response to therapy	Vincent *et al.* (2004)
6	76M	MoS	4835	Improved initially with PE, died 11 months later. A small lung tumour found at autopsy	Liguori *et al.* (2001)
7	41M	MoS	516	Improved with IgG immunoadsorption and cyclophosphamide	Antozzi *et al.* (2005)
8	NK	NMT	<100	Good response to PE	M. Bonifati (unpublished data)
9	35F	NMT[b]	3047	Improved with PE	C. Buckley (unpublished data)
10	45F	NMT	247	Good response to PE and immunosuppression	Shillito *et al.* (1995) Hart *et al.* (1997)
11	70F	NMT	388	Good response to PE and immunosuppression	Hart *et al.* (2002)
12	42M	NMT	<100	Good response to PE	Hart *et al.* (2002)
13	74M	NMT	112	NK	Hart *et al.* (2002)
14	34F	NMT	130	NK	
15	18F	NMT	<100	Good response to PE	N. Wood (unpublished data)
16	50F	NMT	218	Not available	
17	18F	NMT	160	Improved with PE and carbamazepine	Hart *et al.* (2002)

NMT = neuromyotonia; MoS = Morvan's syndrome; LE = limbic encephalitis; PE = plasma exchange; NK = not known. [a]Normal: <100 pM; [b]some anxiety was present before treatment but neuropsychology and MRI were normal.

Kv1.6 was co-transfected with a rat *Kcnab2/Kvβ2* construct (DNA ratio Kv1.1/Kv1.2 to Kv1.4 and Kv1.6 to Kvβ2 1 : 4), both in the pRGB4 vector, provided by Dr James Trimmer (Manganas and Trimmer, 2000). Cells were also transfected with *Kcna4/Kv1.4* or *Kvβ2* alone as a control. After incubation with transfection complex for 40–48 h cells were washed and processed for immunocytochemistry or immunoblotting.

Immunoblots

Tissues were harvested from freshly killed mice and either frozen or lysed directly in ice-cold 50 mM Tris, pH 7.0, 1% SDS, and 0.1 mM phenylmethylsulphonyl fluoride (PMSF) (Sigma, St. Louis, MO, USA), followed by a brief sonication on ice (Fisher Scientific, Houston, TX, USA). For cell lysates, transfected and untransfected HeLa cells were washed in cold PBS and then lysed (ice-cold 0.01 M Tris–HCl, pH 7.4, 0.1 M NaCl, 0.002 M EDTA, 0.5% NP40 and 0.5 mM PMSF). Proteins (25 μg from tissue; 100 μg from cells) were electrophoresed on a 12% SDS–polyacrylamide gel and transferred to a Hybond-C extra membrane (Amersham, Piscataway, NJ, USA) over 1 h using a semi-dry transfer unit (Pharmacia Biotech, UK). After blocking (5% powdered skim milk and 0.5% Tween-20 in Tris-buffered saline) for 1 h the blots were incubated overnight at 4°C with either human sera (1 : 500–1 : 5000), mouse monoclonal antibodies against Kv1.1, Kv1.2 or Kvβ2 (Upstate Biotechnologies, Lake Placid, NY, USA), or rabbit antisera against Kv1.1, Kv1.2, Kv1.4, Kv1.6 (Alomone Labs, 1 : 500–1000) or Kvβ2 (Abcam, Cambridge, UK). After washing and incubation in peroxidase-coupled secondary antibodies (Jackson, diluted 1 : 3000–5000), the blots were visualized by enhanced chemiluminescence (Amersham).

Immunocytochemistry

For immunocytochemistry, HeLa cells grown in 4-chamber slides (Nalge Nunc, Hereford, UK) were washed in PBS, fixed in acetone at −20°C for 10 min and blocked with 5% BSA in PBS containing 0.1% Triton for 1 h. Rabbit antisera against Kv1.1, Kv1.2, Kv1.4, Kv1.6 (Alomone, diluted 1 : 200) or Kvβ2 (Abcam, diluted 1 : 300) were combined with each of the sera from neuromyotonia/LE/MoS patients or normal controls (diluted 1 : 350), or mouse anti-calnexin (Abcam, diluted 1 : 100) in blocking solution. Cells were incubated overnight at 4°C, washed, incubated with appropriate secondary antibodies and imaged as above. In addition, confocal microscopy (Leica DMR, Leica Microsystems, Heidelberg) was used to confirm co-localization of some sera with Kv subunits in HeLa cells. To determine whether human sera labelled the expressed subunits, in each experiment with Kv1.1/Kv1.4, Kv1.2/Kv1.4 or Kv1.6/Kvβ2 double transfection, as well as Kv1.4 or Kvβ2 single transfection, at least 50 cells expressing the respective subunit at the cell membrane were examined.

To demonstrate that co-transfected Kv1 alpha subunits were localized on the cell surface, cells expressing the same subunit combinations as above were not permeabilized (no triton, no acetone), but were fixed in 2% paraformaldehyde followed by incubation with some of the human sera (diluted as above) combined with a fluorescein-conjugated ShK toxin (200 nM; Bachem AG, Bubendorf, Switzerland), which binds with high affinity to the outer vestibule of Kv1 channels. Cells were washed, incubated with rhodamine-conjugated anti-human secondary antibodies and visualized as above.

Statistical analysis

The percentage of cells showing double labelling with the respective specific rabbit antiserum and the human serum was determined for each of the patient and control sera. The mean binding (%) and standard error was obtained from three independent experiments. The mean % binding of LE sera to different Kv1 subunits was compared with that of neuromyotonia sera using the Mann–Whitney *U*-test. The Wilcoxon Signed Ranks Test was used to compare the % binding of LE or neuromyotonia sera to different types of Kv1 subunits.

Results

We immunostained sciatic nerve teased fibres, spinal cord, cerebellum and hippocampus from adult mouse with sera from patients with neuromyotonia, MoS or LE (Table 1). In each case, we combined the human sera with rabbit antiserum against different Kv1 subtypes and compared with sera from five normal controls. All experiments were performed at least three times. Representative results, as shown in the figures, were scored (−, +, ++, +++) and the results summarized in Table 2. All sera were tested on peripheral myelinated axons, but five neuromyotonia sera were not tested on the other tissues.

Neuromyotonia, MoS and LE sera co-localize with Kv1.1/Kv1.2 in peripheral myelinated axons

Antibodies to Kv1.2 labelled the juxtaparanodal region of myelinated axons as expected (Wang *et al.*, 1993; Arroyo *et al.*, 1999). Kv1.1 was also present at juxtaparanodes, but not Kv1.6 or Kv1.4 (data not shown). Control sera showed only background staining, whereas sera from LE (5 out of 5), MoS (1 out of 2) and neuromyotonia (5 out of 10) patients showed different degrees of staining in the juxtaparanodal region (Fig. 1 and Table 2). Similar findings were obtained with sections of fixed mouse sciatic nerve as well as teased fibres and sections of unfixed nerves (data not shown). As described with the rabbit Kv1.2 antiserum (Arroyo *et al.*, 1999), the sera that showed the strongest juxtaparanodal labelling also labelled the juxtamesaxons (aligned with the glial inner mesaxon) and the juxtaincisures (apposing the incisures of the myelin sheath), and co-localized with Kv1.1 and Kv1.2 (Rasband *et al.*, 1998; Arroyo *et al.*, 1999).

Neuromyotonia, MoS and LE sera co-localize with Shaker-type K^+ channels in the CNS

For binding to juxtaparanodes in the CNS, we double-labelled mouse spinal cord and brain with human sera and rabbit antisera against Kv1.1, Kv1.2, Kv1.4 or Kv1.6. In the spinal cord, Kv1.1 and Kv1.2 subunits were localized at the juxtaparanodes of myelinated axons (Fig. 2A and B), including large diameter fibres in the white matter and smaller diameter fibres in the white and grey matter (Arroyo *et al.*, 2001). Kv1.6 was expressed in the perikarya of neurons in the grey matter (Fig. 2C and D) as described previously (Matus-Leibovitch *et al.*, 1996), while Kv1.4 was expressed at low levels mainly in the white matter (data not shown). In the white matter, S3, S6 and S8 showed a consistent and strong juxtaparanodal staining, co-localizing with Kv1.1 and Kv1.2 (Table 2; Fig. 2B and data not shown), while other sera showed weaker or absent staining. In the grey matter, S7 labelled motoneuron perikarya, overlapping with Kv1.6-immunoreactivity (Fig. 2D). Weak staining of motoneurons was also seen with serum S8, while all other sera showed background staining (data not shown).

Since Kv1.1, Kv1.2 and Kv1.6 are distinctly localized in cerebellar layers, we also immunostained sections of mouse cerebellum. As reported previously (McNamara *et al.*, 1993; Sheng *et al.*, 1994; Laube *et al.*, 1996; Koch *et al.*, 1997; Chung *et al.*, 2001), Kv1.2 was more prominent than Kv1.1 in the pinceau, the basket cell terminals surrounding the Purkinje cell initial segments; Kv1.1 (but not Kv1.2) was also localized to the granule cell layer; Kv1.6 was mainly localized at the Purkinje cell perikarya and weakly in the granular cell layer; Kv1.1 and, less prominently, Kv1.2 and Kv1.6 were localized in the molecular layer; Kv1.4 was expressed weakly at the Purkinje cell layer, much less in the other layers, and not in pinceau (Fig. 3, Supplementary Fig. 8A and data not shown).

S1–6 and S9 consistently labelled pinceau co-localizing with Kv1.1 and Kv1.2 (Fig. 3B and C), while S8 and S10–12 showed weak or no detectable labelling. Three sera labelled the Purkinje cell perikarya, co-localizing with Kv1.6 subunits (Fig. 3D and E and data not shown). Many sera, mostly from LE and MoS patients, also labelled the granule cell layer and the molecular layer where Kv1.1 and, to a lesser degree, Kv1.6 are expressed. These results suggest that binding by many sera, particularly from LE patients, can be accounted for by Kv1.1. However, S7 from a MoS patient appeared to co-localize selectively with Kv1.6.

Only sera from LE patients bind to hippocampal axon terminal areas

As in previous reports, Kv1.1, Kv1.2 and Kv1.4 were mostly concentrated in subfields with excitatory axon terminals: in the molecular layer of the dentate gyrus (DG), corresponding to the terminals of the medial perforant path; in the mossy fibre zone of CA3 where granule cell axons project; and in the stratum radiatum of CA3 and CA1, where the Schaffer collaterals terminate (Sheng *et al.*, 1993, 1994; Wang *et al.*, 1994; Rhodes *et al.*, 1997; Monaghan *et al.*, 2001). Kv1.1 was more prominently expressed in the mossy fibre zone of CA3, where Kv1.2 is absent, and in the stratum radiatum and oriens of CA1. Kv1.6-immunoreactivity was mainly found in the perikarya of granular and pyramidal cells and in the stratum radiatum in the whole CA area (Figs 4 and 5, Supplementary Fig. 8B and data not shown).

Sera S1–5, all from LE patients, labelled specifically the hippocampal axon terminal areas, co-localizing with Kv1.1, but also partly overlapping with Kv1.2 (Table 2). In the DG, for example, all LE sera labelled the middle third of the molecular layer, co-localizing with Kv1.1 and Kv1.2, but also the inner third where Kv1.1, but not Kv1.2, is expressed (Fig. 4B and C and data not shown). In CA3, all LE sera (especially S4 and S5) labelled the mossy fibre layer, which expresses Kv1.1 but not Kv1.2 (Monaghan *et al.*, 2001) (Fig. 5C and D). In contrast, the neuromyotonia patients' sera did not show any specific labelling of these hippocampal areas, whereas S7 showed selective staining of neuronal

Table 2 Binding of LE, MoS and neuromyotonia sera to different areas of the nervous system

Patient[a]/phenotype	Sciatic nerve	Spinal cord		Cerebellum				Hippocampus								
	JP	WM- JP	GM-LMN	GC	Pinceau	PC	Mol	DG			CA3			CA1		
								Mol-ot/mt	Mol-it	GC	Py	MF	Rd	Or	Py	Rd
	Kv1.1[b] Kv1.2	Kv1.1 Kv1.2	Kv1.6> Kv1.2	Kv1.1 Kv1.6	Kv1.2> Kv1.1	Kv1.6	Kv1.1 Kv1.6 Kv1.2	Kv1.1 Kv1.2 Kv1.4	Kv1.1 Kv1.4	Kv1.6> Kv1.1	Kv1.6> Kv1.1 Kv1.2	Kv1.1 Kv1.4	Kv1.2 Kv1.1 Kv1.4	Kv1.1 Kv1.4 Kv1.2	Kv1.6> Kv1.1	Kv1.1 Kv1.4 Kv1.2
1/LE	++	+	−	++	++	−	+	+	+	+	+	+	−	+	+	−
2/LE	+	+	−	++	+++	−	+	+	+	−	−	+	−	+	−	+
3/LE	+++	+++	−	++	+++	−	++	+	++	++	+	+	++	++	+	+
4/LE	+	−	−	++	++	−	++	++	++	++	+	+++	+	+	+	+++
5/LE	+	−	−	+	++	−	+	++	++	++	+	++	+	+	++	−
6/MoS	+++	+++	−	++	++	−	++	++	+	−	−	−	−	−	−	−
7/MoS	−	−	+++	+	−	+++	+	−	−	+++	+++	−	−	−	+++	−
8/NMT	++	++	+	+	+	++	−	−	−	−	−	−	−	−	+	−
9/NMT	++	−	−	+	++	+	+	−	−	−	−	−	−	−	−	−
10/NMT	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11/NMT	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−
12/NMT	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
controls (n=5)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

JP = juxtaparanodes; WM = white matter; GM = grey matter; LMN = lower motoneurons; PC = Purkinje cells; GC = granule cell layer; Mol = molecular layer (ot: outer third; mt: middle third; it: inner third); DG = dentate gyrus; Py = pyramidal cell layer; MF = mossy fibre zone; Or = stratum oriens; Rd = stratum radiatum. [a]This table does not include NMT patients 13–17, whose sera were negative for juxtaparanodal staining and were not studied further for tissue binding; [b]the *Shaker*-type K^+ channel alpha subunits prominently expressed in each area are indicated. +++: very strong labelling; ++: consistent labelling; +: weak or partial labelling; −: no difference from normal control /background labelling only.

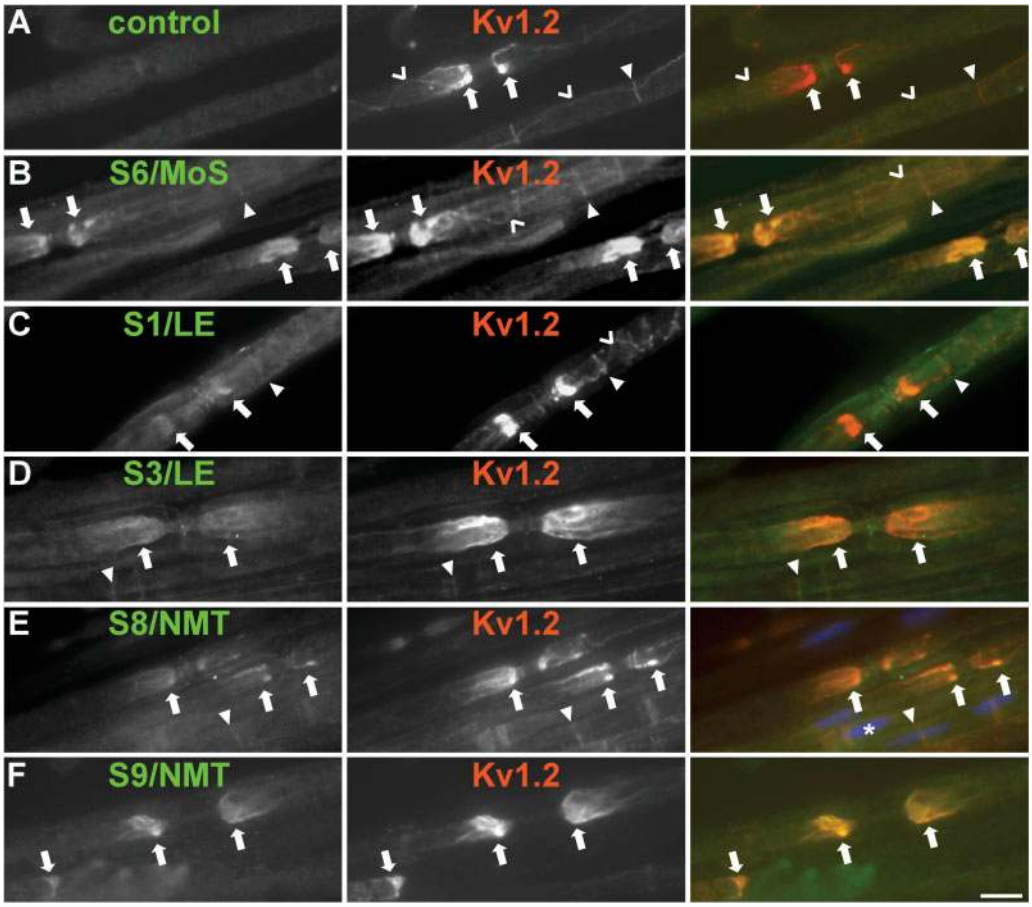

Fig. 1 Neuromyotonia, MoS and LE sera bind to juxtaparanodes of PNS myelinated axons. These are images of paraformaldehyde (PFA)-fixed teased fibres from adult mouse sciatic nerve, labelled with sera from a normal/control (**A**), or patients with MoS (**B**), LE (**C** and **D**) or neuromyotonia (NMT) (**E** and **F**) (green; left column), a rabbit antiserum against Kv1.2 (red; middle column), and DAPI (blue; right column) to visualize Schwann cell nuclei (asterisk). Merged images are shown in the right column. Kv1.2-immunoreactivity is present at juxtaparanodes (arrows), juxtaincisures (arrowheads) and juxtamesaxons (open arrowheads) of myelinated axons. The MoS, LE and NMT sera all label juxtaparanodes, and some label juxtaincisures, whereas the normal control serum only shows minimal staining. Scale bar = 10 μm.

Immunoblot analysis of neural tissues and HeLa cells

To investigate further the antigenic targets of the LE, MoS and neuromyotonia sera, we performed immunoblots of adult mouse brain, spinal cord and sciatic nerve, probed with specific antisera to Kv1.1, Kv1.2, Kv1.4, Kv1.6 and Kvβ2 subunits, as well as with nine patients' sera (S1–9), which were all high-titered (516–7082 pM) except for S8. We also immunoblotted extracts of HeLa cells transfected with specific Kv subunits (*see* below). None of the multiple bands seen on the immunoblots corresponded to those recognized by the rabbit Kv1-specific antisera and no patient's serum showed evidence of specific binding (Fig. 6 and data not shown).

Binding of neuromyotonia, MoS and LE sera to shaker-type K^+ subunits expressed in HeLa cells

To demonstrate the subunit specificity of LE/MoS/neuromyotonia sera, we used HeLa cells that were transiently transfected with plasmids in order to express human Kv1.1, Kv1.2 and Kv1.6 α subunits, respectively. Two days after transfection, cells were immunostained with specific rabbit antisera combined with patient or control sera. However, Kv1.1-, Kv1.2- and Kv1.6-immunoreactivity appeared to be mainly localized in the endoplasmic reticulum (ER), as confirmed by double-staining with the ER marker calnexin and none of the patient or control sera labelled these cells (Fig. 7 and data not shown).

In order to increase expression of mature Kv subunits at the cell surface, we used co-transfection with Kvβ2 or Kv1.4 (Shi *et al.*, 1996; Manganas and Trimmer, 2000). Co-expression with Kv1.4 appeared to result in cell surface labelling in about one-half of Kv1.1- and Kv1.2-positive cells (Fig. 7C–E and I–K), but had no effect on Kv1.6-positive cells (results not shown). Co-expression with Kvβ2 appeared to increase the surface localization of Kv1.6 (Fig. 7O–Q), but had little effect on Kv1.1 or Kv1.2 (data not shown). Cells with surface localization of Kv1.1, Kv1.2 or Kv1.6 could be labelled with different patient sera (Fig. 7C–E, I–J, O and

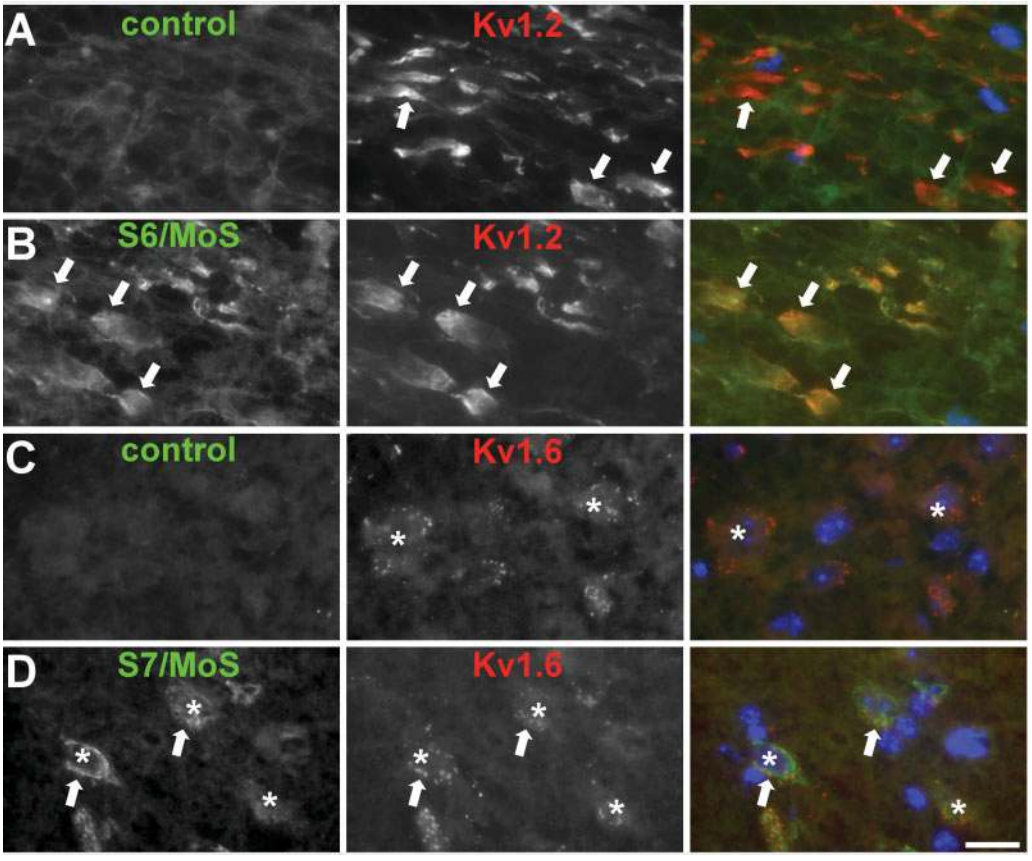

Fig. 2 MoS sera bind to spinal juxtaparanodes or motoneurons. These are images of sections of PFA-fixed mouse spinal cord white matter (**A** and **B**) or grey matter (**C** and **D**), labelled with normal/control or MoS sera (S6 or S7), as indicated, a rabbit antiserum against Kv1.2 (**A** and **B**) or Kv1.6 (**C** and **D**), and DAPI (blue; right column). Merged images are shown in the right column. S6 (**B**) labels the juxtaparanodes (arrows) of myelinated fibres in the white matter, co-localizing with Kv1.2. S7 (**D**) labels motoneurons (nuclei are marked by asterisks) and partially co-localizes with Kv1.6 (arrows). The control serum shows minimal staining in both white (**A**) and grey matter (**C**). Scale bar = 10 μm.

Table 3). Only cells labelled by the corresponding specific rabbit antibodies to Kv1.1, 1.2 or 1.6 were labelled with patient sera, and no patient's serum labelled cells expressing Kv1.4 or Kvβ2 alone (Fig. 7 and data not shown). Surface expression of the co-transfected Kv1 subunit combinations and binding by some of the sera was also demonstrated by double labelling of unpermeabilized cells with fluorescein-conjugated ShK toxin, which binds with high affinity to the outer vestibule of Kv1 channels (Supplementary Fig. 9).

To quantify these results, we calculated the percentage of cells ($n > 50$) with surface Kv1.1, Kv1.2 or Kv1.6 that bound each patient's serum. (Table 3). All sera from patients with LE (S1–5) labelled Kv1.1-expressing cells (19.7–38.0%) and bound to a higher percentage of Kv1.1 than Kv1.2 or 1.6-positive cells ($P = 0.05$), whereas sera from patients with neuromyotonia or MoS labelled significantly fewer Kv1.1-positive cells (0–10.7%) than Kv1.2-positive cells (5.55–28.7%). The percentage of Kv1.1-expressing cells bound by LE sera was significantly higher than that bound by NMT sera ($P = 0.00033$), whereas the percentage of Kv1.2- or Kv1.6-expressing cells bound by LE and neuromyotonia sera did not differ. In contrast, neuromyotonia sera labelled significantly more Kv1.2- than Kv1.1- or Kv1.6-expressing cells ($P = 0.0027$). In agreement with the above results, Kv1.6-expressing cells were labelled strongly by MoS S7 (31.3%) and only weakly by three LE and four neuromyotonia sera (1.33–5.33%). The second MoS serum (s6) labelled strongly Kv1.2- and to a lesser degree Kv1.1- but not Kv1.6-expressing cells. These results demonstrated that all 17 sera that we examined bind to one or more of the Kv1.1, Kv1.2 or Kv1.6 subunits when they are expressed on the cell surface and additionally confirm that LE sera predominantly bind Kv1.1.

Discussion

Potassium channel antibodies are increasingly found in patients with a range of acquired neurological disorders. Here, using immunofluoresence of tissue and transfected cells, we demonstrate that sera from these patients probably bind to mature cell-surface channels, that sera from LE patients bind preferentially to Kv1.1 channels and that sera from neuromyotonia or MoS patients bind relatively more strongly to Kv1.2 or Kv1.6 channels. Although these results

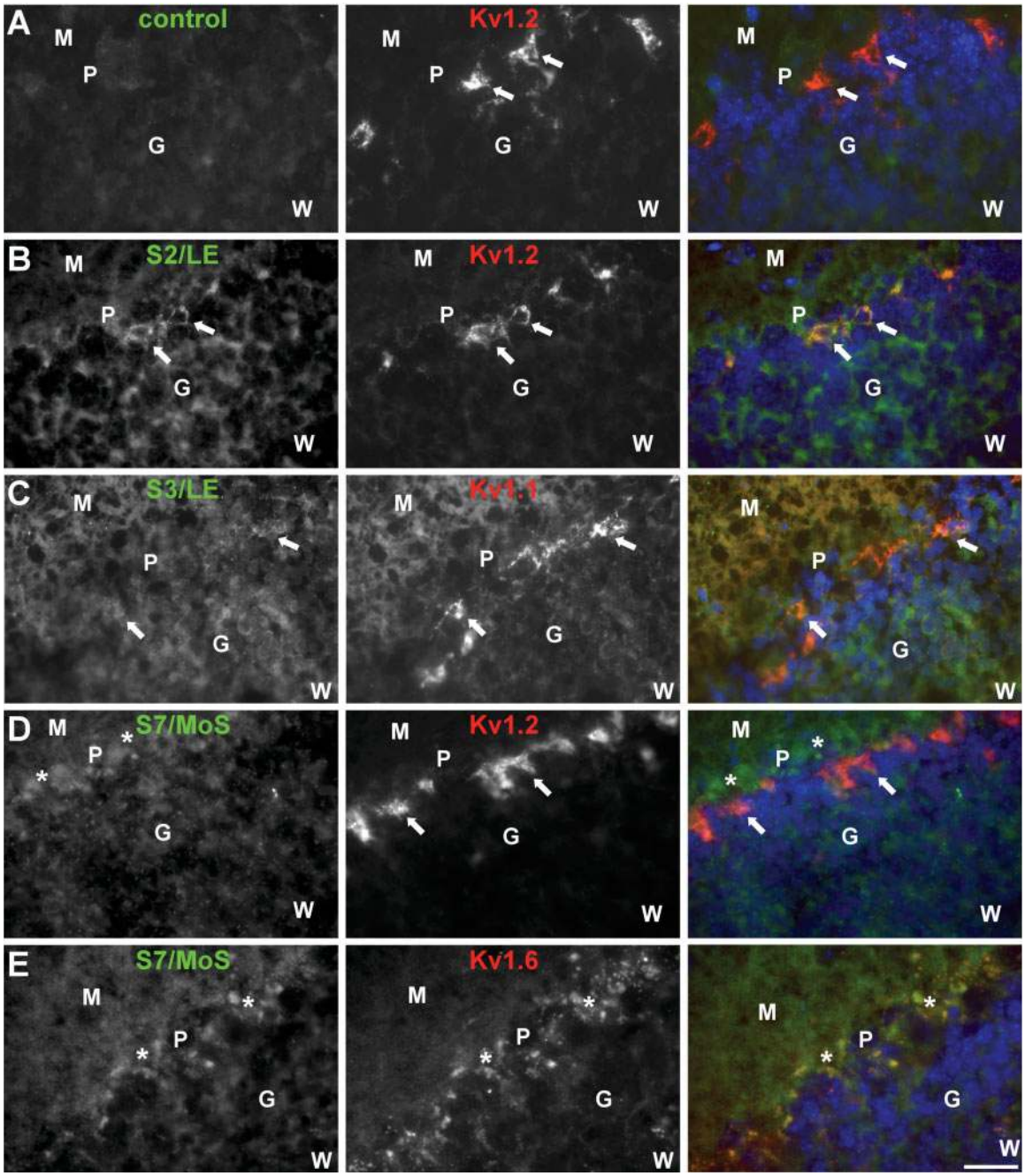

Fig. 3 LE and MoS sera co-localize with *Shaker*-type K^+ channels in the cerebellum. These are images of sections of PFA-fixed mouse cerebellum, labelled with a normal/control serum (**A**), S2 (**B**) and S3 (**C**), from patients with LE, and S7 (**D** and **E**; from a patient with MoS), combined with a rabbit antiserum against Kv1.2 (**A**, **B** and **D**), Kv1.1 (**C**) or Kv1.6 (**E**), and DAPI (blue; right column). Merged images are shown in the right column, where DAPI-stained nuclei demonstrate the different layers (M: molecular layer; P: Purkinje cell layer; G: granule cell layer; W: white matter). S2 and S3 label the pinceau (arrows) co-localizing with Kv1.2 (**B**) and Kv1.1 (**C**) subunits; both S2 and S3 label the granule cell layer where Kv1.1 (**C**) but not Kv1.2 (**B**) is expressed; S3 more than S2 labels the molecular layer, which expresses Kv1.1 more than Kv1.2 (**B** and **C**). In contrast, S7 does not label the pinceau (arrows in **D**), and instead labels most intensively the Purkinje cell perikarya (asterisks in **D** and **E**), where it co-localizes with Kv1.6 (**E**). The control serum (**A**) shows minimal staining. Scale bar = 20 μm.

do not fully explain the clinical phenotypes of these patients, they illuminate for the first time how the antigenic specificity of these autoantibodies might determine the various manifestations of these diseases.

Examination of multiple areas with high expression of *Shaker*-type K^+ channels showed specific binding in at least one area with most sera, including two 'negative' or low-titer sera. This is the first time that binding of serum antibodies

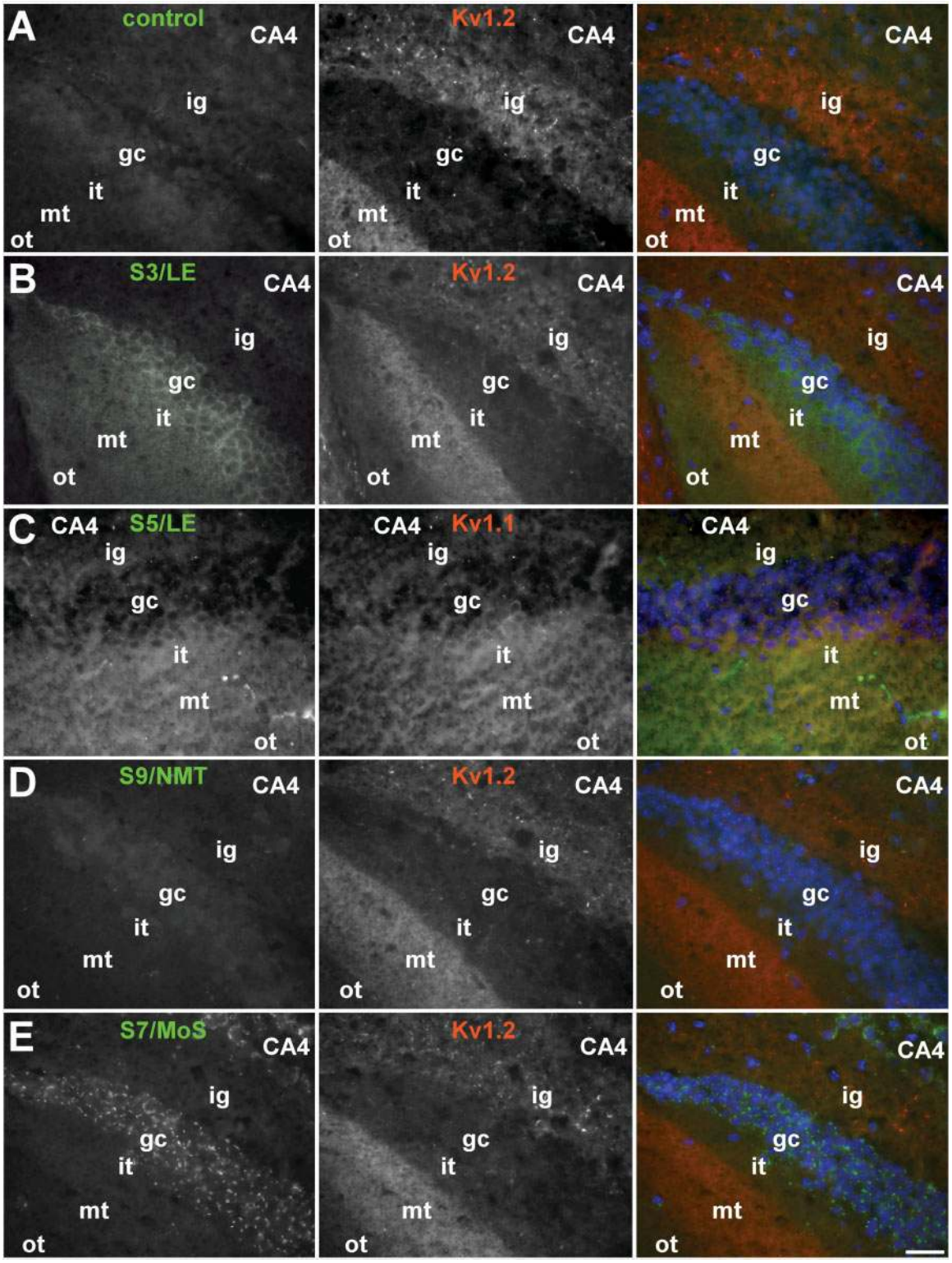

Fig. 4 LE sera co-localize with Kv1.1 in the DG. These are images of mouse hippocampal DG double-labelled with S3 (**B**) and S5 (**C**) from LE patients, S9 (**D**; from a NMT patient) and S7 (**E**; from a MoS patient), or a normal control serum (**A**), as indicated (left column), and a rabbit antiserum against Kv1.2 (**A**, **B** and **D**, **E**) or Kv1.1 (**C**) (middle column). Merged images are shown in the right column, where staining of cell nuclei with DAPI (blue) demonstrates the different layers (ot: outer third, mt: middle third, it: inner third of the molecular layer; gc: granule cell layer; ig: infragranular layer; and CA4). The LE sera (S3 and S5; **B** and **C**) show labelling of the molecular layer, co-localizing with Kv1.1 (**C**) more than Kv1.2 (**B**), which is restricted to the middle third of the molecular layer. The NMT serum (S9) stains only background (**D**), as does the normal control (**A**), while MoS serum (S7) labels exclusively perikarya of granule cells and CA4 neurons (**E**) but not the molecular or infragranular layers. Scale bar = 50 μm.

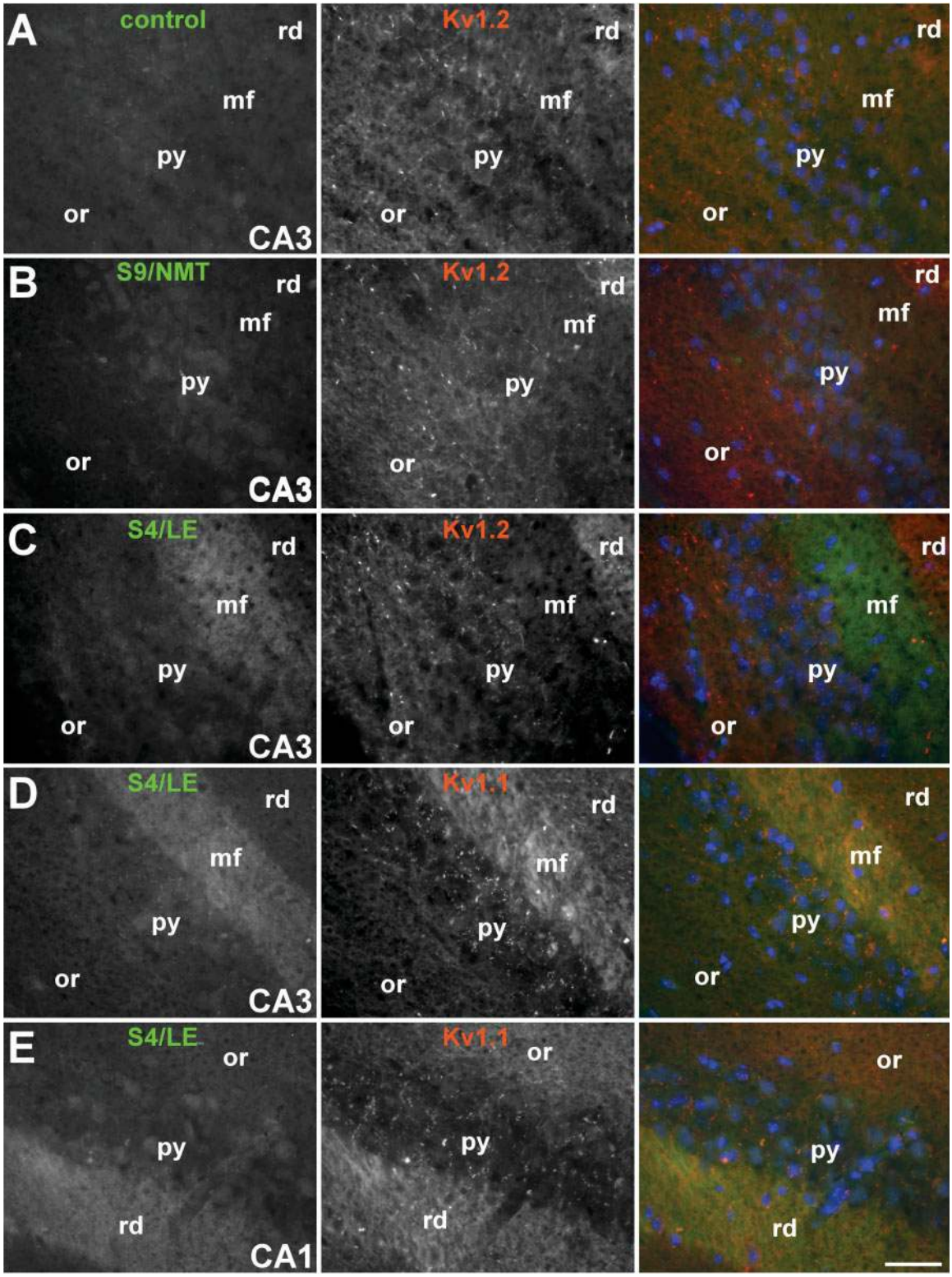

Fig. 5 LE sera co-localize with Kv1.1 in the CA3 and CA1 hippocampal areas. These are images of mouse hippocampal CA3 (**A**–**D**) and CA1 (**E**) fields double-labelled with S9 (**B**; from a NMT patient), S4 (**C**–**E**; from a LE patient), or a normal control serum (**A**), as indicated (left column), and rabbit antisera against Kv1.2 (**A**–**C**) or Kv1.1 (**D** and **E**) (middle column). Merged images are shown in the right column. Cell nuclei are visualized with DAPI (blue) demonstrating the different layers (or: stratum oriens; py: pyramidal cell layer; mf: mossy fibre zone; rd: stratum radiatum). The LE serum (S4) stains intensely the mossy fibre zone in CA3 (**C** and **D**) and the stratum radiatum in CA1 (**E**) co-localizing with Kv1.1 (**D** and **E**) but not with Kv1.2 (**C**). The NMT serum (S9) stains only background (**B**), as does the normal control (**A**). Scale bar = 50 μm.

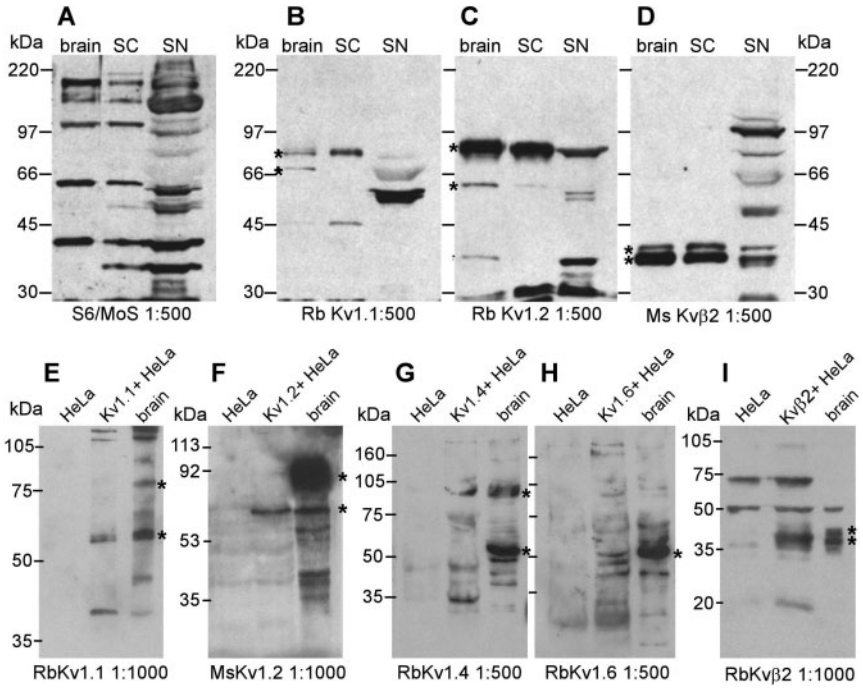

Fig. 6 Immunoblots of *Shaker*-type K^+ channel subunits. Panels (**A**–**D**) show immunoblots of membranes isolated from brain, spinal cord (SC) and sciatic nerve (SN) (100 μg of protein loaded in each lane) probed with (**A**) S6 (from a MoS patient; 1 : 500), (**B**) a rabbit antiserum against Kv1.1 (1 : 500), (**C**) a rabbit antiserum against Kv1.2 (1 : 500) and (**D**) a mouse antibody to Kvβ2 (1 : 500). The bands obtained with S6 (~175, 145, 114, 63, 42 and 35 kDa) do not correspond to the expected sizes of Kv1.1 (~80 and 66 kDa), Kv1.2 (~88 and 60 kDa), Kv1.6 (~50 kDa), Kv1.4 (~96 kDa) or Kvβ2 (~41 and 38 kDa). Panels (**E**–**I**) are immunoblots of whole brain homogenates and lysates of untransfected HeLa cells or cells transfected with different *Shaker*-type K^+ channels subunits, as indicated. Note that the recombinant subunits expressed in transfected cells correspond to some but not all of the isoforms found *in situ*, including Kv1.1 (~66 but not 80 kDa; **E**); Kv1.2 (~60 but not 88 kDa; **F**); Kv1.4 (~96 but not 50 kDa; **G**); Kv1.6 (~50 kDa; **H**) and Kvβ2 (~38 kDa; **I**). Putative specific bands are indicated with asterisks.

to juxtaparanodal areas of myelinated axons is shown. Because juxtaparanodes are enriched in Kv1.1 and Kv1.2 (Wang *et al.*, 1993), and may contribute to the repolarization of axons (Zhou *et al.*, 1998; Vabnick *et al.*, 1999), diminished function would probably lead to hyperexcitability, as described in loss-of-function mutations in both mice and humans (Browne *et al.*, 1994; Smart *et al.*, 1998; Zerr *et al.*, 1998; Chiu *et al.*, 1999; Eunson *et al.*, 2000). Since Kv1.1 and Kv1.2 subunits are co-localized at juxtaparanodes, the specificity of each serum for one or the other subunit, or both, could not be demonstrated by this approach. However, not all neuromyotonia sera showed juxtaparanodal labelling, and it was also seen in sera from LE patients, in whom neuromyotonia is not a clinical feature, raising the question of which specificity is unique to LE patients. Moreover, binding to Kv1.2 more than to Kv1.1 expressed by transfected cells, seen with all 10 neuromyotonia sera, correlates better with the clinical phenotype of neuromyotonia than does juxtaparanodal labelling.

LE sera, as well as having the highest titers by the ^{125}I-α-dendrotoxin immunoprecipitation assay, were distinguished from neuromyotonia sera by their patterns of CNS immunostaining: LE sera stained relevant regions that contain Kv1.1 but not Kv1.2, including the cerebellar granule cell layer, the inner third of the hippocampal molecular layer and the CA3 mossy fibre zone. The cerebellar granule cell layer contains Kv1.6, and the inner third of hippocampal molecular layer and the CA3 mossy fibre zone contain Kv1.4, but Kv1.1 is also localized in each of these sites (Sheng *et al.*, 1994; Laube *et al.*, 1996; Koch *et al.*, 1997; Rasband *et al.*, 1998; Chung *et al.*, 2001, 2005). The labelling of the cerebellar molecular layer and pinceau, the middle and outer thirds of the hippocampal molecular layer, the stratum radiatum of CA1 and CA3 and juxtaparanodes, could result from either Kv1.1-reactive or Kv1.2-reactive antibodies in LE sera. Overall, the observed regions of staining with the five LE sera can be accounted for by their reactivity with Kv1.1. Our results extend the report of Buckley *et al.* (2001), who observed staining of the middle third of the hippocampal molecular layer with one LE serum, although they attributed this staining to Kv1.2. The neuropil staining by another LE serum (Case 5 in Ances *et al.*, 2005) also corresponds to the Kv1.1 expression pattern, but this patient was not tested by the immunoprecipitation assay.

Confirmation of Kv1 specificity of the sera came from immunocytochemistry on transfected HeLa cells expressing Kv1.1, Kv1.2 or Kv1.6 subunits. Labelling HeLa cells required co-expression with Kv1.4 or Kvβ2, as subunits that were retained in the ER were not labelled. This suggests that the sera may be specific for the mature proteins, recognizing

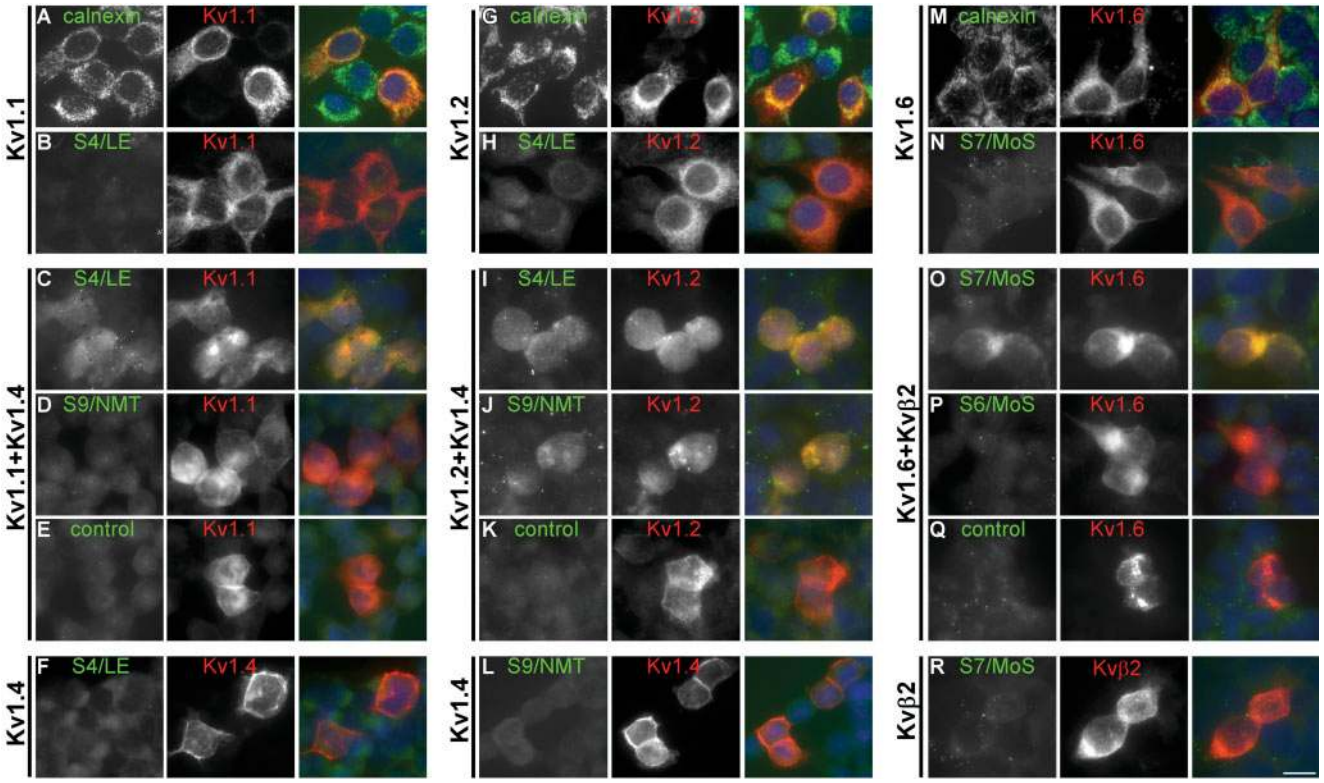

Fig. 7 Expression of *Shaker*-type K^+ channel subunits in HeLa cells. These are images of HeLa cells transiently transfected to express Kv1.1 (**A** and **B**), Kv1.1 + Kv1.4 (**C**–**E**), Kv1.4 (**F** and **L**), Kv1.2 (**G** and **H**), Kv1.2 + Kv1.4 (**I**–**K**), Kv1.6 (**M** and **N**), Kv1.6 + Kvβ2 (**O**–**Q**) or Kvβ2 (R) subunits, as indicated. The left columns (green) show staining with the ER marker calnexin (**A**, **G** and **M**) or with patient or control sera, as indicated. The middle columns (red) show labelling with a rabbit antiserum against Kv1.1 (**A**–**E**), Kv1.2 (**G**–**K**), Kv1.6 (**M**–**Q**), Kv1.4 (**F** and **L**) or Kvβ2 (**R**) demonstrating the presence of the corresponding Kv subunit in some of the transfected cells. Merged images, including DAPI staining, are shown in the right columns. Note that in cells expressing only Kv1.1, Kv1.2 or Kv1.6, the corresponding proteins are localized in the ER, as demonstrated by their co-localization with calnexin (**A**, **G** and **M**), that Kv1.4 appears to be localized to the cell membrane (**F**), and that S4 (from a LE patient) and S7 (from a MoS patient) do not label cells expressing intracellularly retained Kv1.1 (**B**), Kv1.2 (**H**) or Kv1.6 (**N**) subunits. When co-transfected with Kv1.4, Kv1.1 and Kv1.2 are expressed at the cell surface. S4 (**C**) but not S9 (**D**), or the control serum (**E**), binds to cells expressing Kv1.1 at the surface. Cells co-transfected with Kv1.2 and Kv1.4 are labelled by both S4 (**I**) and S9 (**J**), but not by the control serum (**K**). Neither S4 nor S9 labels cells expressing only Kv1.4 (**F** and **L**). S7 labels cells co-transfected with Kv1.6 and Kvβ2, which express Kv1.6 at the surface (**O**), in contrast to S6 (from another MoS patient; **P**) or the control serum (**Q**). S7 does not bind to Kvβ2 expressed alone (**R**). Scale bar = 10 μm.

post-translational modifications such as N-glycosylation, or tertiary structure that depends on subunit interactions (Shi *et al.*, 1996; Manganas and Trimmer, 2000; Campomanes *et al.*, 2002). The feature that most clearly distinguished LE from neuromyotonia was the greater proportion of Kv1.1- versus Kv1.2-positive cells; this is the first time that this distinction has been noted, and provides the first explanation for why some patients get CNS symptoms without peripheral nerve involvement.

The results confirm the high sensitivity of immunocytochemical methods, previously estimated ~80–90% using an oocytes expression assay for the same Kv1 subunits but looking mainly at neuromyotonia sera. That study found reactivity predominantly, but variously, with Kv1.2 and Kv1.6, and little with Kv1.1 (Hart *et al.*, 1997). Using a wider range of patient sera, including those from LE, the HeLa cell assay revealed reactivity of all sera to one or more of these subunits (Table 3), including three neuromyotonia sera that had no detectable antibody titers by the ^{125}I-α-dendrotoxin immunoprecipitation assay. Overall, there was little correlation between the immunoprecipitation assay titers and the binding data (Tables 1 and 3), perhaps partially explained by the fact that the rabbit brain extracts contain mostly Kv1.1 and Kv1.2, and little Kv1.6 (Clover L, Vincent A, manuscript in preparation), and by the higher affinity that α-dendrotoxin has for Kv1.2 than for Kv1.1 or Kv1.6 (Stuhmer *et al.*, 1989). The higher sensitivity of the cell binding assay may also be due to the higher concentration of channels in transfected cells compared with tissue. It remains to be determined whether these sera are comprised of many individual antibodies that have specific activity to different subunits, or that individual antibodies cross-react with multiple subunits. In either case, our data show that 'polyreactive' sera are associated with LE and MoS, not just with neuromyotonia, and provide clues to phenotypic associations.

Table 3 Binding of LE, MoS and neuromyotonia sera to *Shaker*-type K^+ channel α subunits expressed in HeLa cells

Patient/ phenotype	%Kv1.1/Kv1.4*-expressing cells labelled with patient serum (mean ± SE)	%Kv1.2/Kv1.4*-expressing cells labelled with patient serum (mean ± SE)	%Kv1.6/Kvβ2*-expressing cells labelled with patient serum (mean ± SE)
1/LE	24.0 ± 7.2	6.67 ± 3.1	0
2/LE	30.0 ± 12	14.7 ± 3.1	2.67 ± 1.2
3/LE	38.0 ± 13	12.0 ± 2.0	3.33 ± 1.2
4/LE	31.3 ± 6.4	10.0 ± 2.0	1.33 ± 1.2
5/LE	19.3 ± 4.1	5.33 ± 1.2	0
6/MoS	10.7 ± 6.4	28.7 ± 3.1	0
7/MoS	0	0	31.3 ± 8.3
8/NMT	2.67 ± 1.2	12.0 ± 2.0	5.33 ± 2.3
9/NMT	0.67 ± 1.2	28.7 ± 4.2	4.67 ± 1.2
10/NMT	0	8.67 ± 3.1	0
11/NMT	0	7.33 ± 1.2	0
12/NMT	0	4.00 ± 4.0	0
13/NMT	1.33 ± 1.2	5.33 ± 2.3	0
14/NMT	0	6.00 ± 2.0	4.67 ± 1.2
15/NMT	0	8.00 ± 2.0	0
16/NMT	0	9.33 ± 1.2	3.33 ± 1.5
17/NMT	0	5.33 ± 1.2	0
Controls (n=4)	0	0	0

HeLa cells were co-transfected with Kv1.1 or Kv1.2 and Kv1.4 or with Kv1.6 and Kvβ2 (DNA ratios 1 : 4). The percentage of specific binding was measured in three separate experiments as follows: only cells with apparent surface expression of Kv1.1, Kv1.2 or Kv1.6 were counted (>50 cells/serum/experiment), and the proportion of these expressing cells that were also labelled with each serum was determined. The mean and standard error (SE) are shown here. *None of the patient or control sera labelled cells ($n > 50$) expressing Kv1.4 alone or Kvβ2 alone.

Patient sera did not appear to label Kv1.1, Kv1.2 or Kv1.6 in immunoblots of lysates from nerve, brain or transfected cells probably because immunoblotting, in general, substantially reduces the antigenicity for pathogenic antibodies that recognize complex membrane antigens (A. Vincent, unpublished data). For instance, antibodies to K^+ channels (Hart *et al.*, 1997) or P/Q-type voltage-gated calcium channels (Mason *et al.*, 1997) do not bind well to recombinant or denatured proteins, in contrast to antibodies against cytoplasmic constituents such as Hu (Dalmau *et al.*, 1990) or Ma2 (Sutton *et al.*, 2000). One exception was reported in a patient with IgM (as opposed to IgG) antibodies against a *Shaker*-type K^+ channel (Arimura *et al.*, 1997), possibly Kv1.6 given that the band obtained in the immunoblot was around 50 kDa.

Because Kv1.1 is localized in hippocampal regions that have been implicated in excitability and memory (Johnston *et al.*, 2000), dysfunction of Kv1.1 channels could result in the typical manifestations of LE: seizures, agitation, hallucinations and memory impairment (Buckley *et al.*, 2001; Thieben *et al.*, 2004; Vincent *et al.*, 2004). Although we found no reactivity of LE (or any other) sera for 'mature' Kv1.4 subunits in the cell membranes of transfected HeLa cells, we cannot exclude the unlikely possibility that these sera may react with an epitope occurring exclusively in Kv1.1/Kv1.4 heterotetramers, which are highly expressed in the crucial hippocampal subfields (Monaghan *et al.*, 2001).

Our data point to a relationship between Kv1.1 reactivity and LE, and Kv1.2 reactivity and neuromyotonia, but some questions remain, including the subunit specificity associated with MoS. One MoS serum (S6), from a patient with marked CNS, autonomic and peripheral involvement (Liguori *et al.*, 2001), showed a similar pattern to typical neuromyotonia sera: stronger reactivity to Kv1.2 than to Kv1.1. The other MoS serum (S7), with less marked CNS symptoms, showed strong and exclusive reactivity to Kv1.6 in HeLa cells and in different CNS areas, as reported previously (Antozzi *et al.*, 2005). Thus, it is possible that autoantibodies to Kv1.6 contribute to neuromyotonia, but we found only 4 out of 10 neuromyotonia sera that labelled Kv1.6-expressing HeLa cells, in contrast to the results of Hart *et al.* (1997). Further, how reactivity to Kv1.6 could cause neuromyotonia is unclear. Although Rasband *et al.* (1999) reported some Kv1.6-positive juxtaparanodes in the optic nerve, we did not detect juxtaparanodal staining in our material. It remains to be shown whether Kv1.6 dysfunction in motoneuron somata (Fig. 2) could cause neuromyotonia, or whether Kv1.6 is present on the motor nerve terminal.

The finding that sera from both neuromyotonia and LE patients bind to Kv1 channels in the periphery and CNS indicates that other factors may predispose to CNS and PNS manifestations. For the CNS, the permeability of the blood–brain barrier to the antibodies is an important consideration. Although some neuromyotonia and most LE patients have increased IgG or oligoclonal bands in CSF (Newsom-Davis and Mills, 1993; Vincent *et al.*, 2004), suggesting intrathecal antibody production, in general, oligoclonal bands are matched by serum bands, indicating that K^+ channel antibodies probably penetrate the blood–brain barrier. This has been shown for antibodies to glutamate receptors in Rasmussen's encephalitis (McNamara *et al.*, 1999), and is more likely than intrathecal synthesis since serum K^+ channel antibody levels are always higher than those in the CSF (Vincent *et al.*, 2004; Clover L and Vincent A, unpublished data). Furthermore, patient sera labelled different areas in the cerebellum, as they did in the hippocampus, but cerebellar symptoms or MRI signal changes in the cerebellum are uncommon in LE patients (Vincent *et al.*, 2004). This suggests that some CNS areas such as the hippocampus, may be more accessible to circulating Kv1 antibodies, or they may be more prone to hyperexcitability once Kv1 channel function is compromised, perhaps owing to expression of multiple types of K^+ channels in each area, playing different roles in regulating neuronal excitability.

For the PNS, the permeability of the septate-like paranodal junctions to autoantibodies may be an important factor in acquired hyperexcitability and requires further study. Animal models in which the juxtaparanodal concentration of Kv1.1 and Kv1.2 is impaired, including mice deficient in

TAG-1 (Poliak *et al.*, 2003; Traka *et al.*, 2003) or Caspr2 (Poliak *et al.*, 2003), have no hyperexcitability of peripheral nerves. The small amount of Kv1 channels remaining at the juxtaparanodes may still have adequate activity (Poliak *et al.*, 2003), or other potassium channels expressed in myelinated axons (Devaux *et al.*, 2003, 2004) may functionally compensate for the loss of juxtaparanodal Kv1.1 and Kv1.2. Similar mechanisms may be relevant in other areas of the nervous system and their clarification could provide clues to therapeutic possibilities both in acquired and inherited forms of neuronal hyperexcitability.

Acknowledgements

This work was supported by the National Multiple Sclerosis Society (USA) (RG3457A2/1 to K.A.K.), the NIH (RO1 NS43174 to S.S.S. and the DANA Foundation (grant to A.V)). We thank Drs Olav Pongs for *KCNA1/Kv1.1*, *KCNA2/Kv1.2* and *KCNA6/Kv1.6* expression vectors, James Trimmer for *KCNA4/Kv1.4* and *Kcnab2/Kvβ2* expression vectors and antibodies, and Bruce Nicholson and Klaus Willecke for HeLa cells. We are grateful to Drs Rocco Liguori, Carlo Antozzi, Camilla Buckley, Marco Bonifati and Prof. Nick Wood for sera and clinical information from their patients. We also thank Xenia Alevra and Nicoletta Kokkoni for technical assistance.

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