

SHORT REPORT

An improved diagnostic assay for Lambert-Eaton myasthenic syndrome

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Abstract

A new immunoprecipitation assay has been established for detecting antibodies to voltage-gated calcium channels (VGCCs) in Lambert-Eaton myasthenic syndrome (LEMS), using ^{125}I - ω -conotoxin MVIIC, which binds to P-type VGCCs, to label extracts of human cerebellum. Fifty six of 66 serum samples (85%) from patients with clinically and electrophysiologically definite LEMS were positive for the presence of VGCC antibodies, defined as a titre >3 SD above the mean for the healthy controls (n = 10). All disease controls (n = 40) were negative. This sensitive immunoassay should prove valuable in the diagnosis of LEMS.

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Lambert-Eaton myasthenic syndrome (LEMS) is an autoimmune disease, often associated with small cell lung cancer (SCLC), in which antibodies are directed against voltage-gated calcium channels (VGCCs) situated on the presynaptic nerve terminal.¹ The VGCCs may be classified by their electrophysiological characteristics into at least four subtypes (T, L, N, and P).^{2,3} We have previously detected antibodies to N-type VGCCs in a proportion of patients with LEMS using ω -conotoxin GVIA (ω -CgTx), purified from the fish hunting cone snail (*Conus geographus*), which blocks neuronal N-type VGCCs.⁴ Similar assays have been described by Sher and colleagues⁵ and Lennon and Lambert.⁶ In our series, however, less than 50% of serum samples were clearly positive and some autoimmune disease controls gave equivocal results.⁴ Recent evidence indicates that P-type VGCCs are present in some SCLC lines,⁷ are responsible for the control of neurotransmitter release at the mammalian neuromuscular junction,⁸ and may be the targets for the autoantibodies.^{9,10} ω -Conotoxin MVIIC (ω -CmTx), synthesised from a sequence derived from a different cone

snail (*Conus magus*),¹¹ binds to P-type VGCCs in cerebellar extracts, and the binding sites were precipitated by three of four serum samples from patients with LEMS.¹² We report here the results of a radioimmunoassay for 66 LEMS serum samples using ^{125}I - ω -CmTx to label extracted VGCCs.

Methods and patients

Synthetic ω -conotoxin MVIIC was obtained from the Peptide Institute Inc (European Distributor, Scientific Marketing, UK). The toxin was iodinated with ^{125}I as previously described;¹³ the specific activity was 75-150 Ci/mmol. Human cerebellum was obtained seven and 28 hours postmortem from two patients with ischaemic heart disease (aged 74 and 62 years respectively). It was frozen at -70°C until required. The contralateral cerebellum was normal or showed age related changes only on routine histology. Safety precautions were taken throughout. The tissue was homogenised in 25 mM Tris-HCl/5 mM HEPES buffer, pH 7.4, containing 0.32 M sucrose, 1 μM pepstatin, 2 μM leupeptin, 0.1 mM phenylmethanesulphonyl fluoride, and 20 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor, and centrifuged. The membrane pellets were resuspended in the same buffer with 2% digitonin and rotated for an hour. The supernatant was separated by centrifugation at 13 000 rpm for 10 minutes. Cerebellum extract (25 μl) containing about 30 fmol of binding sites, was labelled with ^{125}I - ω -CmTx for two hours (1.5 nM) and incubated overnight with serum (1.25-10 μl diluted 1:10) from patients with LEMS or controls. Excess goat antihuman IgG serum was then added to precipitate serum antibodies. Each sample was centrifuged and the pellets washed twice with 20 mM phosphate buffer pH 7.4 containing 0.1% Triton X-100, before counting. All steps were performed at 4°C . The specific binding was calculated from the total ^{125}I - ω -CmTx precipitated minus the non-specific binding determined by performing the assay in the presence of excess unlabelled ω -CmTx (100 nM). Serum samples were available from 66 patients with typical clinical and EMG features of LEMS. The EMG criteria comprised (a) a reduced amplitude of the resting compound muscle action

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potential (CMAP) in abductor digiti minimi (<8.5 mV) and (b) an increment in CMAP amplitude after 15 seconds maximum voluntary contraction of the muscle >100% of resting CMAP.¹⁴ Twenty two patients had histologically proved small cell lung carcinoma (SCLC), and 29 patients had been followed up for more than five years without signs of SCLC (no cancer detected, NCD). The SCLC status of the remaining 15 patients, followed up for less than five years, was regarded as uncertain (SU). The 50 controls were 10 healthy subjects, and 10 patients each with either SCLC without neurological symptoms, myasthenia gravis, other neurological diseases, or rheumatoid arthritis/systemic lupus erythematosus.

Results

The figure (A) shows quantitative precipitation of ¹²⁵I- ω -CmTx-VGCC from digitonin extracted cerebellum from two patients with LEMS. A healthy control serum showed non-specific binding only. To determine antibody titres routinely, 10 μ l of serum was tested first and those samples precipitating >10 fmol of

¹²⁵I- ω -CmTx binding sites were retested using 2.5 μ l. Results are expressed as pmol specific ¹²⁵I- ω -CmTx binding sites precipitated/litre of serum after subtraction of non-specific binding (see methods).

Fifty six of 66 LEMS samples (85%) were positive for the presence of VGCC antibodies, defined as a titre >40 pM (3SD above the mean for the healthy controls; figure (B), dotted line). The mean titres in the three clinical groups (SCLC, NCD, SU) did not differ significantly. The proportions of patients positive for antibody in the three groups were 90%, 76%, and 93% respectively. The antibody titres for all disease control samples (n = 40) were negative (<40 pM).

Discussion

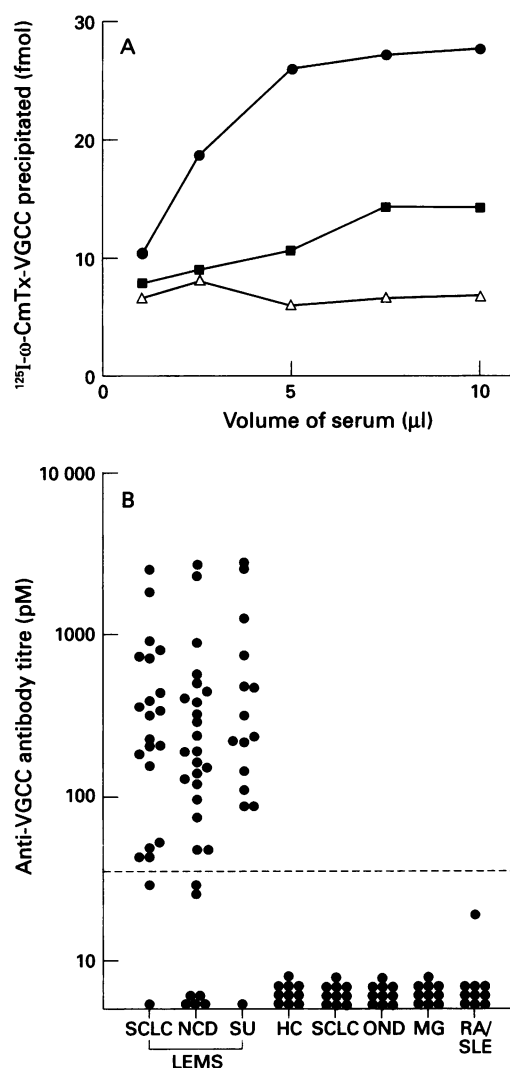
In our previous study⁴ we showed that antibodies could be detected using ¹²⁵I- ω -CgTx to label N-type VGCCs, but this assay yielded positive titres in only 44% of patients with LEMS. The role of anti-N-type VGCC antibodies is uncertain, but they might contribute to the disturbance of the autonomic nervous system in LEMS as N-type VGCCs are concerned in transmitter release from some autonomic nerves.¹⁵

The use of P-type VGCCs should provide a better diagnostic assay because this channel subtype is responsible for neurotransmitter release at the mammalian neuromuscular junction.⁸ Also LEMS IgG blocked potassium stimulated Ca²⁺ flux in a human SCLC cell line, leading to the suggestion that P-type VGCCs might be the principal target for LEMS IgG.^{9,10} Moreover, similar lines have been shown to express mRNA for P-type VGCCs.⁷ In our present study we have detected positive titres in 85% of 66 patients with LEMS, using ¹²⁵I- ω -CmTx. Although ω -CgTx also binds to cerebellar extracts, less than 25% of the serum samples were positive when ¹²⁵I-CgTx was substituted for ¹²⁵I-CmTx (unpublished data), further suggesting that the ω -CmTx-binding VGCCs are a more important antigenic target in LEMS. Human cerebellum extract was used as a source of the VGCCs; cerebellum from other species, human SCLC lines, or neuronal cell lines may provide an alternative source. This new assay should prove useful in the diagnosis and monitoring of disease progression.

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(A) Precipitation of ¹²⁵I-CmTx-labelled VGCCs extracted from human cerebellum by serum samples from two patients with LEMS (●; ■) and one healthy control (△); (B) Serum antibodies in LEMS and controls measured by immunoprecipitation. In this case non-specific binding of each serum, measured in the presence of excess ω -CmTx, has been subtracted. Antibody titres were considered positive if greater than 40 pM (mean + 3 SD for the healthy controls; dashed line). SCLC = Small cell lung carcinoma; NCD = no cancer detected; SU = status uncertain; HC = healthy controls; OND = other neurological disease; MG = myasthenia gravis; RA/SLE = rheumatoid arthritis/systemic lupus erythematosus.



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Hughlings Jackson and the Holmes-Adie tonic pupil

The names of Gordon Holmes¹ and W James Adie^{2,3} are traditionally attached to the syndrome of myotonic pupils and tendon areflexia. Some 50 years earlier in 1881, mydriasis with pupillary paralysis was described clearly by Hughlings Jackson.⁴ In 1914 Oloff found negative Wasserman reactions in blood and CSF in an 18 year old boy with tonic pupils, thereby showing that syphilis—previously implicated—was not the cause. Harriman and Garland⁵ credited earlier papers dating to 1902 (Strassburger, Saener) that described tonic pupils.

Hughlings Jackson⁴:

"A woman aged 26 was sent to see me simply because the right pupil was much larger than the left. It had been so three years . . . the right pupil was dilated and absolutely motionless to light, and also during accommodation, yet the accommodation itself on this side was perfect; this was severely tested by Mr Couper . . . this case at first puzzled me . . . It occurred to me to test the knees. Neither I nor Mr Couper found the smallest trace of the knee phenomenon. Several times did I pertinaciously inquire for other symptoms of tabes; there were no other symptoms of any kind . . . Dr Buzzard . . . confirmed the above observations."

Sir Gordon Holmes¹:

"Frequently no change in the size of the pupil was visible immediately on convergence, but when this was maintained for a few seconds the pupil slowly and gradually grew smaller, till its diameter equalled or was even narrower than that of the normal eye. The rate of contraction varied very much . . . When contracted the pupil remains constant and when convergence is relaxed it dilates slowly."

Adie³ described 19 patients, 13 with absent tendon reflexes, and noted 44 reported cases of tonic pupil. In an exemplary clinical essay, he outlined four incomplete forms (the last would not now be accepted).

1. The complete form—typical tonic pupil and absence of reflexes.
2. Incomplete forms: a) tonic pupil alone; b) atypical phase of the tonic pupil alone (iridoplegia"; internal ophthalmoplegia"); c) atypical phases of the tonic pupil with absent reflexes; d) absent reflexes alone."

Adie did not claim originality, recognising descriptions from 1902. Holmes's work is not acknowledged in Adie's *Brain* paper. Adie commented on the past misconstrued attribution to syphilis: "A perversion of nervous activity" of the vegetative nervous system was, he thought, the cause.

It was the London ophthalmologist, James Ware (1756-1815), however, who furnished one of the earliest depictions in 1813.⁶

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