

CALCIUM-CHANNEL ANTIBODIES IN THE LAMBERT-EATON SYNDROME AND OTHER PARANEOPLASTIC SYNDROMES

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Abstract Background. Voltage-gated calcium channels in small-cell lung carcinomas may initiate autoimmunity in the paraneoplastic neuromuscular disorder Lambert-Eaton syndrome. The calcium-channel subtype that is responsible is not known.

Methods. We compared the effects of antagonists of L-type, N-type, and P/Q-type neuronal calcium channels on the depolarization-dependent influx of calcium-45 in cultured carcinoma cells. Serum samples from patients with various disorders were tested for reactivity with P/Q-type channels solubilized from carcinoma and cerebellar membranes and N-type channels from cerebral cortex.

Results. P/Q-type calcium-channel antagonists were the most potent inhibitors of depolarization-induced ⁴⁵Ca influx in cultured small-cell carcinoma cell lines. Anti-P/Q-type calcium-channel antibodies were found in serum from all 32 patients with the Lambert-Eaton syndrome

and a diagnosis of cancer and in 91 percent of the 33 patients with the Lambert-Eaton syndrome without cancer. Anti-N-type calcium-channel antibodies were found in 49 percent of the 65 patients with the Lambert-Eaton syndrome. Lower titers of anti-P/Q-type and anti-N-type calcium-channel antibodies were found in 54 percent of 70 patients with a paraneoplastic encephalomyeloneuropathic complication of lung, ovarian, or breast carcinoma, 24 percent of 90 patients with cancer but no evident neurologic complications, 23 percent of 78 patients with sporadic amyotrophic lateral sclerosis, and less than 3 percent of 69 patients with myasthenia gravis, epilepsy, or scleroderma.

Conclusions. The high frequency of P/Q-type calcium-channel antibodies found in patients with the Lambert-Eaton syndrome implies that antibodies of this specificity have a role in the presynaptic pathophysiology of this disorder. (N Engl J Med 1995;332:1467-74.)

PARANEOPLASTIC syndromes are most often associated with small-cell lung carcinoma, a relatively common neuroendocrine neoplasm. The autoimmune neurologic syndromes may reflect host immune responses against neuron-like components of the tumor cells¹ (Fig. 1). An example is the Lambert-Eaton syndrome, a disorder of neuromuscular transmission caused by antibodies that impair the presynaptic release of acetylcholine.^{10,11}

Acetylcholine is released from storage vesicles in the nerve ending in response to an action potential. This mechanism requires the regulated influx of calcium through voltage-gated channels in nerve terminals. In the Lambert-Eaton syndrome, these calcium channels are the target of pathogenic autoantibodies.^{10,11} Cultured small-cell lung carcinoma cells exhibit voltage-activated calcium-channel activity.¹² A report that IgG from patients with the Lambert-Eaton syndrome interferes with this activity¹³ focused attention on subtypes of calcium channels in small-cell lung carcinoma.^{2-4,14,15} The subtype of a calcium channel depends on its α_1 subunit (Fig. 2), which contains the voltage sensor, antagonist-binding sites, and cation pore. Auxiliary subunits include $\alpha_2\delta$ and β (also γ for L-type channels of muscle and "95 K" for N-type channels).¹⁶⁻¹⁹ The calci-

um channels in small-cell carcinomas were initially reported to be L-like¹³⁻¹⁵ and N-like,^{4,14,15} because of pharmacologic sensitivities to dihydropyridines and a snail-derived neurotoxin, ω -peptide GvIA. A molecular classification of calcium channels, based on DNA sequences of α_1 subunits,¹⁶ led to the detection of RNA transcripts for L, N, and P/Q subtypes of calcium channels (Fig. 2) in small-cell carcinomas.²⁻⁴ The variety of calcium channels in tumors might provoke a diversity of autoantibodies that react with a particular channel subtype or several subtypes. Antibodies against extracellular segments of calcium channels in neurons could potentially cause neurologic syndromes.

Toxins from various venoms are useful for analyzing the diverse calcium channels that initiate the release of neurotransmitters.^{18,20,21} The P/Q subtype is probably the predominant mediator of neuromuscular transmission. In mice, evoked acetylcholine release is blocked at the P/Q type of synapse by a cone-snail ω -peptide called MVIIIC²¹ and a funnel-web-spider polyamine called FTX.²⁰ Antagonists of L-type and N-type channels have no effect.

We report evidence that small-cell lung carcinoma cell lines from patients with or without neurologic autoimmunity have high-affinity receptors for MVIIIC, a P/Q-type calcium-channel antagonist.^{18,21-24} Almost all the patients with the Lambert-Eaton syndrome, whether or not they had evidence of cancer, had serum antibodies to these high-affinity receptors for MVIIIC. About half the patients also had antibodies to receptors for the N-type calcium-channel antagonist GvIA. Antibodies against calcium channels were also found, at lower frequencies and titers, in patients with paraneoplastic encephalomyeloneuropathies associated with lung, ovarian, and breast cancers; in a minority of pa-

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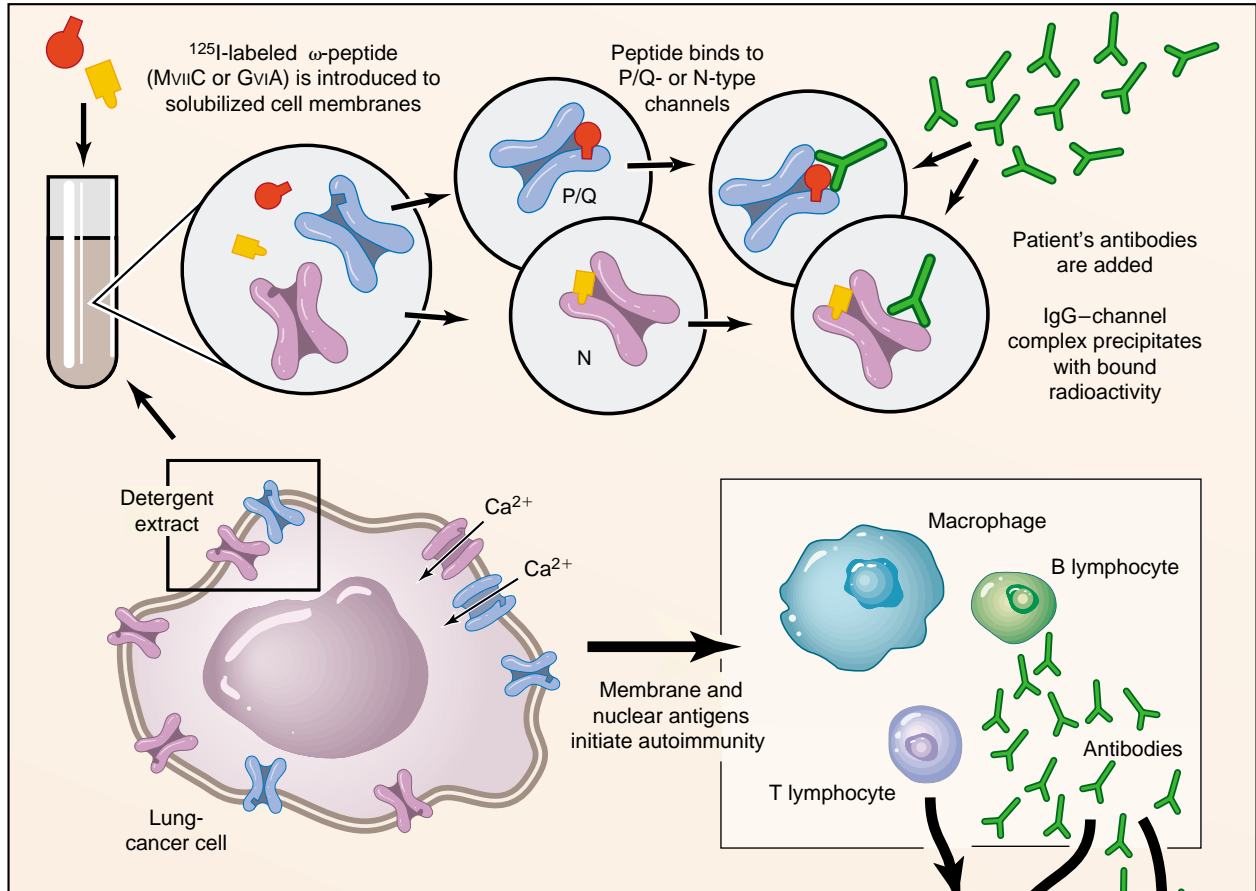
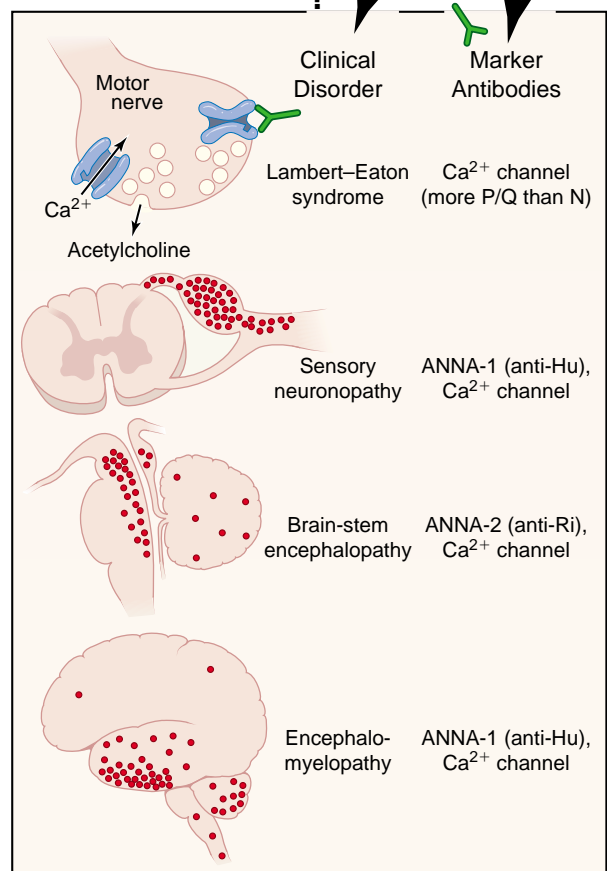


Figure 1. Antibody Assay and Model for the Induction of the Neurologic Spectrum of Autoimmunity.

Small-cell lung carcinomas have antigens in the plasma membrane, nucleus, and cytoplasm that are normally found in neurons of the peripheral and central nervous systems. Examples include the P/Q, N, and L subtypes of voltage-gated calcium channels²⁻⁴ and neuronal nuclear and cytoplasmic proteins (e.g., HuD,⁵ NOVA,⁶ and related RNA-binding proteins). The latter are antigens for paraneoplastic marker antineuronal nuclear autoantibodies type 1 and 2 (ANNA-1 and ANNA-2, also known as anti-Hu and anti-Ri, respectively).^{1,7,8} The focal necrosis that is characteristic of small-cell carcinomas releases tumor proteins and DNA to cells with antigen-presenting potential.⁹ Helper T lymphocytes activated in this fashion might therefore initiate the production of autoantibodies against a macromolecular complex of self-proteins. The assay shown detects anti-P/Q-type calcium-channel antibodies in all patients with paraneoplastic Lambert-Eaton syndrome. Antibodies directed at extracellular epitopes may explain the pathogenicity of serum IgG preparations that impair the depolarization-induced release of acetylcholine at neuromuscular synapses when injected into mice.^{10,11} No causal role is yet ascribed to antibodies against other calcium-channel subtypes. Calcium-channel antibodies complement ANNA-1 and ANNA-2 as markers for encephalomyeloneuropathies that occur with small-cell carcinoma. They also complement ANNA-2 and type 1 anti-Purkinje-cell antibodies as markers for encephalomyeloneuropathies that occur with breast and ovarian carcinomas. The implication, yet to be proved, is of a common immunobiologic basis for all these disorders, which may constitute a spectrum of pathophysiology. Commonly affected regions of the neuraxis are indicated by red dots.



tients with cancer without evident neurologic dysfunction; and in patients with sporadic amyotrophic lateral sclerosis.

METHODS

Small-Cell Lung Carcinoma Cell Lines

The small-cell lung carcinoma cell lines SCC-9 and SCC-15 were established in the neuroimmunology laboratory of the Mayo Clinic and have been characterized in earlier studies.^{14,25-27} Cells were grown in RPMI-1640 medium supplemented with 10 percent calf serum.

Calcium-45 Influx Assays

The depolarization-dependent influx of ⁴⁵Ca, assayed as described elsewhere,²⁶ was determined for each specified condition by exposing tumor cells to 90 mM potassium chloride for one minute at 37°C. Base-line ⁴⁵Ca influx in 4.7 mM potassium chloride was subtracted from influx in 90 mM potassium chloride.

Calcium-Channel Antagonists

The ω -peptide MviiC was synthesized, labeled with iodine-125, and characterized as described previously²²; ω -peptides GviA and

Aga-ivA were obtained from Peninsula Laboratories (Belmont, Calif.) and the Peptide Institute (Osaka, Japan). Nifedipine, a dihydropyridine antagonist of L-type calcium channels, was obtained from Sigma (St. Louis).

Serum Samples

The study protocol was reviewed and approved by the institutional review board at the Mayo Clinic. Patients gave oral consent for their serum to be used in studies of antineuronal antibodies. Serum was obtained from patients with specified neurologic or autoimmune disorders or cancer and from equal numbers of consecutive normal subjects, some of whom had a history of tobacco use, and stored at -20°C.

Preparation and Assays of Receptors for ω -Peptides MviiC and GviA

Membranes from homogenized small-cell lung carcinoma cell lines²⁵ and human cerebellar²⁸ and cerebral cortical²⁹ tissues obtained at autopsy were solubilized for two hours at 4°C in buffer containing digitonin (4.5 percent), HEPES (50 mM), glycerol (20 percent), aprotinin (1 kallikrein inhibitory unit per milliliter), pepstatin A (0.1 μ g per milliliter), and phenylmethylsulfonyl fluoride (2 mM), pH 7.5. The formation of complexes with radioligand, quantitation by

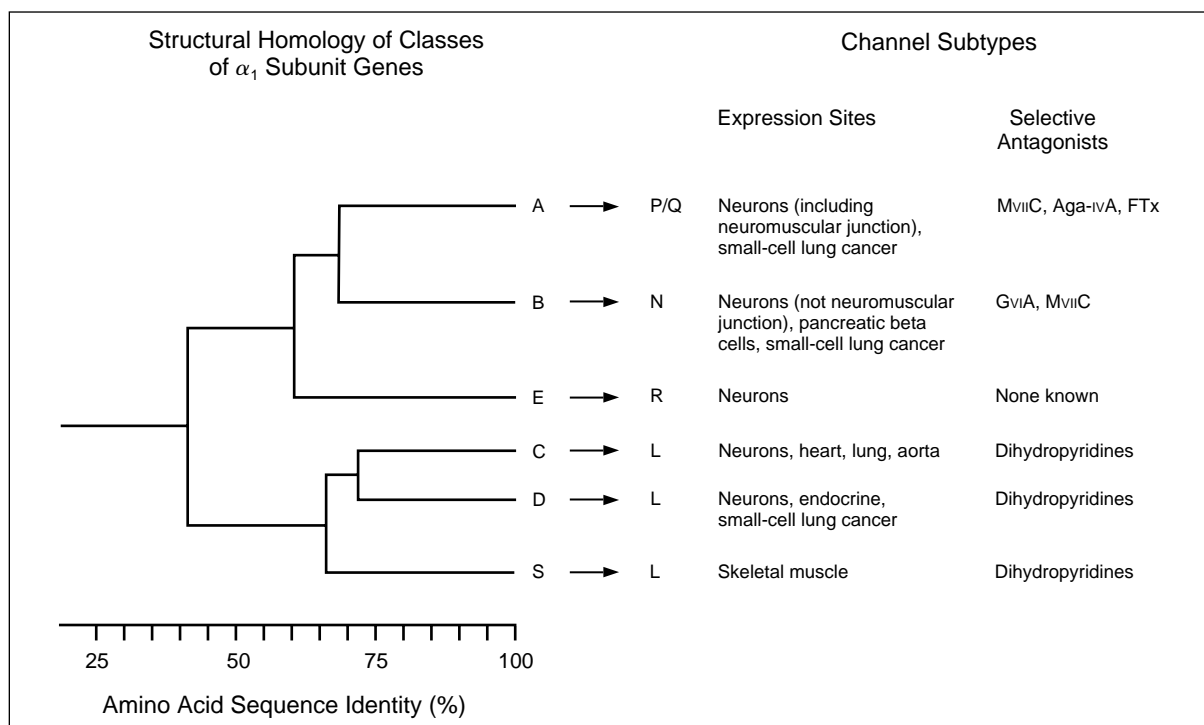


Figure 2. Classification of Mammalian High-Voltage-Activated Calcium Channels.

The subtype of a calcium channel is determined by its α_1 subunit, which is approximately 2000 amino acids long and contains the channel's voltage sensor, antagonist-binding sites, and cation pore. Auxiliary subunits include $\alpha_2\delta$ and β (also γ for L-type channels of muscle and "95 K" for N-type channels).¹⁶⁻¹⁹ The dendrogram was adapted from Zhang et al.¹⁷ The degree of homology between any pair of α_1 subunits is indicated as the percentage of sequence identity for that pair at a branch point.

The selectivity of ω -peptides GviA and MviiC for the N and P/Q subtypes depends on assay conditions. ¹²⁵I-labeled MviiC has a higher affinity for P/Q-type channels than for N-type channels under the conditions used in our study for binding and immunoprecipitation of channels solubilized from human-brain tissue. A rat monoclonal antibody against a variable-region peptide of α_1 class B sequence immunoprecipitated 100 percent of GviA receptors, but less than 0.002 percent of an equivalent amount of solubilized MviiC receptors (unpublished data). By adding excess unlabeled GviA peptide to solubilized receptors before introducing ¹²⁵I-labeled MviiC, we ensured that the antibodies designated as having specificity for P/Q-type channels in patients with the Lambert-Eaton syndrome were not immunoprecipitating N-type channels that potentially bind ¹²⁵I-labeled MviiC.

Class E- or R-type channels are the most recently discovered and least characterized of the high-voltage-activated calcium-channel subtypes. They are resistant to blockade by specific antagonists of the other types of calcium channels. Their physiologic role remains obscure. Dihydropyridines are calcium-channel blockers, such as nifedipine and nicardipine, used to treat hypertension. Data were obtained from Sher et al.,¹⁵ Oguro-Okano et al.,^{2,3} Codignola et al.,⁴ Birnbaumer et al.,¹⁶ Olivera et al.,¹⁸ Uchitel et al.,²⁰ Sugiura et al.,²¹ Hillyard et al.,²² and Kristipati et al.²³

glass-fiber filtration, and immunoprecipitation have been described elsewhere.³⁰ Receptors that formed complexes with ¹²⁵I-labeled MVIIIC or ¹²⁵I-labeled GvIA (20 pM and 150 pM, respectively) were incubated for 16 hours at 4°C with patients' serum samples (5 μl, or 10-fold dilutions) in duplicate in a final volume of 320 μl, before goat antiserum against human immune globulins was added for immunoprecipitation.

RESULTS

Pharmacologic Profile of Calcium-Channel Antagonists

As reported previously,^{13,14,26} brief exposure to high concentrations of potassium chloride stimulates the influx of ⁴⁵Ca into cultured small-cell carcinoma cell lines. Figure 3 shows the pharmacologic sensitivity of depolarization-dependent ⁴⁵Ca influx for SCC-9, a prototypic small-cell lung carcinoma cell line, and SCC-15, a cell line from a patient with the Lambert–Eaton syndrome. The ω-peptide MVIIIC, at micromolar concentrations, reduced potassium chloride–evoked calcium influx by 60 to 90 percent. Micromolar concentrations of another ω-peptide, Aga-ivA, reduced the depolarization-dependent influx of ⁴⁵Ca by 60 percent in these cell lines. GvIA, an ω-peptide antagonist of N-type channels, reduced stimulated ⁴⁵Ca influx minimally (20 to 40 percent at 100 μM), contrary to earlier reports that it had a potent inhibitory effect on small-cell carcinoma calcium channels.^{4,14} Nifedipine reduced stimulated ⁴⁵Ca influx only at millimolar concentrations, well above the submicromolar concentrations that specifically block L-type channels.³¹ These results suggest that calcium channels with P/Q-type properties account for most of the depolarization-dependent influx of calcium in small-cell carcinoma cell lines, regardless of whether the patient from whom the cells originated had a neurologic syndrome.

Quantitation and Immunoprecipitation of ω-Peptide Receptors

To optimize our assay for detecting antibodies against P/Q-type calcium channels,^{28,32} we first tested solubilized membranes from small-cell lung carcinomas and human cerebellum for receptors for MVIIIC using a filtration assay to quantitate binding sites for

¹²⁵I-labeled MVIIIC. SCC-9 membranes yielded approximately 2 fmol of receptors per milligram of protein, and cerebellar membranes approximately 1300 fmol per milligram. The apparent dissociation constant (Fig. 4) (the MVIIIC concentration required to saturate 50 percent of specific receptors) was 75 pM for digitonin-solubilized human cerebellar membranes, a value consistent with the dissociation constant for intact rat and bovine cerebellar membranes.²³ A serum reactive with P/Q-type calcium channels (from a patient with the Lambert–Eaton syndrome, identified in our preliminary studies^{28,32}) immunoprecipitated receptors from both small-cell lung carcinoma and cerebellum. Despite an approximately 50 times greater preference for P/Q-type calcium channels,²³ MVIIIC can bind to both P/Q-type and N-type channels. At the concentrations of radiotracer used in our experiments (approximating the dissociation constant for P/Q channels), virtually all ¹²⁵I-labeled MVIIIC binding was predicted to be to P/Q-type channels.²³ To determine whether N-type calcium channels with a low affinity for ¹²⁵I-labeled MVIIIC were detected in the immunoprecipitation assay, we retested in duplicate serum samples from 56 patients with the Lambert–Eaton syndrome that immunoprecipitated ¹²⁵I-labeled MVIIIC receptor complexes, using a receptor preparation containing a 1400-fold excess of unlabeled GvIA to render N-type channels inaccessible for radiolabeling by ¹²⁵I-labeled MVIIIC. Immunoprecipitation of ¹²⁵I-labeled MVIIIC–receptor complexes was not significantly reduced (the mean [±SE] value obtained with pretreatment was 91 ± 1 percent of the mean value obtained without pretreatment). This indicated specific immunoprecipitation of P/Q-type calcium channels.

Frequency of Anti-P/Q-Type and Anti-N-Type Calcium-Channel Antibodies

We used cerebellum as the source of P/Q-type channels to screen serum samples from the patients (Fig. 5 and Table 1) because of its high density of MVIIIC receptors. N-type channels (GvIA receptors) were obtained from human cerebral cortical membranes.²⁹

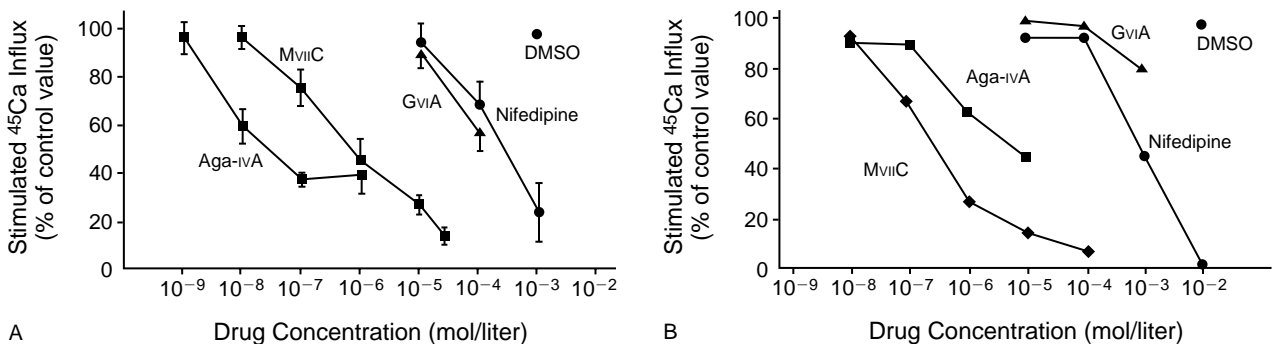


Figure 3. Pharmacologic Sensitivity of Depolarization-Dependent ⁴⁵Ca Influx in Two Lines of Small-Cell Lung Carcinoma Cells. SCC-9 (Panel A) is from a 60-year-old man without paraneoplastic neurologic complications; SCC-15 (Panel B) is from a 59-year-old woman with the Lambert–Eaton syndrome and serum antibodies reactive with neuronal receptors for the ω-peptides MVIIIC (titer, 49 pM) and GvIA (titer, 133 pM). Each point represents the mean value of three to seven experiments done in duplicate, expressed as the percentage of potassium-stimulated ⁴⁵Ca influx (i.e., influx at 90 mM potassium chloride minus influx at 4.7 mM potassium chloride) in the absence of drug. DMSO denotes dimethylsulfoxide, which was used as a vehicle for nifedipine.

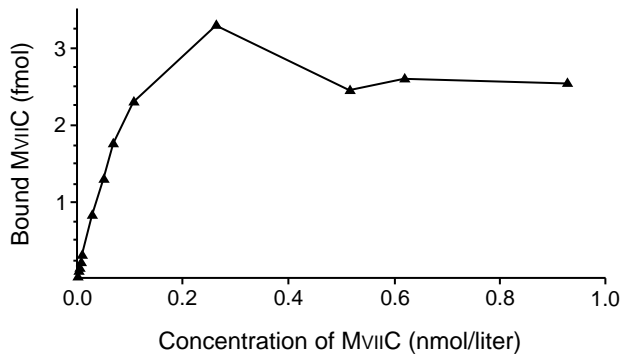


Figure 4. Specific Binding of ^{125}I -labeled MvIIc to Digitonin-Solubilized Membranes Prepared from Cerebellar Cortex Obtained at Autopsy from a Patient without Neurologic Disease.

The binding was determined by filtration with glass-fiber filters; $8.7\ \mu\text{g}$ of protein was incubated with ^{125}I -labeled MvIIc at 4°C for 5.5 hours. The amount of nonspecific binding in the presence of $1.73\ \mu\text{M}$ unlabeled MvIIc was subtracted from the total.

The serum samples of all 32 patients who had the Lambert-Eaton syndrome and cancer were positive for anti-P/Q-type channel antibodies. Among 33 patients with the syndrome but without cancer, 30 (91 percent) had anti-P/Q-type channel antibodies. This high frequency suggests that autoantibodies with specificity for P/Q-type calcium channels have a pathogenic role in the syndrome. Only 1 of 47 normal subjects (2 percent) and 1 of 69 patients (1 percent) with myasthenia gravis, epilepsy, or scleroderma had anti-P/Q-type calcium-channel antibodies.

In results consistent with our earlier studies using N-type channels (solubilized from small-cell carcinomas with the detergent 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate [CHAPS]³⁰ and from cerebral cortical membranes with digitonin^{19,29}), we found antibodies against N-type calcium channels in 19 of 26 patients (73 percent) with the Lambert-Eaton syndrome and primary lung carcinoma (small cell, squamous cell,³⁰ or adenocarcinoma) (Table 1). These antibodies were significantly less common among patients with the Lambert-Eaton syndrome (12 of 33, or 36 percent) who had no evidence of cancer or who had cancers other than lung cancer (1 of 6, or 17 percent; $P < 0.01$ by chi-square analysis).

Serum from patients with cancer and no evident neurologic dysfunction had a relatively low frequency of antibodies against P/Q-type and N-type calcium channels (20 of 71 patients with small-cell lung carcinoma, or 28 percent; and 2 of 19 with ovarian carcinoma, or 11 percent) (Fig. 5 and Table 1). Median values were relatively low in this group (P/Q-type, 43 pM and 72 pM, respectively; and N-type, 67 pM and 52 pM, respectively). Of 70 patients who had small-cell lung or ovarian or breast carcinoma with a paraneoplastic encephalomyeloneuropathic complication but without evidence of the Lambert-Eaton syndrome, 38 (54 percent) had anti-calcium-channel antibodies (19 P/Q-type only, 10 N-type only, and 9 both types). Median values (P/Q-type, 83 pM; and N-type, 179 pM) were between those of patients with the Lambert-Eaton

syndrome and those of patients with cancer and no evident neurologic dysfunction. Anti-calcium-channel antibodies were also found in 18 of 78 patients (23 percent) with amyotrophic lateral sclerosis (Table 1). These findings suggest that antigenically related calcium channels of the P/Q, N, or L type may be targets for pathogenic autoantibodies in disorders other than the Lambert-Eaton syndrome.

DISCUSSION

Our finding of anti-P/Q-type calcium-channel autoantibodies in 95 percent of 65 patients with the Lambert-Eaton syndrome implies that these antibodies are a determinant of presynaptic pathophysiology in this disorder. The low frequency of these antibodies (2 percent) in patients with myasthenia gravis or scleroderma and in normal subjects supports the clinical usefulness of detecting antibodies with brain-derived antigens that have high-affinity receptors for ω -peptides.

Different calcium-channel α_1 subunits have regions of considerable amino acid sequence homology (Fig. 2), which presumably represent homologies of structure and antigenicity. About half the patients with the Lambert-Eaton syndrome had antibodies reactive with

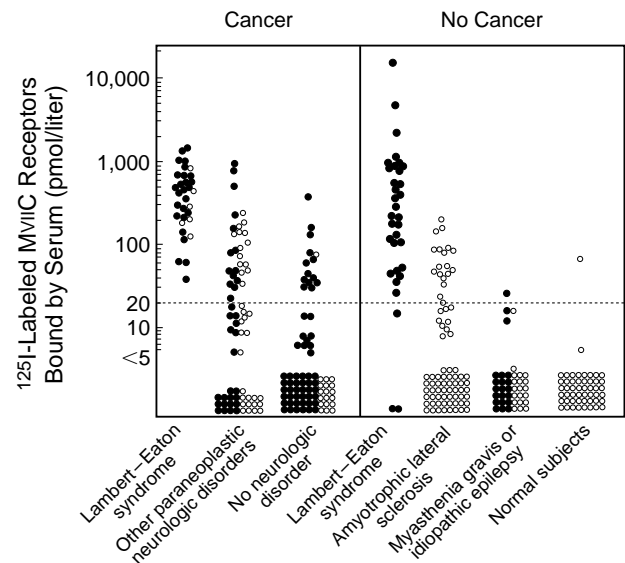


Figure 5. Distribution of Values for P/Q-type Calcium-Channel Antibodies in Serum Samples from Patients with Various Types of Cancer and Neurologic Disorders and from Normal Subjects.

The horizontal line at 20 pM represents the upper limit of the normal range (2 SD above the mean value for 47 normal subjects). Patients with cancer and the Lambert-Eaton syndrome had primary lung carcinoma (●) or some other cancer (○); patients with paraneoplastic encephalomyeloneuropathies had small-cell lung carcinoma (or ANNA-1 or ANNA-2 autoantibody as a marker of small-cell lung carcinoma¹) (●) or ovarian or breast carcinoma (or PCA-1 or ANNA-2 as a corresponding marker autoantibody¹) (○); patients with cancer and no evident neurologic dysfunction had small-cell lung carcinoma (●) or ovarian carcinoma (○). Patients with no evidence of cancer had the Lambert-Eaton syndrome, amyotrophic lateral sclerosis, or myasthenia gravis (●) or idiopathic epilepsy (○), or they were normal subjects matched to patients with the Lambert-Eaton syndrome for age, sex, and smoking habits. Results for 28 patients with scleroderma were all negative and are not shown.

Table 1. Frequency of Antibodies to P/Q-Type and N-Type Voltage-Gated Calcium Channels in Patients with Various Neurologic, Neoplastic, and Autoimmune Disorders and in Normal Subjects.

GROUP AND DIAGNOSIS	NO. OF PATIENTS	P/Q-TYPE POSITIVE		N-TYPE POSITIVE*		P/Q- OR N-TYPE POSITIVE PERCENT
		PERCENT	MECHAN VALUE	PERCENT	MECHAN VALUE	
			<i>pM</i>		<i>pM</i>	
Patients with the Lambert–Eaton syndrome						
Lung cancer	26	100	499	73	256	100
Other cancer	6	100	250	17	56	100
No cancer	33	91	300	36	165	91
Patients with paraneoplastic encephalomyeloneuropathies†	70	40	83	27	179	54
Control patients with cancer						
Small-cell lung carcinoma	71	18	43	22	67	28
Ovarian carcinoma	19	5	72‡	5	52‡	11
Control patients with neurologic diseases						
Amyotrophic lateral sclerosis	78	22	55	13	39	23
Myasthenia gravis	21	5	26‡	0	<20	5
Epilepsy	20	0	<20	0	<20	0
Control patients with an autoimmune disease						
Scleroderma	28	0	<20	4	45‡	4
Normal subjects	47	2	59‡	0	<20	2

*Patients with the Lambert–Eaton syndrome and lung cancer were more frequently seropositive than patients with the Lambert–Eaton syndrome and another type of cancer or no evidence of cancer ($P=0.0017$ by the chi-square test). Of the 26 cases of lung cancer, 25 were small-cell carcinoma and 1 was adenocarcinoma. The other cancers consisted of one case each of mastocytosis, Hodgkin's lymphoma and renal carcinoma, mixed parotid tumor, and carcinomas of colon, uterus, and skin.

†An antineuronal nuclear antibody marker of small-cell lung, ovarian, or breast carcinoma^{1,5-8,33} was found in 68 of the 70 patients with paraneoplastic encephalomyeloneuropathies. In most of the patients, detection of these autoantibodies (known as type 1 and 2 antineuronal nuclear autoantibody and type 1 anti–Purkinje-cell cytoplasmic antibody, or anti-Hu, anti-Ri, and anti-Yo)¹ expedited the diagnosis of cancer. Associated carcinomas consisted of small-cell lung (29 patients), ovarian (20 patients), breast (10 patients), and uterine cervix (1 patient). Of 10 patients without a documented tumor, 6 (positive for type 1 or 2 antineuronal nuclear autoantibody) had a history of tobacco use, and 1 (positive for type 1 anti–Purkinje-cell cytoplasmic antibody) was seropositive for the ovarian carcinoma antigen, CA-125. Predominant neurologic manifestations were subacute cerebellar ataxia (34 patients), limbic or midbrain encephalopathies, myelopathies (10 patients), and sensorimotor or autonomic peripheral neuropathies (26 patients).

‡Value is for one subject only.

N-type calcium channels. This finding may reflect immunity against homologous regions of α_1 subunits of N-type and P/Q-type calcium channels. However, in some serum samples we found evidence of independent responses to N-type and P/Q-type calcium channels. In 15 patients, antibodies with specificity for N-type calcium channels were present in higher titers than antibodies of P/Q-type specificity. No pathophysiologic role has been ascribed to antibodies with specificity for N-type calcium channels. Various frequencies have been reported in patients with the Lambert–Eaton syndrome.^{29,30,34,35} The findings in the present study confirm our earlier finding that the detection of N-type calcium-channel antibodies in a patient with the Lambert–Eaton syndrome increases the likelihood of discovering an underlying primary lung cancer. In contrast, nearly all patients with the Lambert–Eaton syndrome, with or without a tumor, are seropositive for anti–P/Q-type calcium-channel antibodies.

Immunoprecipitation assays of the type used in this study can detect autoantibodies with and without pathogenic potential.¹⁹ In living neurons, most of the structure of the calcium-channel protein is inaccessible to

antibodies because it is largely sequestered in the plasma membrane and cytoplasm (Fig. 1). Solubilization with detergent, however, would render more antigenic sites accessible in vitro. Using Western blotting, Rosenfeld et al.³⁶ found that three of seven patients with the Lambert–Eaton syndrome had antibodies to human-brain calcium-channel β subunits, which are predicted to be cytoplasmic.¹⁹ Leveque et al.³⁷ suggested that the primary target of Lambert–Eaton antibodies might be the neurotransmitter-vesicle protein synaptotagmin rather than the calcium-channel protein itself. Synaptotagmin is exposed in the synapse transiently during exocytosis of transmitter, and in certain detergents it associates noncovalently with calcium-channel proteins. In agreement with the observations of Leveque et al. on rat-brain GVIA receptors extracted in Triton X-100,³⁷ we found that human-brain GVIA receptors (and MVIIIC receptors) in CHAPS detergent were immunoprecipitated by a mouse antisynaptotagmin monoclonal antibody (MAb 48, provided by Dr. Louis Reichardt, University of California, San Francisco). However, in digitonin, the detergent that we have found optimal for the immunoprecipitation of calcium channels by IgG from patients with the Lambert–Eaton syndrome,^{28,32} neither GVIA nor MVIIIC receptors were appreciably immunoprecipitated by antisynaptotagmin antibody.

Small-cell lung carcinoma and other cancers are sometimes complicated by paraneoplastic neurologic disorders other than the myasthenic syndrome (Fig. 1). We studied 70 patients with an assortment of these other paraneoplastic neurologic disorders. Most patients had a marker antibody in their serum appropriate to their tumor (Table 1). Patients with encephalopathy, cerebellar ataxia, myelopathy, or peripheral neuropathy had type 1 or type 2 antineuronal nuclear autoantibodies. These serologic markers of small-cell carcinoma¹ are directed at nuclear antigens in neurons and tumors.^{1,5-7} Female patients with breast carcinoma who had midbrain encephalitis and myelopathy also had type 2 antineuronal nuclear autoantibodies,^{1,6,8} and female patients with ovarian or breast carcinoma who had subacute cerebellar ataxia had type 1 anti–Purkinje-cell cytoplasmic antibodies.^{1,33} In 38 of these patients (54 percent), we detected anti–P/Q-type or anti–N-type calcium-channel antibodies.

It has not yet been shown that antibodies against a

particular subtype or extracellular segment of calcium channel can impair neuromuscular transmission. Nor is it known whether any antibody (or T cells) against calcium channels (Fig. 1) can actually cause a paraneoplastic encephalomyeloneuropathy. Evidence might be obtained experimentally by injecting rats or mice³ with antibodies of a defined calcium-channel specificity (affinity-purified or monoclonal) or by immunizing animals with antigenic polypeptides of α_1 -subunit sequences. The different neurologic presentations of seropositive patients in the three groups with cancer (the Lambert-Eaton syndrome, encephalomyeloneuropathy, and apparent neurologic normality) (Fig. 5) might be explained by polyclonality of an individual patient's immune responses and by a multiplicity of epitopes immunogenic for B cells in any given calcium-channel subtype. An example is a patient we studied who had cerebellar ataxia related to ovarian carcinoma but no detectable neuromuscular transmission defect.³⁸ She had serum antibodies reactive with N-type calcium channels (titer, 322 pM), but negligible P/Q-type antibodies (titer, 16 pM). In contrast is a patient who had clinical and electrophysiologic signs of the Lambert-Eaton syndrome without evident central nervous system problems or cancer; this patient had serum antibodies reactive with P/Q-type calcium channels at 918 pM, which was 16 times higher than the level of N-type calcium-channel antibodies (57 pM). In mice injected with the IgG fraction of serum from that patient,¹¹ a profound neuromuscular transmission defect developed.

Smith et al.³⁹ reported that 75 percent of patients with sporadic amyotrophic lateral sclerosis and 75 percent of patients with the Lambert-Eaton syndrome have antibodies that bind to L-type calcium channels purified from skeletal muscle. Muscle is not primarily involved in the pathophysiology of either disorder. Llinas et al.⁴⁰ reported that IgG prepared from the serum of a patient with amyotrophic lateral sclerosis affected P-type calcium-channel activity in dissociated cerebellar Purkinje cells. We found P/Q-type and N-type calcium-channel antibodies in a minority of the 78 patients with amyotrophic lateral sclerosis we studied. This result may also reflect the detection of antibodies that cross-react with multiple calcium-channel subtypes (Fig. 2).

These observations provide evidence of the antigenic relatedness of different classes of high-voltage-activated calcium channels. Although antibodies against cytoplasmic epitopes are not anticipated to be pathogenic, they might serve as markers of either antitumor immunity or a neurologic spectrum of autoimmunity (Fig. 1). On the other hand, as illustrated by the Lambert-Eaton syndrome, antibodies specific for calcium-channel epitopes that are exposed in discrete anatomical regions may have a pathogenic role in both paraneoplastic and nonparaneoplastic neurologic disorders.

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REFERENCES

1. Lennon VA. Paraneoplastic autoantibodies: the case for a descriptive generic nomenclature. *Neurology* 1994;44:2236-40, 2412-5.
2. Oguro-Okano M, Griesmann GE, Wieben ED, Slaymaker S, Snutch TP, Lennon VA. Molecular diversity of neuronal-type calcium channels identified in small cell lung carcinoma. *Mayo Clin Proc* 1992;67:1150-9.
3. Oguro-Okano M, Griesmann GE, Kryzer T, Wieben ED, Lennon VA. N-type (class B) neuronal Ca^{2+} channels expressed in small cell lung carcinomas of patients with and without Lambert-Eaton myasthenic syndrome (LES). *Soc Neurosci Abstr* 1993;19:1755.
4. Codignola A, Tarroni P, Clementi F, et al. Calcium channel subtypes controlling serotonin release from human small cell lung carcinoma cell lines. *J Biol Chem* 1993;268:26240-7.
5. Szabo A, Dalmau J, Manley G, et al. HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and Sex-lethal. *Cell* 1991;67:325-33.
6. Buckanovich RJ, Posner JB, Darnell RB. Nova, the paraneoplastic Ri antigen, is homologous to an RNA-binding protein and is specifically expressed in the developing motor system. *Neuron* 1993;11:657-72.
7. Kiers L, Altermatt HJ, Lennon VA. Paraneoplastic anti-neuronal nuclear IgG autoantibodies (type I) localize antigen in small cell lung carcinoma. *Mayo Clin Proc* 1991;66:1209-16.
8. Luque FA, Fumeaux HM, Ferziger R, et al. Anti-Ri: an antibody associated with paraneoplastic opsoclonus and breast cancer. *Ann Neurol* 1991;29:241-51.
9. Looser P, Laissue JA. Extrazelluläre Deoxyribonukleinsäure in kleinzelligen Bronchuskarzinomen. *Schweiz Med Wochenschr* 1980;110:1342-6.
10. Fukunaga H, Engel AG, Lang B, Newsom-Davis J, Vincent A. Passive transfer of Lambert-Eaton myasthenic syndrome with IgG from man to mouse depletes the presynaptic membrane active zones. *Proc Natl Acad Sci U S A* 1983;80:7636-40.
11. Lambert EH, Lennon VA. Selected IgG rapidly induces Lambert-Eaton myasthenic syndrome in mice: complement independence and EMG abnormalities. *Muscle Nerve* 1988;11:1133-45.
12. McCann FV, Pettengill OS, Cole JJ, Russell JAG, Sorenson GD. Calcium spike electrogenesis and other electrical activity in continuously cultured small cell carcinoma of the lung. *Science* 1981;212:1155-7.
13. Roberts A, Perera S, Lang B, Vincent A, Newsom-Davis J. Paraneoplastic myasthenic syndrome IgG inhibits $^{45}Ca^{2+}$ flux in a human small cell carcinoma line. *Nature* 1985;317:737-9.
14. De Aizpurua HJ, Lambert EH, Griesmann GE, Olivera BM, Lennon VA. Antagonism of voltage-gated calcium channels in small cell carcinomas of patients with and without Lambert-Eaton myasthenic syndrome by autoantibodies omega-conotoxin and adenosine. *Cancer Res* 1988;48:4719-24.
15. Sher E, Biancardi E, Passafaro M, Clementi F. Physiopathology of neuronal voltage-operated calcium channels. *FASEB J* 1991;5:2677-83.
16. Birnbaumer L, Campbell KP, Catterall WA, et al. The naming of voltage-gated calcium channels. *Neuron* 1994;13:505-6.
17. Zhang JF, Randall AD, Ellinor PT, et al. Distinctive pharmacology and kinetics of cloned neuronal Ca^{2+} channels and their possible counterparts in mammalian CNS neurons. *Neuropharmacology* 1993;32:1075-88.
18. Olivera BM, Miljanich GP, Ramachandran J, Adams ME. Calcium channel diversity and neurotransmitter release: the omega-conotoxins and omega-agatoxins. *Annu Rev Biochem* 1994;63:823-67.
19. Lennon VA. Serological diagnosis of myasthenia gravis and the Lambert-Eaton myasthenic syndrome. In: Lisak R, ed. *Handbook of myasthenia gravis*. New York: Marcel Dekker, 1994:149-64.
20. Uchitel OD, Protti DA, Sanchez V, Cherksey BD, Sugimori M, Llinas R. P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. *Proc Natl Acad Sci U S A* 1992;89:3330-3.
21. Sugiura Y, Woppmann A, Miljanich GP, Ko C-P. A novel ω -conopeptide for the presynaptic localization of calcium channels at the mammalian neuromuscular junction. *J Neurocytol* 1995;24:15-27.
22. Hillyard DR, Monje VD, Mintz IM, et al. A new Conus peptide ligand for mammalian presynaptic Ca^{2+} channels. *Neuron* 1992;9:69-77.
23. Kristipati R, Nadasdi L, Tarczy-Hornoch K, et al. Characterization of the binding of omega-conopeptides to different classes of non-L-type neuronal calcium channels. *Mol Cell Neurosci* 1994;5:219-28.
24. Wheeler DB, Randall A, Tsien RW. Roles of N-type and Q-type Ca^{2+} channels in supporting hippocampal synaptic transmission. *Science* 1994;264:107-11.
25. Cunningham JM, Lennon VA, Lambert EH, Scheithauer B. Acetylcholine receptors in small cell carcinomas. *J Neurochem* 1985;45:159-67.
26. Williams CL, Lennon VA. Activation of M₁ muscarinic acetylcholine receptors inhibits voltage-dependent calcium influx in small cell lung carcinoma. *J Biol Chem* 1990;265:1443-7.
27. *Idem*. Activation of muscarinic acetylcholine receptors inhibits cell cycle progression of small cell lung carcinoma. *Cell Regul* 1991;2:373-81.
28. Lennon VA, Kryzer T, Griesmann GE, et al. P-like Ca^{2+} channels in human cerebellum and small cell lung carcinoma bind ω -conotoxin-MVIIIC and are immunoprecipitable by Lambert-Eaton myasthenic syndrome (LES) IgGs that react highly with N-type Ca^{2+} channels. *Soc Neurosci Abstr* 1993;19:1753.

29. Lennon VA. Reactivity of Lambert-Eaton autoantibodies with voltage-gated calcium channel complex of human cerebral cortex. *Ann Neurol* 1990;28:281.
30. Lennon VA, Lambert EH. Autoantibodies bind solubilized calcium channel-omega-conotoxin complexes from small cell lung carcinoma: a diagnostic aid for Lambert-Eaton myasthenic syndrome. *Mayo Clin Proc* 1989;64:1498-504.
31. Triggle DJ, Rampe D. 1,4-Dihydropyridine activators and antagonists: structural and functional distinctions. *Trends Pharmacol Sci* 1989;10:507-11.
32. Lennon VA, Kryzer T, Griesmann GE, Miljanich G, Ramachandran J, Lambert EH. Human neuronal P-like calcium channels (omega-conotoxin-MVIIIC-receptors) are immunoprecipitated by serum IgG of patients with various central and peripheral nervous system disorders, with and without evidence of tumor. *Muscle Nerve* 1994;Suppl 1:S22. abstract.
33. Furneaux HM, Rosenblum MK, Dalmau J, et al. Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. *N Engl J Med* 1990;322:1844-51.
34. Sher E, Gotti C, Canal N, et al. Specificity of calcium channel autoantibodies in Lambert-Eaton myasthenic syndrome. *Lancet* 1989;2:640-3.
35. Leys K, Lang B, Johnston I, Newsom-Davis J. Calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. *Ann Neurol* 1991;29:307-14.
36. Rosenfeld MR, Wong E, Dalmau J, et al. Cloning and characterization of a Lambert-Eaton myasthenic syndrome antigen. *Ann Neurol* 1993;33:113-20.
37. Leveque C, Hoshino T, David P, et al. The synaptic vesicle protein synaptotagmin associates with calcium channels and is a putative Lambert-Eaton myasthenic syndrome antigen. *Proc Natl Acad Sci U S A* 1992;89:3625-9.
38. O'Suilleabhain PE, Lambert EH, Lennon VA. Anti-voltage-gated calcium channel antibodies in women with paraneoplastic cerebellar degeneration (PCD) and seropositivity for type 1 anti-Purkinje cell antibody (PCAb1⁺). *Neurology* 1994;44:Suppl 2:A157. abstract.
39. Smith RG, Hamilton S, Hofmann F, et al. Serum antibodies to L-type calcium channels in patients with amyotrophic lateral sclerosis. *N Engl J Med* 1992;327:1721-8.
40. Llinas R, Sugimori M, Cherksey BD, et al. IgG from amyotrophic lateral sclerosis patients increases current through P-type calcium channels in mammalian cerebellar Purkinje cells and in isolated channel protein in lipid bilayer. *Proc Natl Acad Sci U S A* 1993;90:11743-7.