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Lambert–Eaton sera reduce low-voltage and high-voltage activated Ca^{2+} currents in murine dorsal root ganglion neurons

(calcium channels/calcium conductance/potassium currents/transmitter release/active zones)

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ABSTRACT Voltage-gated Ca^{2+} channels are categorized as either high-voltage activated (HVA) or low-voltage activated (LVA), and a subtype (or subtypes) of HVA Ca^{2+} channels link the presynaptic depolarization to rapid neurotransmitter release. Reductions in transmitter release are characteristic of the autoimmune disorder, Lambert–Eaton syndrome (LES). Because antibodies from LES patients reduce Ca^{2+} influx in a variety of cell types and disrupt the intramembrane organization of active zones at neuromuscular synapses, specificity of LES antibodies for the Ca^{2+} channels that control transmitter release has been suggested as the mechanism for disease. We tested sera from four patients with LES. Serum samples from three of the four patients reduced both the maximal LVA and HVA Ca^{2+} conductances in murine dorsal root ganglion neurons. Thus, even though LES is expressed as a neuromuscular and autonomic disorder, our studies suggest that Ca^{2+} channels may be broadly affected in LES patients. To account for the specificity of disease expression, we suggest that incapacitation of only a fraction of the Ca^{2+} channels clustered at active zones would severely depress transmitter release. In particular, if several Ca^{2+} channels in a cluster are normally required to open simultaneously before transmitter release becomes likely, the loss of a few active zone Ca^{2+} channels would exponentially reduce the probability of transmitter release. This model may explain why LES is expressed as a neuromuscular disorder and can account for a clinical hallmark of LES, facilitation of neuromuscular transmission produced by vigorous voluntary effort.

Lambert–Eaton myasthenic syndrome (LES) is a human autoimmune disorder characterized by autonomic dysfunction and muscle weakness resulting from reduced neurotransmitter release (1, 2). At neuromuscular junctions, transmitter release occurs at discrete sites called active zones (3, 4). In freeze-fracture views of neuromuscular synapses, intramembrane particles, which are thought to include Ca^{2+} channels, are organized in linear arrays at active zones (5–7). The hypothesis that LES antibodies target the Ca^{2+} channels that control transmitter release arose from the observation that LES antibodies decrease the number of active zones and disrupt active zone organization in mice and humans (8–10). Further support for this hypothesis has been based on the demonstration that LES antibodies decrease Ca2+ influx in transformed cells such as small cell lung carcinoma (11, 12), rat (13) and human (14) neuroblastoma, and rat thyroid cells (15), as well as in secretory cells such as adrenal chromaffin (16–18) and rat pituitary cells (19). It is uncertain that these cells, which are either transformed or involved in hormonal secretion, express the same kinds of Ca^{2+} channels that are contained in normal neurons (20). Here, we have investigated the specificity of LES antibodies for Ca^{2+} channels in murine dorsal root ganglion (DRG) neurons.

DRG neurons express low-voltage activated (LVA) and high-voltage activated (HVA) Ca^{2+} currents. The LVA current appears to arise from a single kind of channel, referred to as T-type (21). On the basis of electrophysiological and pharmacological criteria, the HVA currents in DRG neurons are categorized as L-, N-, and P-type, plus at least one more type that is not yet well defined (22, 23). Depending on the species and synapse in question, various HVA channels have been reported to control neurotransmitter release. For example, N-, P-, and Q-type channels have been implicated in controlling transmitter release in the mammalian central nervous system (24, 25). In rat postganglionic sympathetic neurons (26) and chicken preganglionic parasympathetic neurons (27), N-type channels appear to play the dominant role in controlling transmitter release, whereas at rodent neuromuscular junctions, both P-type (mice; ref. 28) and N-type (rats; ref. 29) channels are reportedly involved.

Here, we report the effects of LES serum on normal neurons. In our experiments, LVA and HVA Ca^{2+} currents were measured in cultured DRG neurons after a 24-hr exposure to serum from patients with LES and in parallel-control neurons. The ability of serum samples to reduce LVA and HVA Ca^{2+} conductances in DRG neurons was consistent with the electromyographic diagnosis of LES and indicated that LES antibodies act on several Ca^{2+} channel types. The generalized reduction in Ca^{2+} current raises important questions as to why the LES antibodies impair neurotransmitter release from motor and autonomic nerve terminals without significantly affecting muscle or endocrine organs whose function also depends critically on Ca^{2+} channels. Preliminary accounts of some of these findings have been presented previously (30, 31).

MATERIALS AND METHODS

Serum Preparation. Serum obtained from four LES patients was dialyzed (exclusion of ≥100 kDa) for 24 hr against culture medium at a sample-to-dialysate ratio of approximately 1:100, with one dialysate change at about 8 hr (12). Initial experiments for Patient One used whole serum without dialysis; because no obvious differences were observed between whole and dialyzed serum, the results for this patient were pooled and averaged. Serum was added to the culture medium at approximately 1:20 dilution and incubated with the cells overnight.

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Abbreviations: LES, Lambert–Eaton syndrome; DRG, dorsal root ganglion; HVA, high-voltage activated; LVA, low-voltage activated; G_{maxL}, maximal conductance of the LVA current; G_{maxH}, maximal conductance of the HVA current; CMAP, compound muscle action potential; BAPTA, bis(2-aminophenoxy)ethane-*N*,*N*,*N'*,*N'*-tetraacetate.

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DRG Cultures. DRG were removed from 1- to 3-day-old mouse pups, which had been anesthetized and decapitated, and placed in oxygenated rodent physiological saline (146 mM NaCl/5 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/10 mM Hepes/11 mM glucose, pH 7.4). The isolated ganglia were incubated for 20 min at 35°C in 1 ml of oxygenated Pipes buffered saline (120 mM NaCl/5 mM KCl/1 mM $CaCl_2$/1 mM $MgCl_2$/25 mM glucose/20 mM Pipes, pH 7.0) containing 0.5% Type XI trypsin (Sigma) and 0.01% DNAse I (Sigma). After incubation, ganglia were triturated with a fire-polished pipette in "mouse spinal medium" (2 mM glutamine/10 mM $NaHCO_3$/1 mM pyruvate/10 mM Hepes), 100 μg/ml streptomycin, 60 μg/ml penicillin, 10% (vol/vol) fetal bovine serum, 5% horse serum, 5% chicken embryo extract, and 80% MEM with Earle's salts (pH 7.4; GIBCO). Dissociated cells were plated onto 35-mm dishes coated with poly-L-lysine (4–15 kDa; 1 mg/ml in 0.15 M boric acid, pH 8.4) and maintained at 37°C in humidified 95% air and 5% CO_2.

Dissociated DRG cells from a single animal were cultured on several dishes. The dishes were divided into parallel control and test groups. Test dishes were incubated overnight with test serum from one of four patients who showed indications of LES. Parallel control dishes were incubated overnight with normal human serum, which was obtained from consenting volunteers. Ca^{2+} or K^+ currents were recorded from control and test serum-treated cells 12–24 hr after initial plating. Experiments were repeated for each test serum sample so that test serum-induced effects were measured in DRG neurons from at least two animals. Current measurements from neurons in a given serum sample were pooled, averaged, and compared with averages of measurements from parallel control cells.

Clinical Measurements. Compound muscle action potentials (CMAPs) elicited by supramaximal nerve stimulation were measured from the rested, warmed abductor pollicus brevis and abductor digiti minimi muscles of the hand before treatment was begun. Facilitation of the CMAPs was measured as the percent change in CMAP amplitude following maximum voluntary contraction of the tested muscles for 10 sec, divided by the amplitude of the preexercise CMAP.

Ionic Currents. The results of the clinical tests were revealed to those performing measurements of ionic currents only after completion of all experiments. The whole cell patch-clamp configuration (32) was used to record ionic currents from DRG neurons at room temperature (≈20°C) using a DAGAN 3900 patch-clamp amplifier equipped with a model 3911 whole-cell expander. The patch electrodes (3–4 MΩ) were made from soda-lime glass and coated with wax to reduce capacitance. Linear components of leak and capacitive currents were removed from test currents by digital subtraction of scaled control currents elicited by 20-mV hyperpolarizations from the holding potential (−80 mV). Currents were electronically filtered at 1 kHz (8-pole Bessel filter) before sampling by the computer. To normalize for differences in total membrane area, current densities were calculated by dividing total current by the linear capacitance of the cell. Data are expressed as mean ± SEM. Least squares fits were computed with NFIT software (Island Products, Galveston, TX).

To measure Ca^{2+} currents, recording electrodes contained 140 mM cesium aspartate, 5 mM $MgCl_2$, 10 mM Cs_2EGTA, and 10 mM Hepes (pH 7.4), and the extracellular recording medium contained 10 mM $CaCl_2$, 1 μM tetrodotoxin, 145 mM tetraethylammonium chloride, and 10 mM Hepes (pH 7.4). Cesium in the electrode and tetraethylammonium in the extracellular medium blocked K^+ currents and tetrodotoxin in the external solution eliminated Na^+ currents.

To measure K^+ currents, recording electrodes contained 140 mM KCl, 5 mM $MgCl_2$, 10 mM K_2EGTA, and 10 mM Hepes (pH 7.4), and the external solution contained 146 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 3 μM tetrodotoxin, and 10 mM Hepes (pH 7.4). To evaluate possible contributions of Ca^{2+}-activated K^+ currents, 10 mM K_2bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetate (BAPTA) was used instead of K_2EGTA in the patch pipette in some experiments.

Table 1. Clinical electromyography of the four patients whose sera were used in this study

Serum sample	Summed CMAP, mV	Facilitation, %
Patient One	3.4	143
Patient Two	1.0	1400
Patient Three	1.6	670
Patient Four	14.3	47

RESULTS

Serum was obtained from four patients with clinical and electromyographic evidence of abnormal neuromuscular transmission (Table 1). All four patients exhibited decrementing muscle responses to repetitive nerve stimulation at 3 Hz. The degree of neuromuscular block in each patient was assessed by measuring the resting CMAP amplitude in two hand muscles and the degree of facilitation produced in each muscle by maximal voluntary contraction for 10 sec. Responses are considered normal if the sum of the CMAP amplitudes recorded from these muscles at rest is greater than 9.8 mV and there is less than 10% facilitation. Patients One, Two, and Three exhibited findings characteristic of LES: summed, initial CMAP amplitudes ranging from 1.0 to 3.6 mV and facilitation ranging from 143% to 1400% (Table 1). For Patient Four, the summed, initial CMAP amplitude was within the normal range, but the degree of facilitation, although abnormal, was less than 100%, the level usually taken as being diagnostic of LES (Table 1). Patient Four has evidence of an overlap between myasthenia gravis and LES by virtue of having antibodies characteristic of both conditions (33).

Control and test serum-treated cells displayed transient and sustained Ca^{2+} currents separable into LVA and HVA components (Fig. 1). The LVA currents were typical for T channels (21), reaching a maximum at test potentials between −20 and −30 mV (Figs. 2 and 3), and inactivating rapidly (Fig. 1). The HVA currents, which displayed both inactivating and sustained components (Fig. 1), were maximal at test potentials between

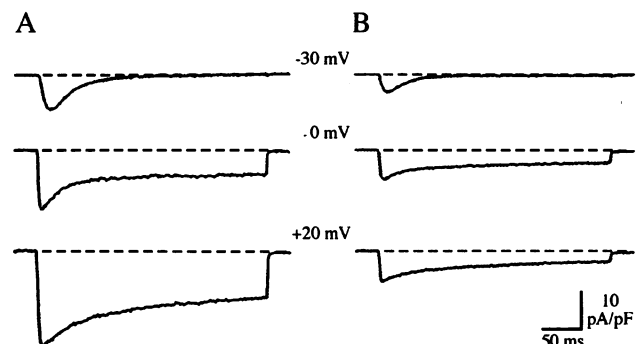

FIG. 1. Voltage-gated Ca^{2+} currents in a control (*A*) and a LES serum-treated (*B*) DRG neuron. Ca^{2+} currents were elicited by 300 msec depolarizations to varying test potentials from a holding potential of −80 mV. Currents elicited by three test potentials are shown for a cell treated with control serum and a cell treated with LES serum. For both cells, a typical, LVA, T-type current was elicited by depolarization to −30 mV, and an HVA current displaying transient and sustained components was elicited by depolarizations to 0 or +20 mV. The currents in the control and LES serum-treated cells had similar kinetics. Current amplitudes are expressed as pA/pF to normalize for differences in cell size.

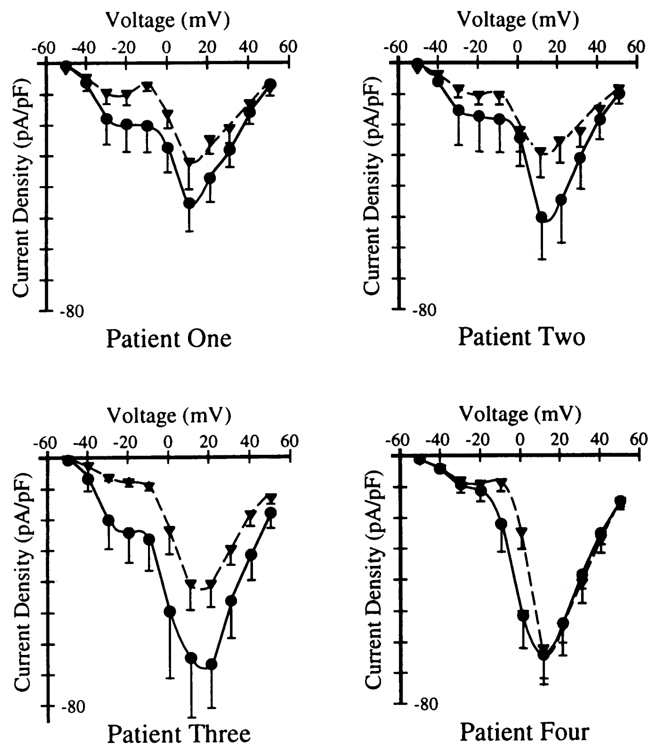

FIG. 2. Ca^{2+} current density as a function of test potential in parallel control (●) and test serum-treated (▼) DRG neurons. Densities were determined from measurements of peak current at each test potential and thus represent the sum of all the Ca^{2+} current components. The smooth curves are spline fits. Except for Patient Four, average current density was smaller at every test potential in cells treated with test serum than in the parallel control. Currents were measured in 16, 16, 20, and 25 cells treated with serum from Patients One through Four, respectively, and in 8, 8, 12, and 17 parallel control cells, respectively.

+10 and +20 mV (Figs. 2 and 3). In principle, the effects of LES serum might best be determined by comparing Ca^{2+} currents from a single cell before and after serum treatment. However, previous work has shown that more than 4 hr of LES antibody exposure is necessary to inhibit neuromuscular transmission (13, 34). Because it was not feasible to measure Ca^{2+} currents in the same cell before and after a prolonged incubation with test serum, current densities were compared between parallel populations of neurons exposed for 24 hr to control or test sera. Compared with parallel-controls, sera from Patients One, Two, and Three decreased the average current density at nearly all test potentials, but serum from Patient Four did not (Fig. 2). Changes in current density were not accompanied by qualitative changes in the kinetics and voltage dependence of Ca^{2+} currents.

DRG Ca^{2+} current densities varied greatly from cell to cell. This variability likely arises from cell-to-cell differences in the density of functional Ca^{2+} channels as well as from variability in the voltage dependence of channel activation. Because of the large variability in the current density, the test serum-induced decrease at any given potential was not always statistically significant even for Patients One, Two, and Three. However, a comparison of current density at a discrete test potential does not provide a statistical measurement of serum effects on current densities at other test potentials. To take into account serum effects at a range of potentials, the current density versus voltage relationship for each cell was fitted based on the assumption of ohmic open channel behavior of two populations of channels (LVA and HVA), each obeying the Boltzmann relationship for channel open probability (Fig. 3). This fit provides an estimate of the maximal conductances

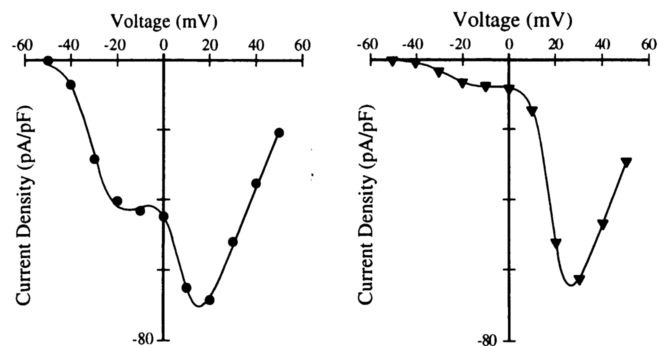

FIG. 3. Analytical fit of the current–voltage relationship for a control (●) and a test serum-treated (Patient One; ▼) DRG neuron. For each neuron, the smooth curve represents a least squares fit of the experimental data by the following equation: $I = [G_{maxL} \cdot (V - V_R)]/\{1 + \exp[(V - V_{b1})/k_1]\} + [G_{maxH} \cdot (V - V_R)]/\{1 + \exp[(V - V_{b2})/k_2]\}$, where V is the test potential; V_R is the Ca^{2+} current reversal potential; G_{maxL} and G_{maxH} are the maximum conductances of the LVA and HVA components of total Ca^{2+} current, respectively; V_{b1} and V_{b2} are potentials for half-maximal activation of the two components; and k_1 and k_2 are related to "steepness" of the voltage-dependence of activation. The fitted parameters for the control cell and test serum-treated cell, respectively, were: G_{maxL} = 0.58 and 0.07, V_R = 62.05 and 75.70, V_{b1} = −30.3 and −37.63, k_1 = 5.06 and 6.11, G_{maxH} = 1.08 and 0.57, V_{b2} = 7.74 and 8.07, and k_2 = 4.18 and 4.12. (The data for all variables are expressed in millivolts except for G_{max}, for which the unit is nanosiemens per picofarads).

associated with the LVA (G_{maxL}) and HVA (G_{maxH}) components of total current. The means for G_{maxL} and G_{maxH} were calculated for control and LES-treated neurons and compared (Student's t test). Serum from Patients One, Two, and Three reduced mean G_{maxL} by 54–72% and reduced the mean G_{maxH} by 43–54% (Fig. 4). Serum from Patient Four had no effect on either the HVA or LVA maximal conductances. Maximal conductances were the only parameters of fit found to be significantly different in control and serum-treated cells.

Because the serum-induced changes in Ca^{2+} currents could have resulted from effects of the LES sera on growth of DRG neurons in culture, cell size was estimated from measurements of whole-cell capacitance. Average capacitances were similar for experimental and control cultures (Table 2), indicating that whole-cell currents were compared between neurons of comparable size and that the effects of the LES antibodies on whole-cell currents did not result from changes in neuron growth. To determine the specificity of LES antibodies for disrupting voltage-gated Ca^{2+} channel function, K^+ currents were measured in cultured DRG neurons (Fig. 5). Current densities were determined for cultures incubated with serum from Patients One or Three and for parallel cultures incubated with control serum (Fig. 6). At positive potentials, whole-cell K^+ current densities are slightly reduced for cells treated with sera from Patients One and Three (Fig. 6). This reduction was statistically significant only for cells treated with serum from Patient Three. However, the differences were small compared with the LES-induced differences in Ca^{2+} currents. No effect or slight effects on K^+ currents are consistent with the clinical signs of LES because a large reduction in K^+ currents would

Table 2. Comparison of linear capacitance in DRG neuron treated with control and test sera

Serum sample	Range	Mean ± SEM, pF	No. of neurons
Control	22–92	39.2 ± 3.3	69
Patient One	20–72	38.9 ± 3.3	27
Patient Two	20–60	36.8 ± 3.5	16
Patient Three	17–68	42.5 ± 7.3	44
Patient Four	24–97	38.1 ± 3.5	20

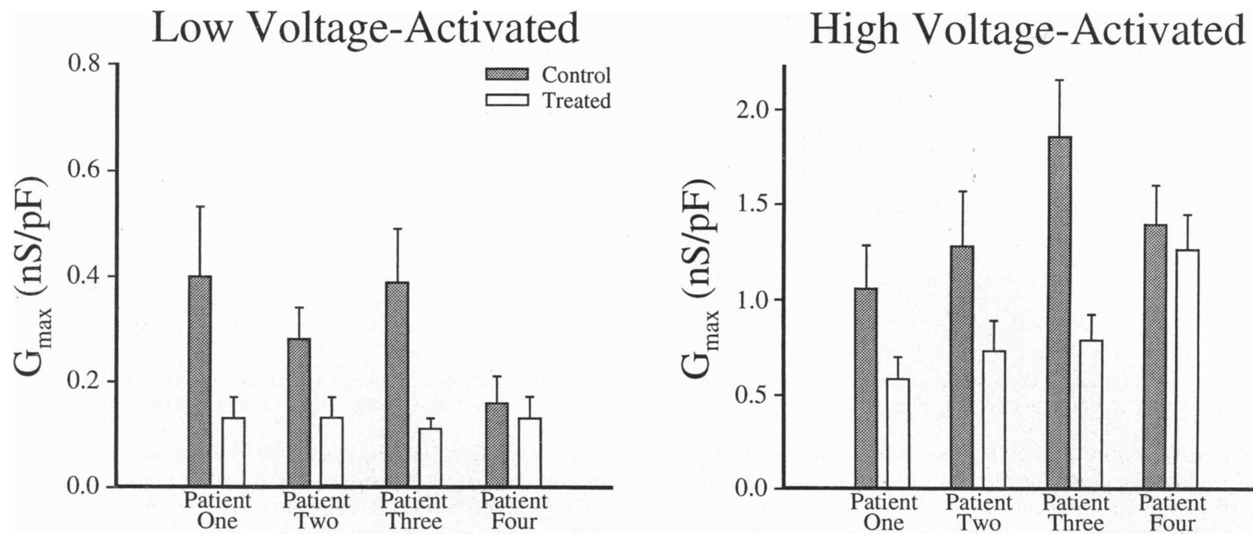

FIG. 4. Maximal LVA (G_{maxL}) and HVA (G_{maxH}) Ca^{2+} conductances for treated and control Ca^{2+} currents. G_{maxL} and G_{maxH} were calculated for each cell as described (Fig. 3). Sera from Patients One, Two, and Three reduced the maximal LVA and HVA Ca^{2+} conductances ($P < 0.05$). Serum from Patient Four had no significant effect. There was no statistically significant ($P < 0.05$) difference between control and test serum-treated values of any of the parameters V_R, V_{b1}, V_{b2}, k_1, and k_2.

prolong the presynaptic depolarization and tend to counteract the effect of reduced Ca^{2+} current.

In principle, the observed reduction in K^+ current could be secondary to the LES-induced decrease in Ca^{2+} current if Ca^{2+}-activated K^+ currents contribute appreciably to the total measured K^+ currents. However, the difference between experimental and control K^+ currents increased as a function of test depolarization (Fig. 6), even for test potentials strong enough (greater than +20 mV) to cause a decrease in Ca^{2+} current amplitude. Furthermore, the substitution of BAPTA for EGTA in the patch pipette little affected K^+ current density (at +50 mV, K^+ density was 318 ± 38 pA/pF with EGTA and 345 ± 40 pA/pF with BAPTA). Thus, the small reduction in K^+ current by LES antibodies was not a secondary consequence of the reduction in Ca^{2+}-activated K^+ current.

DISCUSSION

The present study uses measurements of whole-cell currents in murine DRG neurons to investigate the specificity of LES antibodies for neuronal Ca^{2+} channel subtypes. Sera from patients with clinical electromyographic findings of LES reduced both HVA and LVA Ca^{2+} conductances by more than 40%. A lack of specificity has also been reported for LES antibody effects on LVA and HVA currents in a human neuroblastoma cell line (14).

HVA Ca^{2+} currents are generally believed to control transmitter release, but in various preparations, the channel type of primary importance has been described as N, P, and Q (24–29). Rat DRG neurons express L (18%), N (43%), P (23%) and other (18%) HVA Ca^{2+} currents (23). If mouse DRG neurons are similar, a complete elimination of N current would be necessary to account for the observed reduction in HVA current. Additionally, LES antibodies appear to reduce both N and L currents in human neuroblastoma cells (14). Thus, LES sera appear to cause a generalized decrease in the number, availability, or single-channel conductance of many types of Ca^{2+} channels. However, other kinds of voltage-gated channels do not appear to be targeted because we found that LES sera which decreased neuronal Ca^{2+} conductance density had little effect on K^+ currents.

Although additional studies are necessary to determine if LES antibodies equally affect pharmacologically identifiable components of HVA currents, our findings raise questions about the targeting of Ca^{2+} channels by LES antibodies. One possibility is that LES antibodies represent a polyclonal immune response to a number of different Ca^{2+} channel antigens. Alternatively, the antibodies might be produced in response to a single antigen. In the latter case, the antibodies might target a subunit that is identical in a variety of Ca^{2+} channels, a conserved epitope that is common to channels with differing subunit composition, or a protein that is not integral to the channel but is critical for the function, biosynthesis, or degradation of many Ca^{2+} channel types. In three of the four patients whose sera were used in these studies, the amplitude of the summed, initial CMAP was reduced by about 65–90% compared with the minimal normal value (Table 1; a 9.8-mV summed CMAP is the minimum considered to be normal). This reduction in the summed CMAP amplitude suggests that the number of muscle fibers brought to threshold by the initial activation of the motor nerve was reduced by about 65–90%. In clinical trials (34), Patients One, Two, and Three showed significant improvement upon therapeutic administration of 3,4-diaminopyridine (DAP), which blocks voltage-gated K^+ channels (35) and augments transmitter release by prolonging Ca^{2+} entry (4, 36). DAP provided some less dramatic improvement in Patient Four, whose clinical electrophysiology was ambiguous and whose serology indicated the presence of antibodies characteristic of both LES and myasthenia gravis. The results of our patch-clamp studies are consistent with the clinical observations because serum from Patient Four had little effect on Ca^{2+} currents, and serum from Patients One, Two, and Three reduced HVA Ca^{2+} conductances. The voltage sensitivity and kinetics of the Ca^{2+} conductances remained unchanged in LES-treated DRG neurons, suggesting that LES antibodies block Ca^{2+} channel opening or reduce the number of Ca^{2+} channels. The latter possibility is consistent with evidence from freeze-fracture studies, suggesting that the number of Ca^{2+} channels at active zones is reduced in motor axon terminals exposed to LES antibodies (for review, see ref. 10).

LES manifests itself as a neuromuscular and autonomic disorder characterized by decreased transmitter release. Our studies raise questions about the mechanism and specificity of disease expression since LES antibodies likely reduce Ca^{2+} conductances in a variety of cell types that require Ca^{2+} influx for normal function. Indeed a significant reduction in Ca^{2+} currents in DRGs seems inconsistent with the absence of

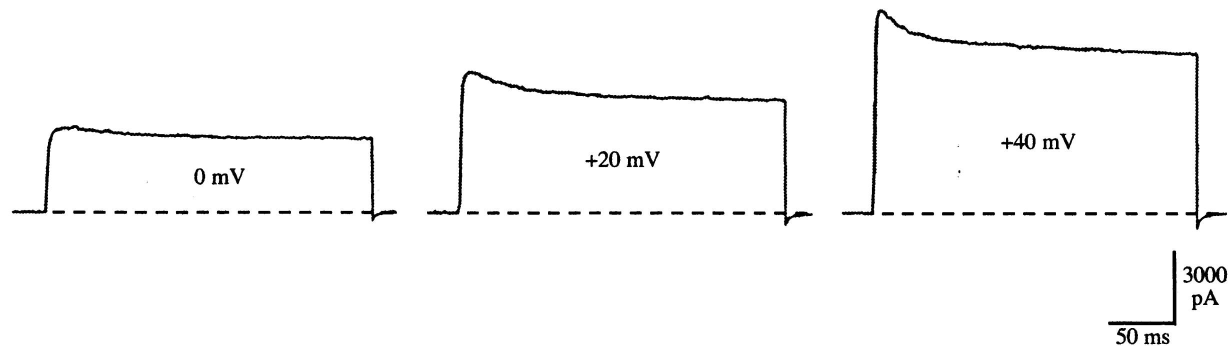

FIG. 5. Representative K^+ currents from a normal DRG neuron (test potentials indicated). At each potential, the current contains inactivating and noninactivating components. K^+ currents in LES serum-treated cells had kinetics like those of the control currents shown here.

sensory dysfunction in LES (37). A possible explanation is that the Ca^{2+} channels responsible for controlling transmitter release from sensory neurons are not accessible to the antibodies *in vivo* because the synaptic terminals lie within the central nervous system. Neuromuscular transmission is resistant to small reductions in transmitter release because vertebrate motor neurons release several times more transmitter than necessary to bring the muscle fiber to threshold (38, 39). Because of this safety factor and the nonlinear summation of the postsynaptic potentials, blockade of 80–90% of the acetylcholine receptors is typically required to reduce the muscle twitch to 50% of normal (38–40). Thus, one would expect that reducing the CMAP amplitude by 50% would require the reduction of the number of quanta released by 80–90%. The relationship between the number of open Ca^{2+} channels and the number of quanta released is expected to be linear if Ca^{2+} channels act independently to regulate this release (41, 42). If the relationship between Ca^{2+} current and the number of quanta released is indeed linear, then reduction of CMAP amplitudes to levels observed in three of the patients whose sera were used in this study (Table 1) would require incapacitation of about 90% of the Ca^{2+} current. This is much larger than the reduction observed for DRG Ca^{2+} conductances in our experiments. Of course, if about half the HVA current in DRG neurons arises from the channels responsible for release at the neuromuscular junction, our results would be consistent with a 90% reduction that is specific for this current. This seems unlikely as discussed above. Perhaps the effects of heterologous antibodies after 24 hr *in vitro* may be less severe than the effects of autologous antibodies on Ca^{2+} channels *in situ*. Since in humans and mice, the decrease in the number of active zone particles (putative Ca^{2+} channels) caused by LES is much smaller than the observed 90% reduction in transmitter release (10), we propose the following model.

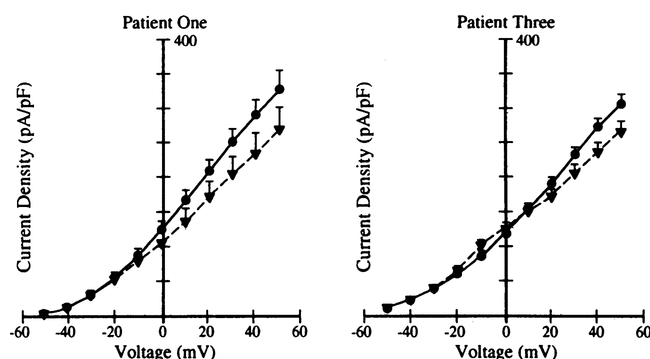

FIG. 6. Normalized, peak K^+ current density as a function of test potential in parallel control (●) and test serum treated (▼) DRG neurons. The smooth curves are spline fits. Currents were measured in 14 and 19 test serum-treated neurons and in 10 and 11 parallel control neurons, for Patients One and Three, respectively. Peak K^+ currents were significantly different ($P \leq .05$) at +40 and +50 mV for Patient Three.

The vulnerability of transmitter release to modest reductions in Ca^{2+} conductance by LES antibodies may result from antibody-induced disruption of the unique organization of the Ca^{2+} channels that act coordinately at active zones to control transmitter release (43, 44). Neuromuscular transmission would be far more sensitive to modest reductions in Ca^{2+} current if overlapping domains of intracellular Ca^{2+} at active zones are important in determining the probability of transmitter release (41, 44). The relationship between the number of open Ca^{2+} channels and the amount of transmitter released would be a power function if several Ca^{2+} channels must open simultaneously to produce these domains (41–45). In this nonlinear model, incapacitating a fraction of the Ca^{2+} channels at active zones could disproportionately reduce the amount of transmitter released. For example, at the squid giant synapse, the relationship between the number of Ca^{2+} channels opened and transmitter release is reported to be a power function with an exponent between three and four (46, 47). If this relationship is also true for the vertebrate neuromuscular junction, reducing the number of Ca^{2+} channels by half would lead to a ≈90% reduction in transmitter release ($1 - 0.5^3 = 0.87$ and $1 - 0.5^4 = 0.94$). Although freeze-fracture studies suggest a decrease in Ca^{2+} channel number in LES (8), our results cannot rule out LES-induced reductions in single-channel conductances. Reducing the unitary conductance by 50% would be equivalent to lowering the $[Ca^{2+}]_o$ by half which, according to the fourth power relationship between transmitter release and $[Ca^{2+}]_o$ (45), would reduce transmitter release by more than 90%. Such nonlinear relationships may explain why transmitter release in the LES patients was severely reduced, yet serum from these patients reduced HVA Ca^{2+} current densities in DRG neurons by less than half.

A short-term increase in strength after vigorous muscle activation is one of the clinical characteristics of LES. Such increases are consistent with presynaptic facilitation, which occurs when the final stimulus in a series releases more transmitter than the initial stimulus (48, 49). If LES reduces the number of competent Ca^{2+} channels at active zones, the initial presynaptic potential might admit insufficient Ca^{2+} to occupy enough cooperatively acting sites to permit transmitter release. If a subsequent depolarization arrives while these sites remain occupied, the probability of transmitter release would be increased (50, 51). Therefore, at active zones where the number of competent Ca^{2+} channels is reduced, facilitation may be necessary for transmitter release to become likely (44). This model is consistent with both the clinical manifestations of LES and the apparent reduction in the number of active zone Ca^{2+} channels.

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