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SELECTIVE ACTION OF MYASTHENIC SYNDROME ANTIBODIES ON CALCIUM CHANNELS IN A RODENT NEUROBLASTOMA × GLIOMA CELL LINE

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SUMMARY

1. The effect of Lambert–Eaton myasthenic syndrome (LEMS) immunoglobulin G (IgG) on Ca^{2+} channels in undifferentiated mouse neuroblastoma × rat glioma hybrid cells (NG 108 15) was studied using the whole-cell patch clamp technique.

2. Sustained inward Ca^{2+} channel currents were evoked by depolarizing pulses from holding potentials of −80 and −40 mV, and were blocked by 5 μM-nitrendipine (L-type currents). Transient inward Ca^{2+} channel currents were activated from a holding potential of −80 mV by small depolarizing steps (T-type currents). Noradrenaline (10 μM) was without effect on transient currents.

3. LEMS IgG selectively reduced sustained (L-type) Ca^{2+} channel current amplitudes evoked from either holding potential used. In the presence of nitrendipine (5 μM), there was no significant effect of LEMS IgG on the remaining transient (T-type) Ca^{2+} channel current amplitudes.

4. Studies of the potential for maximal inward current indicated that voltage sensitivities of both L- and T-type Ca^{2+} channel current amplitudes were unaffected by LEMS IgG, whether recorded in the presence or absence of nitrendipine. LEMS IgG had no significant effect on the time-to-peak or decay of Ca^{2+} channel currents.

5. It is concluded that LEMS IgG acts selectively to cause functional loss of L-type, but not T-type, Ca^{2+} channels in NG 108 15 cells. Any effect of LEMS IgG on N-type channels (not present in these undifferentiated cells) was not studied here. LEMS IgG also acts at motor nerve terminal Ca^{2+} channels leading to muscle weakness. Thus antigenic similarities must exist between L-type channels in NG 108 15 cells and Ca^{2+} channels at motor nerve terminals.

INTRODUCTION

The Lambert–Eaton myasthenic syndrome (LEMS) is a disorder of neuromuscular transmission associated with small-cell carcinoma of the lung in 60% of cases

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(O'Neill, Murray & Newsom-Davis, 1988). Patients show skeletal muscle weakness due to a reduction in the evoked release of acetylcholine from motor nerve terminals (Lambert & Elmqvist, 1971). The disorder is autoimmune in nature and its main electrophysiological features can be passively transferred to mice by injections of immunoglobulin G (IgG) purified from LEMS patients' plasma (Lang, Newsom-Davis, Wray, Vincent & Murray, 1981; Lang, Newsom-Davis, Prior & Wray, 1983; Prior, Lang, Wray & Newsom-Davis, 1985). Studies using mice injected with LEMS IgG have also shown that IgG autoantibodies probably exert their effect by causing loss of presynaptic Ca^{2+} channels, hence reducing calcium-dependent transmitter release following nerve stimulation (Lang, Newsom-Davis, Peers, Prior & W.-Wray, 1987*a*). The loss of channels may in turn be caused by cross-linking of adjacent Ca^{2+} channels at the presynaptic nerve terminal (Lang, Newsom-Davis, Peers & W.-Wray, 1987*b*), leading to their subsequent degradation. Moreover, electron microscopic freeze-fracture studies of presynaptic motor nerve terminals from LEMS patients and from mice pre-treated with LEMS IgG have shown a disorganization and reduction in number of active zone particles (thought to be the presynaptic Ca^{2+} channels) (Fukunaga, Engel, Osame & Lambert, 1982; Fukunaga, Engel, Lang, Newsom-Davis & Vincent, 1983). This reduction is preceded by a shortening of distances between adjacent particles (Engel, Fukuoka, Lang, Newsom-Davis, Vincent & W.-Wray, 1987; Fukuoka, Engel, Lang, Newsom-Davis, Prior & W.-Wray, 1987), as might be expected for cross-linking by antibody.

In this study we have investigated more directly the action of LEMS antibodies on Ca^{2+} channels in a neuronal cell line. To do this we have used whole-cell patch-clamp recordings of Ca^{2+} channel currents from the mouse neuroblastoma × rat glioma hybrid cell line NG 108 15. In other neuronal type tissues, Ca^{2+} channels have been resolved into at least three subtypes (L, T and N), distinguished by their gating characteristics and different responses to various drugs and toxins (Nowycky, Fox & Tsien, 1985; Fox, Nowycky & Tsien, 1987*a*, *b*). Concurrent with the present work (for a preliminary account, see Lang, Newsom-Davis, Peers & W.-Wray, 1987*c*), LEMS IgG has been shown to act on L-type channels in chromaffin cells (Kim & Neher, 1988). Here we have firstly investigated channel subtypes found in undifferentiated NG 108 15 cells, and we have further studied possible selective actions of LEMS IgG on the subtypes of Ca^{2+} channels found in these cells.

METHODS

Cell culture and pre-treatment with antibody. Mouse neuroblastoma × rat glioma hybrid (NG 108 15) cells were grown continuously in the laboratory, and when required were plated onto 60 mm culture dishes at a density of about 5×10^5 cells per culture dish. The cells were suspended in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum and 1 × HAT, along with penicillin and streptomycin to prevent microbial infection. No chemical growth factor was added to induce differentiation. They were then kept at 37 °C in a humidified incubator (10% CO_2) for 24 h. During this period cells settled on and adhered to the base of the culture dishes pre-coated with poly-L-lysine (5 μg ml^{-1}). This allowed exchange of the medium for fresh medium containing 2–4 mg ml^{-1} of either LEMS IgG or control IgG. Cells were then maintained in this medium at 37 °C for a further 24–48 h before electrophysiological study. On any one experimental day at least one control and one LEMS IgG-treated culture dish of cells from the same batch were used for study.

IgG from a Guillain–Barre syndrome patient was used as control. The latter syndrome is an inflammatory demyelinating peripheral neuropathy unrelated to LEMS, in which immunological mechanisms have been implicated. It is characterized by reduced motor nerve conduction velocities due to the destruction of myelin and to secondary degeneration of the axon itself. This leads to nerve conduction block in some cases and hence skeletal muscle weakness (for review, see Hughes & Winer, 1984).

LEMS IgG was obtained from a patient with small-cell lung carcinoma. We have previously shown that injection of this patient's IgG into mice caused a reduction (to 27%) of evoked release of acetylcholine at the neuromuscular junction (patient 5 of Lang *et al.* 1983).

All antibody preparations were purified from plasma by the rivanol–ammonium sulphate precipitation method (Horesji & Smetana, 1956).

Solutions used for electrophysiological recordings. To record current flow through calcium channels, the culture medium bathing each dish of cells was exchanged for one of the following composition with barium as the charge carrier (mM): NaCl, 130; KCl, 3; $MgCl_2$, 0·6; $NaHCO_3$, 1·0; HEPES, 10; glucose, 4; $BaCl_2$, 10; tetraethylammonium bromide, 25, as well as tetrodotoxin (2·5 μM). This solution was adjusted to pH 7·3–7·4 with NaOH. Solution exchange was carried out by perfusion of the culture dish with 25 ml of the recording medium to leave a final volume of approximately 4 ml in the culture dish. This volume was replaced approximately once an hour to minimize concentration changes due to evaporation and each culture dish was only used for up to 3 h. When required, nitrendipine was added to the recording medium from stock solution (in ethanol, 5 mg ml^{-1}) to obtain a final concentration of 5 μM-nitrendipine (together with a low concentration of ethanol, 0·06% v/v). All handling of nitrendipine and experiments involving its use were performed under low-intensity sodium vapour light only.

Patch electrodes were filled with a solution of the following composition (mM): CsCl, 140; EGTA, 1·1; $MgCl_2$, 2; $CaCl_2$, 0·1; HEPES, 10, adjusted to pH 7·2–7·3 with CsOH and to approximately 310 mosM with sucrose.

Electrophysiological recordings. Whole-cell patch clamping (Hamill, Marty, Neher, Sakmann & Sigworth, 1981) was carried out using an Axoclamp-2 single-electrode voltage clamp amplifier (Axon Instruments) at a switching frequency of 5–10 kHz. All experiments were carried out at 24–26 °C, and current and voltage traces were recorded on magnetic tape and later analysed by computer after digitization at 5 kHz. The following protocol was used to evoke Ca^{2+} channel currents. Cells were firstly voltage clamped at a holding potential of −80 mV, and 200 ms depolarizing test pulses of increasing amplitude were applied at a frequency of 0·1 Hz. Secondly, the cells were held at −40 mV and similar depolarizing test pulses were applied. Recordings were rejected if the current required to hold the cells varied by more than 5%. Linear leakage and capacitance subtraction were performed for each cell by appropriate scaling of averaged hyperpolarizing steps (and small depolarizing steps of 10 and 20 mV from a holding potential of −80 mV). Each subtracted individual current was measured for peak (i.e. maximum) inward current (I_{peak}), for sustained inward current amplitude (I_{sus}, taken as the current amplitude over the last 8 ms of the step pulse), and for the time-to-peak of the current. For each holding potential and test potential used, measured parameters were averaged for control cells and for LEMS IgG-treated cells. Corresponding test and control values were compared using Student's two-tailed t test, and values shown are means ± standard error (S.E.) of the means.

RESULTS

Types of Ca^{2+} channels in undifferentiated NG 108 15 cells

Using hyperpolarizing (and small depolarizing, see Methods) voltage steps to evoke only leakage currents, current–voltage (I–V) curves were linear, the fitted slope giving the input resistance. This was not significantly affected either by changing the holding potential or by LEMS IgG (Table 1).

Larger depolarizing steps evoked inward Ca^{2+} channel currents, and typical results are shown in Fig. 1, after leakage and capacitance subtraction. From a holding potential of −80 mV, depolarizing pulses to a test potential of, for example, −30 mV

(Fig. 1) evoked transient inward currents which decayed fully within the 200 ms test depolarization. At a holding potential of −40 mV, the transient component was not present (Fig. 1). These properties are expected for T-type Ca^{2+} channels (Nowycky *et al.* 1985).

At a holding potential of −80 mV, as the depolarizing steps were increased to a test potential of, for example, 0 mV (Fig. 1), evoked currents also showed a sustained component, which was also present for depolarizing steps from a holding potential of −40 mV (Fig. 1). The sustained component (unlike the transient component) was blocked by nitrendipine (see below), and accordingly has the properties expected for L-type Ca^{2+} channels (Nowycky *et al.* 1985).

TABLE 1. Effect of LEMS IgG on input resistance of NG 108 15 cells

Holding potential (mV)	Cell input resistance (MΩ)	
	Control	LEMS
−80	123 ± 15 (25)	110·3 ± 9·4 (25)
−40	118 ± 19 (18)	107 ± 16 (17)

Values for input resistance are means ± S.E.M. averaged over the number of cells shown in parentheses.

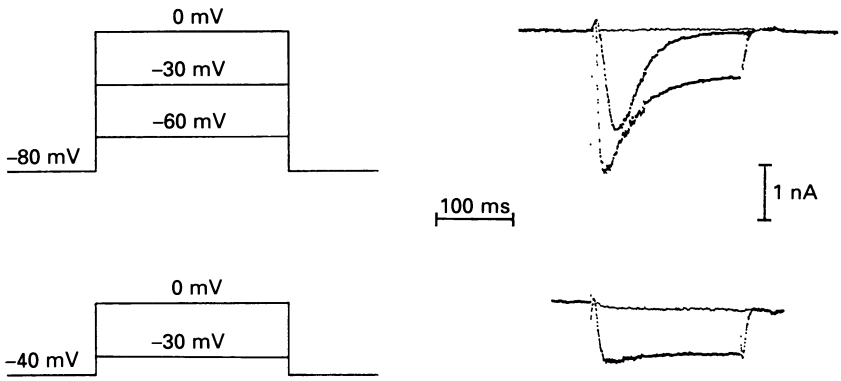

Fig. 1. Ca^{2+} channel currents evoked in NG 108 15 cells. Current shown (right) were evoked in a control IgG-treated cell in response to 200 ms depolarizing test pulses at the potentials shown (left). Upper traces, −80 mV holding potential; lower traces, −40 mV holding potential. Leakage and capacitance currents have been subtracted.

No evidence was found (see below) to indicate the presence of a third, N-type Ca^{2+} channel which would only be expected to be activated (Nowycky *et al.* 1985) by large depolarizations from strongly negative holding potentials.

Effects of LEMS IgG on L-type Ca^{2+} channels

To study the effects of LEMS IgG on L-type Ca^{2+} channels in the absence of T- or N-type currents, depolarizing steps were firstly applied to control and LEMS IgG-treated cells held at −40 mV. Under such conditions, only sustained currents were observed (see above). Figure 2*A* shows the *I–V* relationship averaged from eighteen control and seventeen LEMS IgG-treated cells. Over a wide range of test potentials,

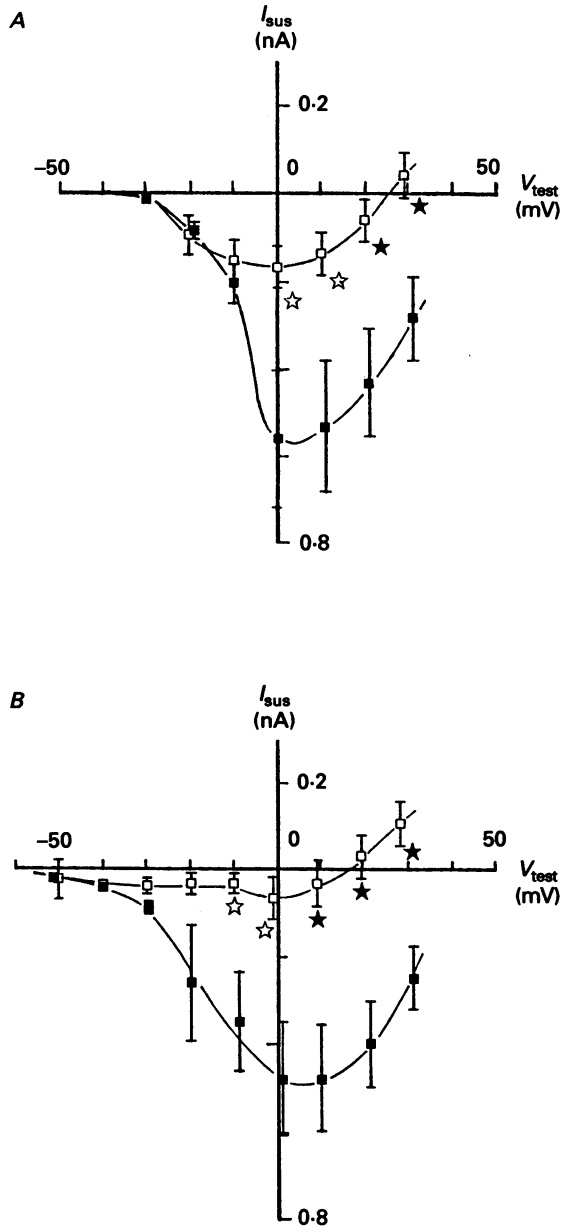

Fig. 2. Plots of sustained current amplitudes (I_{sus}) *versus* test membrane potential (V_{test}) measured in control (■) and LEMS (□) IgG-treated NG 108 15 cells. Currents were evoked from a holding potential of −40 mV in eighteen control and seventeen LEMS IgG-treated cells (*A*) and −80 mV in twenty-five control and twenty-five LEMS IgG-treated cells (*B*). Cells used in *B* included all those used in *A*. Current values shown are means ± S.E.M. (one value per cell at each test potential). Potentials at which each current value is plotted are also mean values, while standard errors in potentials were always so small as to be covered by the symbol in this and all subsequent figures. Curves were fitted by eye. Significant reductions in current by LEMS IgG as compared with controls: ☆ $P < 0{\cdot}02$; ★ $P < 0{\cdot}005$.

LEMS IgG caused significant reductions (see Fig. 2 legend for details) in the sustained current amplitude as compared with controls, indicating an action of the antibody on L-type Ca^{2+} channels.

I–V relationships were also constructed (Fig. 2*B*) for the sustained component of

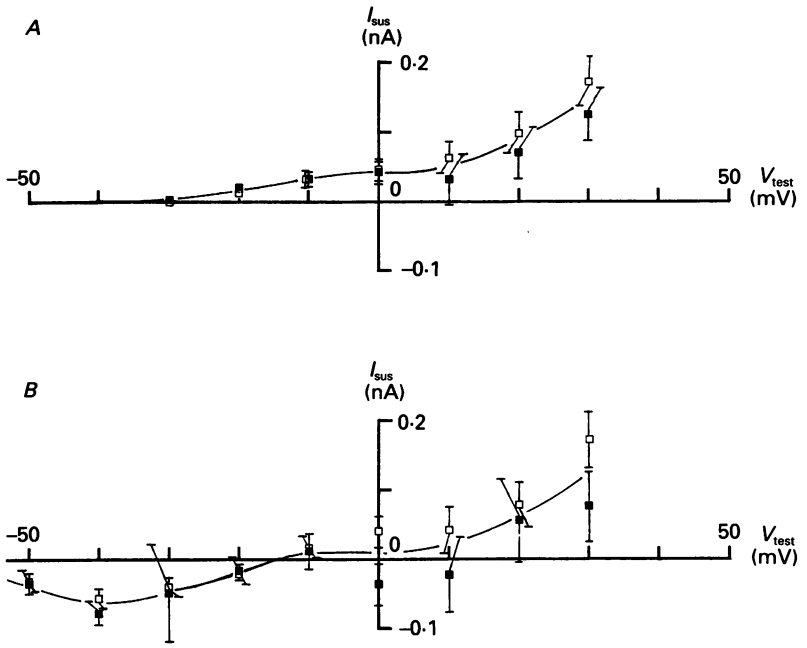

Fig. 3. Plots of sustained current amplitudes (I_{sus}) *versus* test membrane potential (V_{test}) measured in control (■) and LEMS (□) IgG-treated NG 108 15 cells in the presence of nitrendipine (5 μM). Currents were evoked from holding potentials of −40 mV (*A*) and −80 mV (*B*). Values shown are means ± S.E.M. from twenty-four control and twenty-five LEMS IgG-treated cells. LEMS IgG had no significant effect on current amplitude as compared with controls at any test potential studied, and so single curves have been fitted by eye through the data points.

currents evoked at different test potentials from a holding potential of −80 mV in twenty-five control and twenty-five LEMS IgG-treated cells (the transient component is discussed below). Firstly, the sustained currents at −80 mV were similar in magnitude to those evoked at a holding potential of −40 mV (cf. Fig. 2*A*). This lack of inactivation at −40 mV is as expected for L-type currents. Secondly, at a holding potential of −40 mV, LEMS IgG again caused significant reductions (see Fig. 2 legend for details) in the sustained current amplitude as compared with controls over a wide range of test depolarizations.

The effect of LEMS IgG on sustained currents in the presence of nitrendipine (5 μM) was also investigated. From either holding potential employed (−40 mV, Fig. 3*A*, or −80 mV, Fig. 3*B*), sustained inward currents were almost totally absent, consistent with the known blocking effect of nitrendipine on L-type Ca^{2+} channels. In the presence of the drug, sustained outward currents were observed at larger test potentials which were not significantly different between control and LEMS IgG-treated cells at all test potentials studied whether evoked from a holding potential

of −40 or −80 mV. The ionic nature of this outward current was not investigated but is probably due to incomplete block of other ion channels (e.g. K^+ channels) under these experimental conditions (see also Narahashi, Tsunoo & Yoshii, 1987). The small inward sustained currents evoked at low test potentials from −80 mV holding potential (Fig. 3*B*) were presumably due to T-type Ca^{2+} channels being occasionally incompletely inactivated within the 200 ms test depolarizations.

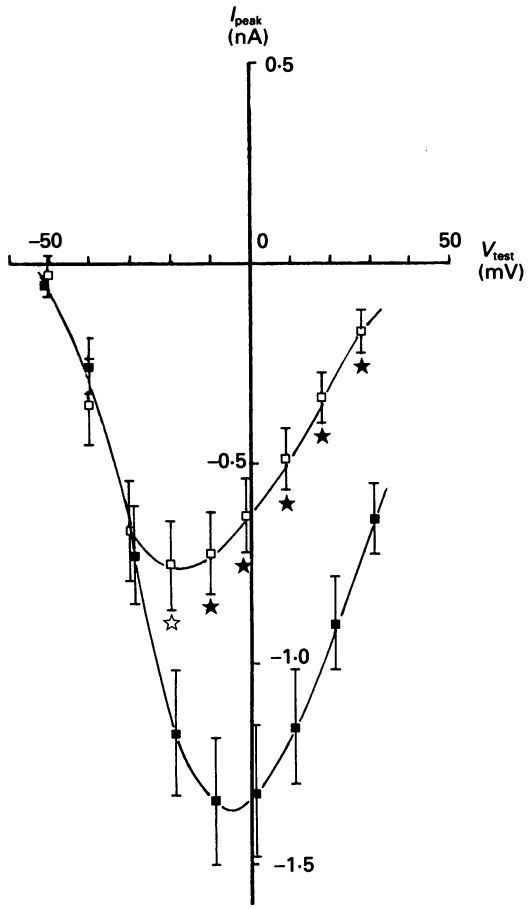

Fig. 4. Plot of peak Ca^{2+} channel current amplitude (I_{peak}) *versus* test membrane potential (V_{test}) measured in twenty-five control (■) and twenty-five LEMS (□) IgG-treated NG 108 15 cells. All currents were evoked from a holding potential of −80 mV and values plotted are means ± S.E.M. Curves were fitted by eye. Significant reductions by LEMS IgG as compared with controls: ☆ $P < 0{\cdot}05$; ★ $P < 0{\cdot}002$.

Effects of LEMS IgG on T-type Ca^{2+} channels

From a holding potential of −80 mV, evoked currents showed a transient component (see above). Over the low range of test potentials (up to −30 mV), LEMS IgG had no significant effect on the peak (transient) Ca^{2+} channel current (Fig. 4). This indicates a lack of effect of LEMS IgG on T-type channels as these are the only

Ca^{2+} channel type expected to be activated at these low test potentials (Nowycky *et al.* 1985). However, at larger test potentials, LEMS IgG caused significant reductions (see Fig. 4 legend for details) in peak inward currents (I_{peak}) as compared with controls, presumably due to the above action of the antibody on L-type channels which would also be activated over this latter range of test potentials.

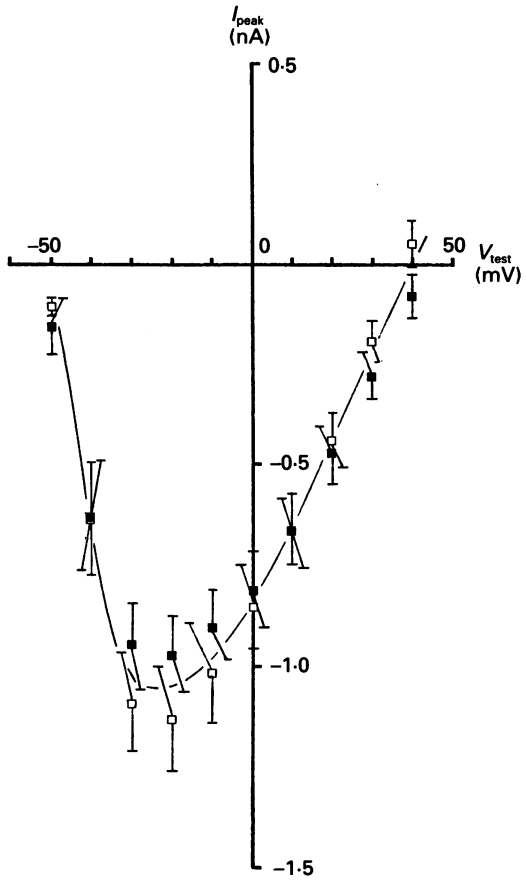

Fig. 5. Plot of peak Ca^{2+} channel current amplitude (I_{peak}) *versus* test membrane potential (V_{test}), measured in twenty-four control (■) and twenty-five LEMS (□) IgG-treated cells. All currents were evoked from a holding potential of −80 mV in the presence of nitrendipine (5 μM). Points plotted are means ± S.E.M. LEMS IgG had no significant effect on current amplitude as compared with controls at any potential studied and so a single curve was fitted by eye through all data points. Data were obtained from the same recordings as in Fig. 3

To investigate this further, nitrendipine (5 μM) was applied in order to block L-type sustained currents (Fig. 3). The transient component of currents evoked from −80 mV was not blocked. Over the whole range of test depolarizations used, LEMS IgG had no significant effect on the amplitude of the transient current (Fig. 5), indicating that LEMS IgG had no effect on T-type Ca^{2+} channels, assuming absence of N-type channels (see below).

Time-to-peaks (holding potential −80 mV) were voltage dependent in both

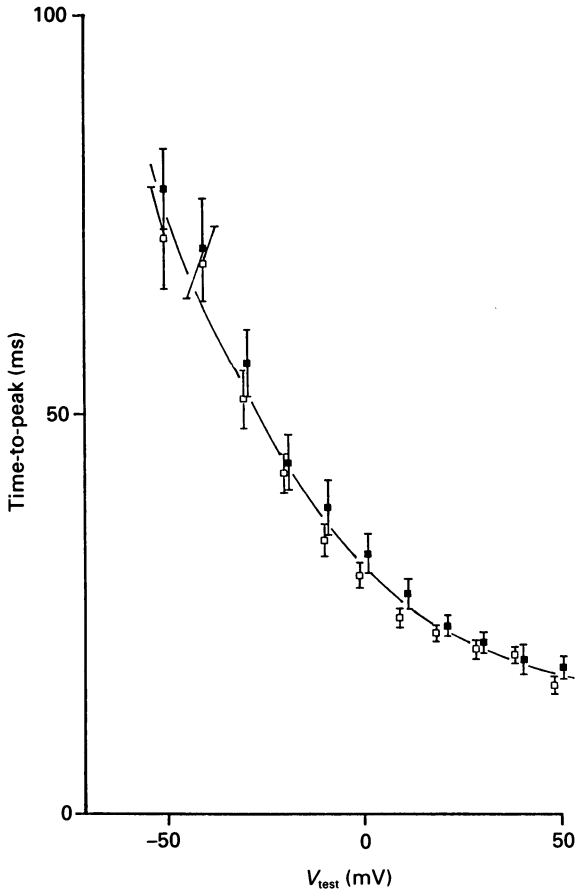

Fig. 6. Plot of time-to-peak of currents (evoked from a holding potential of −80 mV) *versus* test membrane potentials (V_{test}). Values shown are means ± S.E.M. from twenty-five control (■) and twenty-five LEMS (□) IgG-treated cells. LEMS IgG had no significant effect on time-to-peak at any test potential studied; therefore one curve has been fitted (by eye) through all data points. Data were obtained from the same recordings as in Fig. 4.

control and LEMS IgG-treated cells, and LEMS IgG had no significant effect at any test potential studied (Fig. 6). The dominant contribution to the time-to-peaks appeared to come from T-type channels since nitrendipine (5 μM) had no significant effect on time-to-peaks (data not shown, again assuming absence of N-type channels). The data therefore indicate that the antibody did not affect the rate of activation of T-type channels.

The decay phase of the transient current evoked by large depolarizing steps from a holding potential of −80 mV might be expected to show two exponential components if N-type as well as T-type channels are present. However, good fits to the decay phase were obtained with a single exponential only, suggesting absence of N-type channels. Furthermore, for steps to +10 mV, the exponential time constant

for LEMS IgG-treated cells (39·6 ± 2·7 ms, twenty-five cells) was not significantly different from that for controls (46·5 ± 2·7 ms, twenty-four cells). Thus LEMS IgG did not appear to affect the rate of inactivation of T-type channels.

Finally, it is worth mentioning that LEMS IgG did not seem to convert L-type channels into T-type; the very slow rate of inactivation of L-type currents and the magnitude of T-type currents were not increased by LEMS IgG.

Effect of noradrenaline on Ca^{2+} channels in NG 108 15 cells

Noradrenaline inhibits N-type currents in certain cells (Tsien, Lipscombe, Madison, Bley & Fox, 1988). In differentiated NG 108 15 cells, although T-type currents are also affected by noradrenaline, the *main* effect is on currents resembling N-type (Docherty & McFadzean, 1989). In order to provide further insight as to whether N-type channel currents are present in the undifferentiated cells studied here, whole-cell currents were recorded in the presence of noradrenaline (10 μM). Nitrendipine (5 μM) was also present throughout to block L-type channels. Inward currents at a holding potential of −80 mV were recorded in a total of twenty-five control and twenty-five LEMS IgG-treated cells and the mean *I-V* relationships for peak (transient) current are shown in Fig. 7.

Firstly, there were no significant differences in these *I–V* relationships (whether for control or for LEMS IgG-treated cells) as compared with those obtained in the absence of noradrenaline (Fig. 5, nitrendipine present). This further supports the idea that N-type channels are not present in the undifferentiated NG 108 15 cells used under these conditions. An alternative explanation could be that α-adrenoreceptors are not present in these cells.

Secondly, in the presence of noradrenaline (and nitrendipine) LEMS IgG had no significant effect on peak transient current as compared with controls at any test potential studied (Fig. 7). Since only T-type channels appear to be involved under these conditions, this confirms our earlier result for lack of action of LEMS IgG on T-type channels.

The effects of LEMS IgG on the voltage sensitivities of whole-cell Ca^{2+} channel currents

At first sight, LEMS IgG seems to alter the voltage sensitivity at positive potentials, shifting apparent reversal potentials (Figs 2 and 4). However, this effect is more apparent than real because of distortion of the *I–V* curves by outward currents which were appreciable at positive potentials (Fig. 3). Indeed, after subtraction of these outward currents, it can be seen that there is no evidence for a shift in reversal potential (Fig. 8).

To quantify any effect of LEMS IgG on the voltage sensitivity of Ca^{2+} channel currents, the test potentials at which inward currents were maximal were measured in control and LEMS IgG-treated cells. These potentials were in the range where outward currents were small (Table 2) and so should be reliable.

For sustained currents at both holding potentials studied (−80 and −40 mV), LEMS IgG had no significant effect on the test potential at which these currents were maximal (Table 2). Thus LEMS IgG appears to simply reduce (to around 25% of

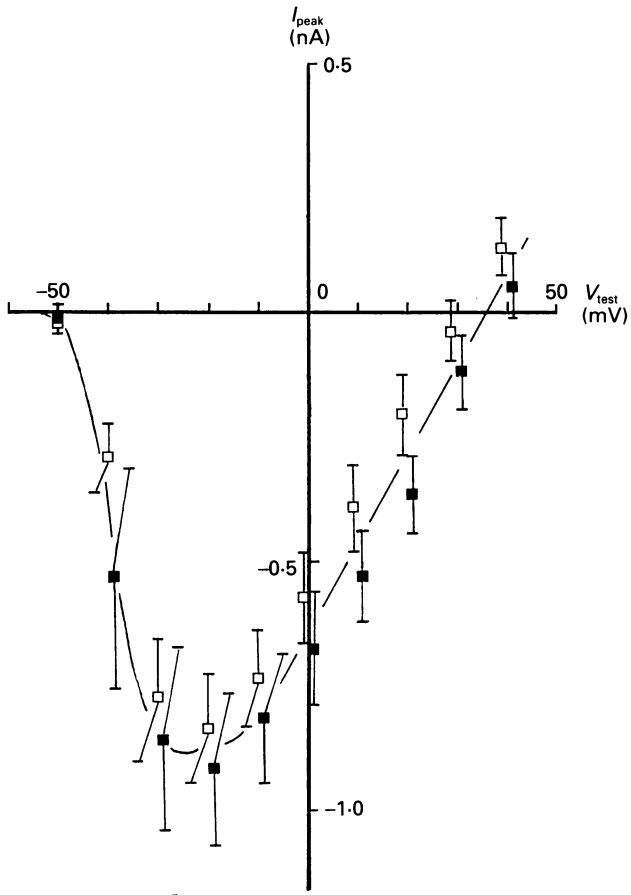

Fig. 7. Plot of peak Ca^{2+} channel current amplitude (I_{peak}) *versus* test membrane potential (V_{test}) measured in twenty-five control (■) and twenty-five LEMS (□) IgG-treated cells. All currents were evoked from a holding potential of −80 mV in the presence of nitrendipine (5 μM) and noradrenaline (10 μM). Points plotted are means ± S.E.M. LEMS IgG had no significant effect on I_{peak} as compared with controls at any test potential studied and so a single curve has been fitted (by eye) through all data points.

controls, Fig. 8*A*) L-type currents without affecting the voltage sensitivity. It is noteworthy that this potential (around 3 mV) was not significantly different (Table 2) at the two holding potentials studied, whether for control or LEMS IgG-treated cells, as expected for these non-inactivating L-type currents over this range of holding potentials.

For transient inward currents, in the presence of nitrendipine to block L-type channels, the test potential at which currents were maximal was not significantly different between controls and LEMS IgG-treated cells (Table 2). This held true also in the additional presence of noradrenaline (Table 2). As argued above, these currents are essentially due to T-type currents; thus LEMS IgG did not affect either

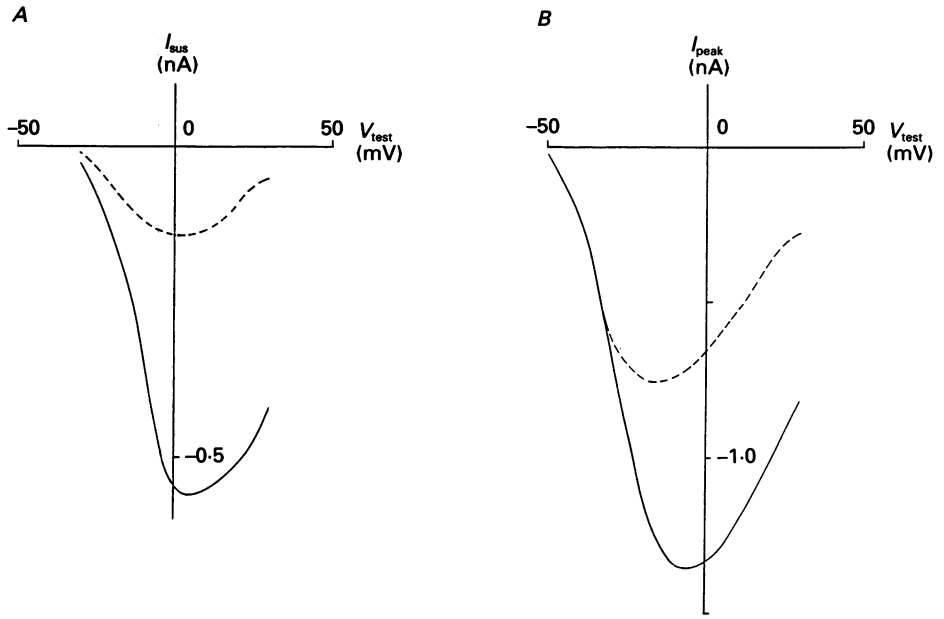

Fig. 8. *I–V* curves for calcium channel currents after correction for outward currents. *A*, I_{sus} averaged over holding potentials of −40 and −80 mV. Currents were corrected by subtracting the average of the smooth curves in Fig. 3 from the average of those in Fig. 2. *B*, I_{peak} at a holding potential of −80 mV. Currents were corrected by subtracting the smooth curve in Fig. 3*B* from those in Fig. 4. Controls, continuous line; LEMS IgG, dashed line.

TABLE 2. Test potentials at which inward Ca^{2+} channel currents were maximal in control and LEMS IgG treated cells

Current	Drug present	Holding potential (mV)	Test potential for maximal Ca^{2+} channel current (mV)	
			Control	LEMS
I_{sus}	—	−40	1·3 ± 1·7 (18)	0·9 ± 1·7 (17)
I_{sus}	—	−80	5·5 ± 2·3 (25)	2·3 ± 3·6 (25)
I_{peak}	—	−80	−7·4 ± 1·9 (25)	−18·7 ± 1·8 (25)
I_{peak}	5 μM-nitrendipine	−80	−21·3 ± 3·1 (24)	−24·2 ± 1·9 (25)
I_{peak}	5 μM-nitrendipine + 10 μM-noradrenaline	−80	−20·1 ± 2·6 (25)	−25·4 ± 1·5 (25)

Values shown are means ± S.E.M., averaged from potentials for maximal current obtained from individual current–voltage plots for each cell (number of cells shown in parentheses).

amplitude (Figs 5 and 7) or voltage sensitivity of T-type channels. These transient (T-type) currents were maximally activated at significantly ($P < 0·001$) more negative potentials (around −23 mV) than for sustained (L-type) currents (around 3 mV, see above). It is interesting that these data further support the idea that N-type channels are absent; firstly transient currents would have been expected to

have been maximal at more positive potentials if N-type channels had been dominantly present; secondly (in the presence of nitrendipine) the potential for maximal current was not significantly affected by the further addition of noradrenaline (Table 2) which would have been expected to affect N-type currents.

In the absence of nitrendipine, for peak currents (I_{peak}), the test potential for maximal current was significantly ($P < 0{\cdot}001$) shifted towards more negative potentials by LEMS IgG as compared with controls (Table 2, Fig. 8*B*). This shift can be understood simply by functional block of L-type channels (potential for maximal current 3 mV, see above) leaving predominantly T-type channels which are maximally activated at more negative potentials (−23 mV, see above). A similar effect occurred when nitrendipine was applied to controls; nitrendipine shifted the potential for maximal current (I_{peak}) to significantly ($P < 0{\cdot}001$) more negative potentials (Table 2, Figs 4 and 5), again presumably due to block of L-type channels. Indeed, this potential for controls in the presence of nitrendipine was not significantly different from that for LEMS IgG-treated cells in the absence of nitrendipine (Table 2).

In summary, using the potential for maximal inward current as an indicator of voltage sensitivity, LEMS IgG appeared to cause reductions in L-type currents without any change in their voltage sensitivity, an action similar to that for nitrendipine.

DISCUSSION

In this study, the whole-cell patch clamp technique has been used in undifferentiated NG 108 15 cells to show that L- and T-type but probably not N-type Ca^{2+} channels are present, and that LEMS IgG selectively blocks L-type, but not T-type, Ca^{2+} channels. The voltage sensitivity and rates of activation and inactivation of these channels were unaffected by LEMS IgG. The results can be understood by a simple functional block of around 75% of the L-type channels by LEMS IgG.

Three subclasses of Ca^{2+} channel (with some features resembling T-, N- and L-type) have been described in chemically differentiated NG 108 15 cells (Docherty, 1988). Gadolinium and noradrenaline have been shown to block selectively a transient component of whole-cell Ca^{2+} currents evoked by large depolarizing pulses from strongly negative holding potentials, suggesting involvement of N-type channels (Docherty & McFadzean, 1987; Docherty, 1988), although T-type channels are also affected to a lesser degree (Docherty & McFadzean, 1989). On the other hand, for the undifferentiated cells used here, evidence from our studies of the transient component (voltage dependence, inactivation kinetics and lack of sensitivity to noradrenaline) taken together pointed to the absence of N-type Ca^{2+} channels. Thus N-type channels seem to appear during the process of differentiation.

Our findings here that LEMS IgG acts on L-type Ca^{2+} channels in NG 108 15 cells and on Ca^{2+} channels at skeletal muscle motor nerve terminals (Lang *et al.* 1987*a*) indicates that some degree of similarity must exist between these channels. However, L-type channels are not involved in transmitter release at the neuromuscular junction since organic Ca^{2+} channel antagonists and agonists do not substantially affect transmitter release (Gotgilf & Magazanik, 1977; Nachshen & Blaustein, 1979;

Bregestovski, Miledi & Parker, 1980; Burges & W.-Wray, 1989). Furthermore, antibodies raised in mice against L-type Ca^{2+} channel protein from rabbit skeletal muscle T-tubules are without effect on transmitter release at the mouse neuromuscular junction (W.-Wray, Peers & Norman, 1987). Thus, it appears that, while L-type Ca^{2+} channels in NG 108 15 cells and motor nerve terminal Ca^{2+} channels are clearly not identical, they must however possess some regions of antigenic similarity (absent for T-type channels) to which LEMS antibody binds.

LEMS IgG also causes functional loss of Ca^{2+} channels in bovine adrenal chromaffin cells (Kim & Neher, 1988), where the Ca^{2+} channels present appear to be of a single type (L). It also acts on dihydropyridine-sensitive Ca^{2+} channels in small-cell carcinoma cells which are thought to be of neuroectodermal origin (Roberts, Perera, Lang, Vincent & Newsom-Davis, 1985). There are suggestions that the antibody may act on parasympathetic nerve endings (see e.g. Lang, Newsom-Davis & W.-Wray, 1988). However, it does not block L-type channels in the heart (Lang *et al.* 1988), nor Ca^{2+} channels in insect skeletal muscle (Pearson, Newsom-Davis, Lees & W.-Wray, 1989). Thus the antibody appears to cross-react preferentially with certain subtypes of neuronal Ca^{2+} channels, which share regions of antigenic similarity. Clearly the antibody shows up differences, not only between T- and L-type channels in the same tissue, but also between L-type channels in different tissues.

In our experiments, thorough exchange of the culture medium (containing LEMS IgG) for the recording medium was carried out. Thus the effects we have observed are due to irreversible actions of LEMS IgG. Similarly, the effect of LEMS IgG on motor nerve terminals and on chromaffin cells is also irreversible. For motor nerve terminals, this irreversible action comes about by loss of Ca^{2+} channels by antibody-mediated cross-linking and degradation (see Introduction) rather than direct pharmacological block (Prior *et al.* 1985). It is unclear whether LEMS IgG also acts by causing degradation of Ca^{2+} channels in NG 108 15 cells or in chromaffin cells. However, direct pharmacological blocking actions may contribute to the relatively fast onset in chromaffin cells, although this could also perhaps be explained by accelerated degradation at the higher temperature used (36 °C).

Finally, results presented in this paper for the action of LEMS IgG on neuroblastoma NG 108 15 cells (selective functional block of L-type but not T-type Ca^{2+} channels) indicate that LEMS IgG may serve as a useful probe in the future study of types of Ca^{2+} channels present in neuronal tissues.

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