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Voltage-gated Ca^{2+} channel in mouse myeloma cells

(neoplastic lymphocyte/patch clamp technique/voltage-dependent inactivation)

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ABSTRACT **Electrical properties of the cell membrane were studied in the neoplastic lymphocyte, mouse myeloma cell line S194, by using the whole-cell patch clamp technique. Inward Ca^{2+} currents due to voltage-gated Ca^{2+} channels were found. The current, which decayed exponentially after reaching a peak, was first activated at about −50 mV and attained its maximum peak amplitude at about −20 mV in a 10 mM Ca^{2+} solution. Outward current was negligible for the potential range more negative than +30 mV. The channel was permeable to Sr^{2+} and Ba^{2+} in addition to Ca^{2+}. Among these species, Sr^{2+} carried the greatest current. The time constants of the decay of the current depended neither on the species nor on the concentration of charge carrier. The steady-state inactivation was observed at potentials more negative than those at which the inward Ca^{2+} current was activated. Thus, we concluded that the inactivation of the channel was mainly voltage dependent. For reasons that are not yet understood, the amplitude of the Ca^{2+} current varied greatly among cells.**

Two lines of evidence suggested the possibility of the Ca^{2+} channel playing a role in the immune response. First, external Ca^{2+} is required at the stage of target cell cytolysis by effector lymphocytes, when the lethal hit is thought to take place (1–3). Second, Ca^{2+} is necessary for lymphocyte proliferation when lymphocytes are stimulated by antigens (4) and mitogens (5–9). Because lymphocytes are small (≈10 μm in diameter), it has been impossible to examine electrical properties of their membranes faithfully by using conventional intracellular microelectrode techniques. Recent introduction of the "gigohm seal" patch clamp technique by Sigworth and Neher (10) enabled us to study membrane properties of those small cells successfully (11, 12). We selected mouse neoplastic lymphocytes, myeloma cell line S194, because they were easily kept under tissue culture condition and because they were slightly larger than normal lymphocytes. We found inward currents carried by the voltage-gated Ca^{2+} channel that displayed voltage-dependent inactivation.

MATERIALS AND METHODS

Mouse myeloma cell line, S194/5.XX0, was cultured in suspension in RPMI 1640 medium with glutamine (Flow Laboratories), containing 10% calf serum. This cell line synthesizes IgA but does not secrete antibodies into the medium (13). The mean diameter of the myeloma cells was 13 μm. The gigohm seal patch clamp technique introduced by Sigworth and Neher (10) was used to study membrane properties. The details of the whole-cell voltage clamp with the patch electrode were similar to those described previously (11). The normal external solution contained 138 mM NaCl, 5 mM KCl, 10 mM $CaCl_2$, 1 mM $MgCl_2$, 17 mM glucose, and 10 mM Hepes (pH 7.4). Other solutions were made by replacing the $CaCl_2$ or NaCl (or both) of the normal solution isosmotically. The patch electrode was filled with a solution containing 150 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, 2.5 mM $CaCl_2$ (pCa ≒ 7), and 10 mM Hepes (pH = 7.4) and had a resistance of 5–15 MΩ. The external solution was continuously perfused after the myeloma cells had settled on the bottom of the dish. Experiments were performed at 24–26°C. Output of the current–voltage converter was filtered at 1 kHz, digitized at sampling intervals of 400 μsec, and stored in a computer. The zero-current membrane potential in the normal solution was −20 to −30 mV. It was likely, however, that the resting potential was actually more negative; this was due to relatively small ratios between the seal (2–15 GΩ) and whole-cell input (0.5–2 GΩ) resistances. In most experiments, voltage clamp was performed by holding the membrane potential in the vicinity of −90 mV. Errors caused by liquid junction potentials were corrected as described (11).

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RESULTS

Inward Current Carried by Ca^{2+}. Transient inward currents were observed when the membrane potential was made positive from the holding potential of −94 mV (Fig. 1*A*). When 10 mM Ca^{2+} in the external solution was replaced with Mn^{2+}, they disappeared almost entirely (Fig. 1*B*). The results show that the transient inward current is Ca^{2+} dependent, and records obtained in Mn^{2+} solution indicate that no other time-dependent currents (such as the delayed outward K^+ current) exist within the range of membrane potentials examined in the present experiment (−50 to +30 mV). Records shown in Fig. 1*C* were obtained by subtracting each current in Fig. 1*B* from that at the same voltage in Fig. 1*A*. We consider these difference currents to represent the most reliable Ca^{2+}-dependent currents; most of the following analyses were performed on these currents.

The amplitude of the inward current increased when the external Ca^{2+} concentration was increased. The peak amplitudes of the currents obtained at 10, 25, and 50 mM Ca^{2+} with the same cell were plotted against the membrane potential in Fig. 2. The current–voltage relationship shifted along the voltage axis in the positive direction as the Ca^{2+} concentration increased. The shift was interpreted to result from the change in the surface potential caused by the changes in divalent-ion concentration. The inward current showed no significant change when the NaCl in the external solution was replaced with tetraethylammonium chloride. These findings indicate that the inward current is carried by Ca^{2+} and that Na^+ does not contribute to the inward current as a charge carrier.

Sr^{2+} and Ba^{2+} also can carry inward currents in these cells; currents obtained with 25 mM Ca^{2+}, Ba^{2+}, or Sr^{2+} from the same cell are shown in Fig. 3*A*. By taking into consideration that there is a 10-mV negative shift in the current–voltage relationship for the Sr^{2+} curve, the time course of the current was similar for the three different charge carriers (see the legend). In contrast, the amplitude depended on the charge carrier. The peak amplitude was plotted against the membrane potential in

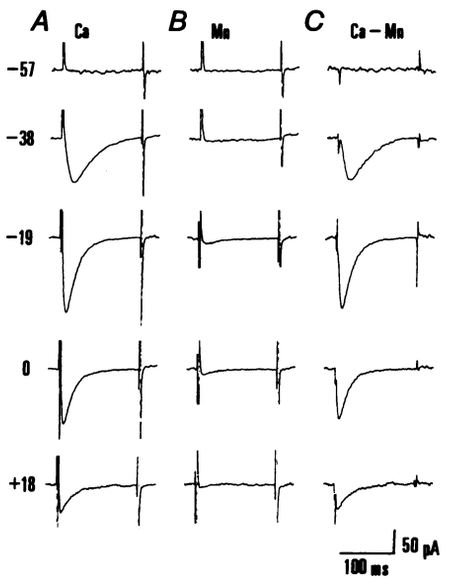

FIG. 1. (*A*) Current records in 10 mM Ca^{2+} solution. Membrane was clamped to the potential (mV) cited on the left of each trace; the holding potential was −94 mV. (*B*) Current records in 10 mM Mn^{2+} solution. All Ca^{2+} in the external solution was replaced by Mn^{2+}. In *A* and *B*, the transients due to the capacitance of the electrode were compensated by an analogue subtraction. (*C*) Current records in *B* were subtracted from current records in *A* to eliminate the distortion of the time course by the capacitative current. However, the subtraction was not perfect, and the initial portion of the record (up to 3–4 msec) could not be analyzed. Although an activation process of greater than first order was suggested, we did not determine the exact value.

Fig. 3*B*, showing that Sr^{2+} carried significantly greater current than did Ca^{2+}; Ba^{2+} also carried more current than Ca^{2+}.

Inactivation Is Voltage Dependent. The decay of the inward current during the maintained voltage pulse approximated a single exponential in Ca^{2+}, Ba^{2+}, or Sr^{2+} solution. The decay could be due to: (*i*) the development of a counteracting outward current (11, 14), (*ii*) Ca^{2+}-induced inactivation of the current (15, 16), or (*iii*) voltage-dependent inactivation similar to that of the Na^+ channel described in the original Hodgkin–Huxley theory (17). The result obtained in Mn^{2+} solution (Fig. 1*B*) and the fact that substitution of tetraethylammonium ion for Na^+ in the external solution did not alter the decay indicate that the

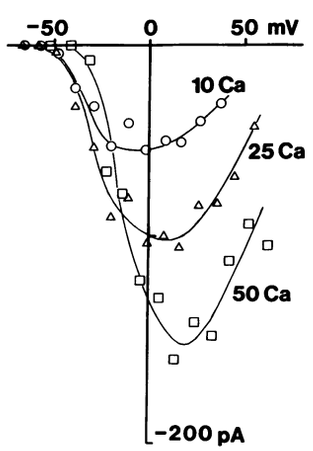

FIG. 2. Current–voltage relationships in 10, 25, and 50 mM Ca^{2+} solutions. The amplitudes of peak inward currents were plotted against membrane potential levels. The solutions of higher Ca^{2+} concentrations were made by replacing NaCl in 10 mM Ca^{2+} solution with $CaCl_2$.

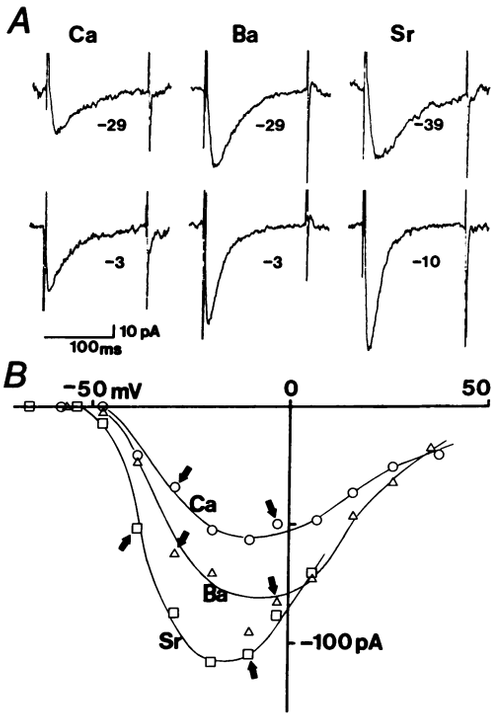

FIG. 3. (*A*) Current records in 25 mM Ca^{2+}, Ba^{2+}, and Sr^{2+} solutions. The membrane potential level (mV) is indicated adjacent to each record. The data from these current records are indicated by arrows in *B*. These are original nonsubtracted records. The decay time course was considered to be roughly the same in either Ca^{2+}, Ba^{2+}, or Sr^{2+} solutions; however, strictly speaking, the decay in Ca^{2+} solution was slightly slower than that in Ba^{2+} or Sr^{2+} solution. We need further experiments to determine if this difference is significant. (*B*) Current–voltage relationships at the peak of the inward current in 25 mM Ca^{2+}, Ba^{2+}, or Sr^{2+} solutions.

decay is probably not due to a delayed K^+ current. The decay time constant was calculated for the cell shown in Fig. 1 and plotted against the membrane potential together with the peak amplitude of the inward current obtained in the same experiment (Fig. 4*A*). These plots show that the time constant decreases as the membrane potential becomes more positive, even after the peak inward current reached its maximum amplitude. This suggests that the decay is not current dependent. This fact, together with the similar decay time course in Ca^{2+}, Sr^{2+}, and Ba^{2+} solutions, excludes the possibilities of Ca^{2+}-induced inactivation and a decay produced by a Ca^{2+}-induced K^+ current (14). Thus, the decay is considered to be the voltage-dependent inactivation. This was further supported by the measurement of the steady-state inactivation (Fig. 4*B*). The inward current obtained from a test pulse to −10 mV was completely suppressed when the membrane potential was held at values more positive than −50 mV. In other words, the Ca^{2+} current was inactivated at membrane potentials more negative than the most negative potential at which it was activated. Therefore, the inactivation could not be due to the Ca^{2+} influx. Voltage-dependent inactivation and a selectivity where Sr^{2+} carries the largest current have been reported also for tunicate and mouse eggs (18, 19).

DISCUSSION

We examined the electrophysiological properties of myeloma cells because we suspected the possible involvement of voltage-gated Ca^{2+} channels in the immune response of lymphocytes (1–9). We have demonstrated that voltage-gated Ca^{2+} channels exist in S194 myeloma cells. However, the implications of the

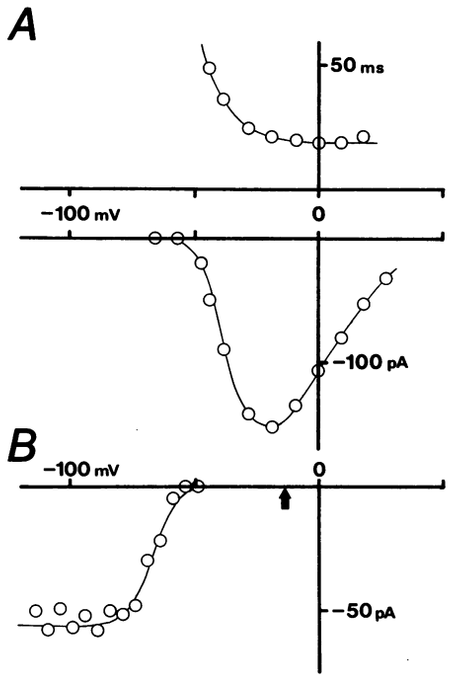

FIG. 4. (*A*) Inactivation time constant and current–voltage relationship in 10 mM Ca^{2+} solution. The time constant (upper plot) and amplitude (lower plot) of peak inward current were measured from the data shown in Fig. 1*C*. (*B*) Steady-state inactivation in 10 mM Ca^{2+} solution. The plot shows the amplitude of peak inward current obtained from a constant test command pulse to −10 mV (arrow) from various holding potentials. This cell is different from the cell in *A*.

Ca^{2+} channel in S194 myeloma cells are not clear at this time. The amount of Ca^{2+} current in a particular cell varied significantly, from 0 to ≈150 pA in 10 mM Ca^{2+} solution; the variation showed almost no correlation to cell size. Preliminary studies indicate that the hybrid cell from S194 myeloma cell and lymphocyte (hybridoma), which secretes monoclonal antibodies, also shows a similar Ca^{2+} current. Because we observed only one type of myeloma cell, the results cannot be considered to represent the properties of myeloma cells in general. We intend to investigate further the cause of the variations in current size and the significance of the Ca^{2+} channel in the immune response.

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