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A voltage-gated calcium channel is linked to the antigen receptor in Jurkat T lymphocytes

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Activation of T lymphocytes results in an increase in intracellular Ca^{2+} due in large part to influx of extracellular Ca^{2+}. Using the patch clamp technique, an inward current in Jurkat T lymphocytes was observed upon depolarization from a holding potential of −90 mV but not from −60 mV. This whole-cell current was insensitive to tetrodotoxin, carried by Ba^{2+}, and blocked by Ni^{2+}. Occupancy of the T lymphocyte antigen receptor increased the current's magnitude. These data suggest that antigen receptor-induced Ca^{2+} entry in T lymphocytes may be mediated by a voltage-regulated Ca channel.

Electrophysiology; T lymphocyte; Calcium channel; Activation; Antigen receptor

1. INTRODUCTION

Activation of T lymphocytes is initiated by the binding of appropriate antigen to the T lymphocyte antigen receptor. One of the early events consequent to engagement of the antigen receptor is an increase in the intracellular concentration of Ca^{2+} ($[Ca^{2+}]_i$), which is critical for T lymphocyte activation [1–3]. This increase consists of an early, transient peak that is independent of extracellular Ca^{2+} and a longer lived plateau that is dependent on influx of Ca^{2+} from outside the cell [2,4,5]. Despite the importance of Ca^{2+} influx, which contributes at least 80% to the overall increase in $[Ca^{2+}]_i$ [3], the mechanism and regulation of Ca^{2+} entry into T lymphocytes is poorly understood.

Initial electrophysiological examination of T lymphocytes found no evidence for voltage-gated Ca channels [6–9]. For this reason, lymphocytes have been placed in a class with other electrically non-excitable cells thought to contain only receptor-operated Ca channels [10]. A receptor-operated channel, once opened by receptor occupancy, would behave as a Ca^{2+}-selective, aqueous pore with Ca^{2+} entering the cell down its electrical and chemical gradients. The role of membrane potential in such a system would be limited to providing the electrical component of the inward gradient. This does not appear to be the case in T lymphocytes because both hyperpolarization and moderate depolarization eliminate receptor-mediated Ca^{2+} entry despite the continued presence of an inward driving force for Ca^{2+} [11,12].

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Using the patch clamp technique, we have identified a voltage-activated Ca^{2+} current in T lymphocytes from three human cell lines including Jurkat. Activation of Jurkat cells by antigen receptor occupancy resulted in an increase in the magnitude of this Ca^{2+} current, suggesting a functional link between the T lymphocyte antigen receptor and a voltage-gated Ca channel.

2. MATERIALS AND METHODS

2.1. *Cell lines*

The T lymphocyte cell lines Jurkat, Molt-4 and HSB were cultured as described previously [13].

2.2. *Electrophysiology*

Cells were allowed to settle to the bottom of a 35 mm Petri dish coated with 0.1% poly-L-lysine (Sigma Chemical, St. Louis, MO) and were maintained at 37°C in an open perfusion micro-incubator (Medical Systems Corp., Greenvale, NY). All recordings were performed using the whole cell, gigaseal patch clamp method [14,15] with microelectrodes of 10–15 MΩ series resistance. In all solutions, K^+ was replaced with Cs^+ to block outward K^+ currents [16]. Unless otherwise noted, bath solutions contained (in mM): NaCl, 110; CsCl, 5; $CaCl_2$, 10; $MgCl_2$, 2.2; D-glucose, 5.6; HEPES, 39 (pH 7.4 with NaOH). The pipette solution contained (in mM): CsCl, 130; $CaCl_2$, 1; NaCl, 20; EGTA, 11; HEPES, 5 (pH 7.3 with NaOH). Both solutions were 290–300 mOsm. Records were low-pass filtered at 5 kHz, and the data were collected, stored and analyzed using the pClamp software program (Axon Instruments, Foster City, CA).

3. RESULTS AND DISCUSSION

The resting membrane potential of T lymphocytes is approximately −60 mV [1,11]. Depolarization of Jurkat cells maintained at 37°C from a holding membrane potential of −60 mV did not evoke inward currents (Fig. 1a). However, in murine [1,11] and human (data not

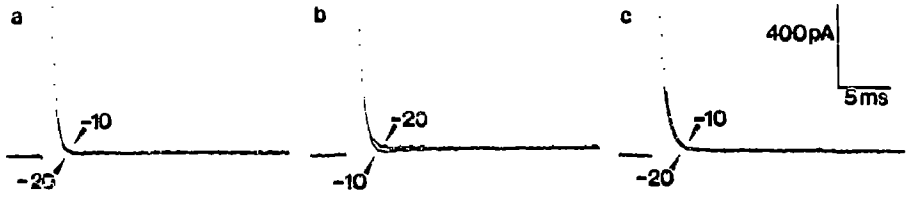

Fig. 1. Whole-cell currents recorded from Jurkat T lymphocytes. (a) Depolarization was to −20 mV (−20) or −10 mV (−10) from a holding potential of −60 mV. (b) Same cell as in (a), but depolarized from a holding potential of −90 mV. (c) Same cell as in (a) and (b), after returning the holding potential to −60 mV. These and subsequent tracings are representative of 75% of more than 100 cells tested.

shown) T lymphocytes,one of the earliest consequences of antigen receptor engagement is hyperpolarization of the membrane potential to −90 mV. As shown in Fig. 1b, depolarization of a Jurkat cell from a holding potential of −90 mV resulted in the appearance of an inward current. This current activated very rapidly and inactivated within 10–20 ms. When the holding potential was returned to −60 mV, the depolarization-induced inward current disappeared (Fig. 1c).

In order to identify the carrier of this inward current, tetrodotoxin was added to the bath solution. Tetrodotoxin, a potent and specific blocker of Na channels [17], had no effect on the inward current (data not shown). Similarly, isosmotic replacement of extracellular Na^+ with tetraethylammonium, which will not traverse Na channels [18], did not affect the inward current (data not shown). It is therefore unlikely that Na channels are responsible for the inward current in Jurkat cells.

Ni^{2+} has been shown to block Ca channels in many systems [16,19] and blocks Ca^{2+} influx in receptor-stimulated Jurkat cells when $[Ca^{2+}]_i$ is measured by fluorometry [20]. When Ni^{2+} was added to the extracellular solution, the inward current (Fig. 2a) was completely blocked (Fig. 2b). This effect of Ni^{2+} was reversed by exchanging the medium containing Ni^{2+} for medium without Ni^{2+} (Fig. 2c). Unlike Ni^{2+}, Ba^{2+} will pass through Ca channels and carry an inward current [16,21]. As shown in Fig. 2d, the depolarization-induced inward current was present when Ca^{2+} in the bath solution was replaced with equimolar Ba^{2+}. The current in response to depolarization with Ba^{2+} present (−112 pA ± 85, $n = 4$) was larger than that seen in medium containing Ca^{2+} (−27.0 pA ± 13.0, $n = 4$, $P < 0.05$). This is consistent with increased Ba^{2+} permeability through Ca channels [22]. The inward current, presumably carried by Ba^{2+}, was blocked by treatment with Ni^{2+} (Fig. 2d). Taken together, the data suggest that the voltage-activated inward current in Jurkat cells is carried by Ca^{2+}.

To determine whether this Ca^{2+} current was unique to Jurkat cells, two other T lymphocyte cell lines, Molt-4 and HSB, were examined. Fig. 3a displays current–voltage curves for the peak, inward current in a single Jurkat cell, showing a depolarization-induced inward current that was eliminated by addition of Ni^{2+} to the bath solution. The current–voltage relation for a Molt-4 cell is shown in Fig. 3b, before and after addition of 1 mM Ni^{2+} to the bath solution. Similar results were obtained for HSB cells (data not shown), indicating that the voltage-activated Ca^{2+} current is common to these three cell lines. Because Jurkat, Molt-4 and HSB are T leukemia cell lines, one explanation for these results is that expression of a voltage-gated Ca channel is a product of leukemic transformation. It is also possible that the channel is expressed in all T lymphocytes and plays a role in the activation of normal T cells.

A previous report described a voltage-activated Ca^{2+} current in 40% of examined Jurkat cells [23]. It is unlikely that the current of that previous work is the same

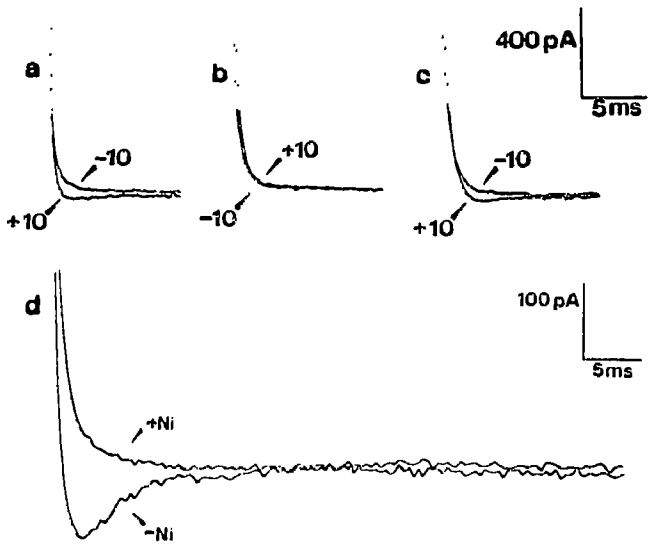

Fig. 2. Effect of Ni^{2+} on the inward current in Jurkat cells. Panels (a), (b) and (c) are from a single Jurkat cell in a bath solution containing 10 mM Ca^{2+}. Panel (d) is a different Jurkat cell in a bath solution containing 10 mM $BaCl_2$ instead of $CaCl_2$. The holding potential for (a), (b) and (c) was −90 mV. (a) The depolarizing steps were to −10 mV (−10) or 10 mV (+10). (b) Same cell as in (a), but following the addition of 1 mM $NiCl_2$ to the extracellular solution. (c) Same cell as in (a) and (b) after exchange of medium containing Ni^{2+} for medium without Ni^{2+}. (d) The holding potential was −100 mV with depolarizing steps to 0 mV. Data were obtained sequentially from a single cell in the absence (−Ni) or presence (+Ni) of 1 mM $NiCl_2$. The upper scale (Y-axis = 400 pA) is for panels (a), (b) and (c). The lower scale (Y-axis = 100 pA) is for panel (d). The tracings are representative of more than 25 cells tested.

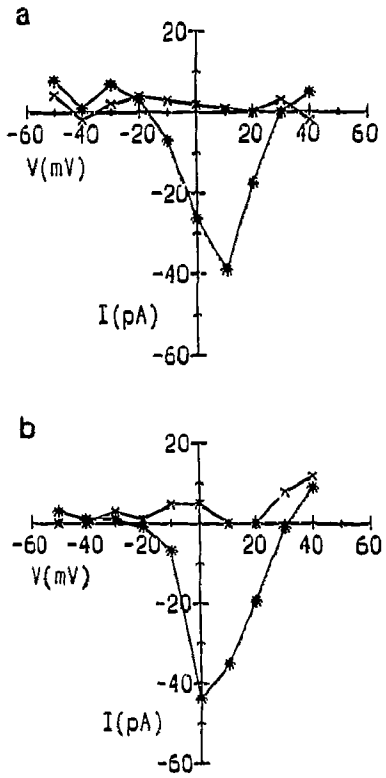

Fig. 3. Effect of Ni^{2+} on the inward current in Jurkat and Molt-4 cells. In the presence of 10 mM Ca^{2+}, the holding potential was −90 mV and depolarizing pulses were applied at 2 s intervals. The largest inward current occurred at approximately 5–6 ms following the depolarizing pulse and steady state current was reached by 48 ms. For all depolarizing pulses, the current at 45–48 ms was subtracted from the current at 5–6 ms to obtain the values shown. (a) Current–voltage relation of a single Jurkat cell before * or after (X) the addition of 1 mM $NiCl_2$ to the bath solution. (b) The current–voltage relation of the inward current of a single Molt-4 cell before * or after (x) the addition of 1 mM $NiCl_2$ to the bath solution. These cells are representative of more than 10 cells tested.

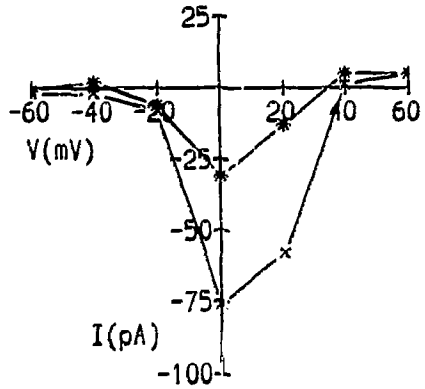

Fig. 4. Effect of anti-CD3 monoclonal antibody, OKT3, on the inward current. The holding potential was −90 mV and depolarizing pulses were applied at 2 s intervals. The largest inward current occurred at approximately 6.5 ms following the depolarizing pulse and steady state current was reached by 45 ms. For all depolarizing pulses, the current at 45 ms was subtracted from the current at 6.5 ms to obtain the values shown. The current–voltage relationship of the inward current of an untreated cell (*) is compared to a cell treated with OKT3 at a concentration of 1 μg/ml (X) added 30 s before rupture of the membrane patch. Each curve is representative of more that 20 cells tested.

one examined in this report. The current in the earlier study was very large and long lasting, properties similar to the L-type Ca channel [23]. In contrast, the Ca^{2+} current reported here is smaller and more transient. Additionally, verapamil, which blocks L-type Ca channels [17] but not T-type Ca channels, has no effect on receptor-stimulated increases in $[Ca^{2+}]_i$ in cytolytic T lymphocytes [11] or Jurkat cells (L.S.G., unpublished observation). The Ca^{2+} current described in the present study is not blocked by verapamil (data not shown). Taken together, these data suggest that the voltage-gated Ca channel examined in this report is more closely related to the T-type Ca channel [22].

To investigate a possible role for a voltage-gated Ca channel in Jurkat cell activation, the Ca^{2+} current in activated and resting Jurkat cells was examined. Anti-CD3 monoclonal antibodies, such as OKT3, activate T lymphocytes in a manner that is similar to relevant antigen [24], causing an increase in $[Ca^{2+}]_i$ that depends in large part on influx [3]. When Jurkat cells were treated with OKT3 before rupturing the plasma membrane to attain the whole-cell configuration, the peak inward current was consistently increased in magnitude compared to control cells (Fig. 4). In response to depolarizing steps to 0 mV, the inward current in cells treated with OKT3 was −56.4 pA ± 20.0 ($n = 6$), while in untreated cells it was −28.7 pA ± 10.6 ($n = 6$, $P < 0.01$). In contrast, the inward current was unaffected by treatment with OKT3 after rupture of the membrane under the patch pipette (data not shown). Because membrane rupture results in dialysis of cellular contents into the electrode solution [25], these results suggest that the effect of antigen receptor stimulation on the voltage-dependence of the inward current is mediated by a diffusible, cytosolic molecule. Nonetheless, it is clear from the data in Fig. 4 that activation of intact Jurkat cells via the antigen receptor results in an increase in the whole cell Ca^{2+} current.

This report describes a receptor-linked, voltage-activated Ca^{2+} current in Jurkat T lymphocytes. Ca^{2+} influx was voltage dependent, although not linear with respect to voltage, suggesting that the current is conducted via a voltage-gated channel similar to that found in electrically excitable cells. These experiments do not provide evidence for any other Ca^{2+} current in these cells, although they do not rule out the presence of a current that is lost when attaining the whole cell configuration. Antigen-receptor stimulation caused an increase in magnitude of the voltage-activated Ca^{2+} current suggesting that this Ca channel is also agonist-dependent. This dual regulation of Ca channels by both voltage and agonist is similar to that seen in cardiac myocytes [26]. In summary, these data suggest that there is a functional link between the T lymphocyte antigen receptor and a

voltage-gated Ca channel which may be responsible for the receptor-induced increase in $[Ca^{2+}]_i$ that is necessary for T lymphocyte activation.

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