Characterization of Ca\(^{2+}\)-activated K\(^{+}\) channels in excised patches of human T lymphocytes

Jos A. H. Verheugen, Regina G. D. M. van Kleef, Marga Oortgiesen, Henk P. M. Vijverberg

Research Institute of Toxicology, Utrecht University, PO Box 80.176, NL-3508 TD Utrecht, The Netherlands

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Abstract. Ca\(^{2+}\)-activated K\(^{+}\) [K(Ca)] channels were studied in excised patches of resting and activated human peripheral blood T lymphocytes. The K(Ca) channel had a single-channel conductance of 50 ± 6 pS in symmetrical high-K\(^{+}\) solutions in the potential range of -100 to -10 mV and was inwardly rectifying at more depolarized potentials. The channel was sensitive to block by charybdotoxin (10 nM) and insensitive to apamin (3 nM). Half-maximum activation occurred at an internal free Ca\(^{2+}\) concentration of 360 ± 110 nM. The concentration-effect curve had a slope factor of 0.83 ±0.12, suggesting a 1:1 interaction of Ca\(^{2+}\) ions with the channel. Ca\(^{2+}\) affects the open time probability of the K(Ca) channels, mainly by modulating the frequency of channel opening. The open probability did not show voltage dependence. The kinetics of the channel could be described assuming one open state and two closed states. The time constant of the exponential describing the open time distribution amounted to 2.8 ± 1.2 ms, whereas the closed time distribution could be described with two exponentials with time constants of 0.2 ± 0.05 ms and 8.0 ± 2.1 ms, respectively. Resting T lymphocytes expressed a low number of channels but the density of channels increased dramatically during chronic phytohaemagglutinin stimulation.

Key words: K\(^{+}\) channel − Calcium − Single channel − Patch clamp − Lymphocyte − Charybdotoxin

Introduction

Activation of T lymphocytes by antigen or mitogenic lectins such as concanavalin A (conA) and phytohaemagglutinin (PHA), which bind to the T cell receptor/CD3 complex, involves a cascade of cellular events eventually resulting in cell proliferation. One of the first changes to occur is the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIns\(_{4,5}\)P) into diacylglycerol and inositol 1,4,5-trisphosphate (Ins\(_{1,4,5}\)P), followed by a rise in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_{i}\)) as a result of both Ca\(^{2+}\) release from internal stores and influx across the plasma membrane (reviewed in [33]). Frequently, [Ca\(^{2+}\)]\(_{i}\) oscillations are observed, which peak in the low micromolar range and disappear when Ca\(^{2+}\) is removed from the extracellular medium [19]. Ca\(^{2+}\) entry is reduced by depolarization [12, 19, 26, 30] and is thought to occur through voltage-independent Ca\(^{2+}\) channels [23, 26]. Although Ca\(^{2+}\) influx would be expected to depolarize the cell, mitogenic stimulation generally causes hyperpolarization ([9, 27, 35, 36]; reviewed in [13]). This hyperpolarization of the membrane potential concomitant with the increase in [Ca\(^{2+}\)]\(_{i}\) is consistent with the opening of Ca\(^{2+}\)-activated K\(^{+}\) [K(Ca)] channels, as originally proposed by Rink and Deutsch [34].

Previous investigations using the whole-cell patch-clamp technique have failed to detect K(Ca) channels in mouse cytotoxic T cells or resting human T cells [3, 10]. Recently, however, single K(Ca) channels have been described in rat thymic and human tonsillar B lymphocytes using excised patch and cell-attached patch-clamp techniques [27, 28]. Two conductance levels were observed in cell-attached recordings (6–8 and 17–25 pS), whereas ion channels in excised patches had a main conductance level of 34 pS. In splenic human B lymphocytes a K(Ca) channel with a single-channel conductance of 26 pS has been described, using whole-cell, cell-attached and inside-out patch-clamp recording. This channel was blocked by charybdotoxin (CTX) and was insensitive to apamin [31].

Grissmer et al. [15] characterized K(Ca) channel properties in the Jurkat human leukaemic T cell line in whole-cell, perforated-patch and cell-attached patch-clamp experiments. The predominant channel present was apamin sensitive, CTX insensitive and had a unitary conductance of 4–7 pS. In addition, a less prevalent K(Ca) channel with a conductance of 40–60 pS, which was blocked by CTX and not by apamin, was observed in these cells. Leonard et al. [25] distinguished three
types of Ca\textsuperscript{2+}-activated channels in excised patches of resting human T lymphocytes. The three types of Ca\textsuperscript{2+}-activated channels had unitary conductances of 11–14 pS, 21–23 pS and 35 pS and were all sensitive to block by CTX as assessed in whole-cell voltage-clamp experiments.

Despite these observations a comprehensive description of the single-channel characteristics of K(Ca) channels in human T lymphocytes is lacking. In the present report, a CTX-sensitive K(Ca) channel, with a conductance of 50±6 pS is characterized in excised patches of resting and PHA-activated human peripheral blood T lymphocytes. It is demonstrated that this K(Ca) channel, which is the prevalent Ca\textsuperscript{2+}-activated conductance in human T lymphocytes, is activated by physiological Ca\textsuperscript{2+} concentrations.

Materials and methods

Cells. Human peripheral blood lymphocytes were isolated from heparinized blood by healthy volunteers using the standard Ficoll-Paque sedimentation technique. The resulting cell population contained more than 75% T lymphocytes as assessed by CD4/CD8 fluorescent labelling. The population contained more than 75% T lymphocytes as assessed by CD4/CD8 fluorescent labelling.

Cells referred to as “resting T cells” were kept overnight at 4°C in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) and the following amino acids (in mM): cysteine · HCl 0.3, L-alanine 0.4, L-aspartate 0.45, L-aspartic acid 0.4, L-proline 0.4 and L-glutamic acid 0.4. Cells were used the day after isolation. Cells referred to as “activated T cells” were incubated in the same medium supplemented with 1 µg/ml PHA (Wellcome, Dartford, England). Cultures were maintained at 37°C in a humidified atmosphere containing 7.5% CO\textsubscript{2}. The medium, including the PHA, was refreshed every 2–3 days. Activated T cells were used at days 1–7.

Electrophysiology and data analysis. Single K(Ca) channel currents were recorded in inside-out and outside-out membrane patches as described by Hamill et al. [18], using an EPC-7 patch-clamp amplifier (List-Elektronik, Darmstadt, Germany). Borosilicate glass pipettes (Clark GC150) with a resistance of 4–6 MΩ were used. After formation of a gigaseal, the attached cell was ruptured by gentle movements of the pipette, resulting in an inside-out patch. To obtain outside-out membrane patches the whole-cell configuration was first established and the pipette was pulled away from the cell. In general, outside-out patches were more difficult to obtain, and older and bigger cells had to be used. All patches were voltage-clamped at a membrane potential of −60 mV, unless stated otherwise. Patches were voltage-clamped at a membrane potential of −60 mV, unless stated otherwise. Patches were alternately superfused with control and test solutions at a flow rate of 350–400 µl/min using two pipettes with a diameter of 100 µm. The opening of the superfusing pipette was always within 50 µm of the patch. Experiments were performed at room temperature (20–24°C). Single-channel currents were low-pass filtered at 3 kHz using an 8-pole Bessel filter and digitized (8 bits; 0.05 ms sample interval; 1024 points/record). For each test condition at least 100 records of 50 ms were recorded at intervals of 550 ms and stored on magnetic disc for off-line analysis. The single-channel amplitude was determined based on the peaks in an amplitude histogram. Transitions between open and closed states were detected using a half-amplitude threshold criterion and a minimum event width of 0.25 ms. Open channel probability (P\textsubscript{o}) was calculated by dividing the sum of all open times by the total observation time. The frequency of channel opening was determined as the ratio between the number of opening transitions and the total observation time. The mean open time of channels in patches with multiple open levels was calculated by dividing the total open time by the total number of closing transitions [20]. Cells were repeatedly superfused with a solution with a free [Ca\textsuperscript{2+}] of 336 nM. Patches showing a trend in P\textsubscript{o} at this concentration were discarded from analysis. For analysis of single-channel amplitude and P\textsubscript{o}, patches with 1–6 multiple open channel levels were used. For analysis of the channel kinetics patches in which only one open level was observed were used. The lifetimes of events that were completed within the duration of the record were divided into classes of approximately equal frequency and presented as frequency density histograms. Exponential time constants were estimated by minimizing χ\textsuperscript{2} using a Levenberg-Marquardt non-linear least-squares algorithm [29]. Concentration-effect curves were fitted by the equation:

\[ E = E_{\text{max}}/[1+(EC50/Ca^{2+})^n] \]

using the Levenberg-Marquardt algorithm. Linearity and regression tests were performed by least-squares regression analysis [6]. Results are presented as mean ± SD, unless stated otherwise.

Solutions. Solutions were prepared from milli-Q/UF purified water (Millipore, Bedford, USA). The bath solution contained (in mM): 145 NaCl, 5 KCl, 1.8 CaCl\textsubscript{2}, 0.8 MgCl\textsubscript{2}, 20 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid (HEPES), adjusted to pH 7.4 with NaOH and to 325–345 mosmol/l with 30 mM glucose. The pipette solution contained (in mM) either (1) 130 K aspartate, 20 KC\textsubscript{1}, 10 NaCl, 10 HEPES, 2 CaCl\textsubscript{2}, 1 MgCl\textsubscript{2}, adjusted to pH 7.25 with KOH and to 315 mosmol/l with 20 mM sucrose, or (2) 150 KCl, 5 HEPES, 0.5 ethylenebis(oxoimino)tetraacetate (EGTA), adjusted to pH 7.2 with KOH and to 315 mosmol/l with 50 mM sucrose. The internal solution for inside-out patches contained (in mM): 120 KNO\textsubscript{3}, 10 HEPES, and either 10 citric acid or 10 EGTA as Ca\textsuperscript{2+} chelator, and various concentrations of Ca(NO\textsubscript{3})\textsubscript{2}, adjusted to pH 7.2 with approximately 30 mM KOH and to 300 mosmol/l with 55 mM sucrose. To avoid contamination with heavy metals, which may affect K(Ca) channels, ultrapure chemicals were used. The final free [Ca\textsuperscript{2+}] of the citrate buffers was measured with Fura-2 (0.1 µM) using a Perkin-Elmer Luminiscence Spectrometer (LS 5) in a 2 ml cuvette. Emission was measured at 510 nm with excitation alternating between 340 and 380 nm. The free [Ca\textsuperscript{2+}] was calculated according to Grynkiewicz et al. [17]. Maximum and minimum fluorescence ratios were determined at each concentration by adding 1 mM CaCl\textsubscript{2} and 10 mM EGTA, successively. A K\textsubscript{D} of 225 nM for Fura-2 was assumed.

Atpamin was purchased from Serva, Heidelberg, Germany and CTX from Research Biochemicals Incorporated, Natick, USA.

Results

Alternate superfusion of inside-out membrane patches of human lymphocytes with control solution (free [Ca\textsuperscript{2+}]< 20 nM) and solutions containing various [Ca\textsuperscript{2+}] revealed the existence of a Ca\textsuperscript{2+}-activated channel, the activity of which increased with increasing [Ca\textsuperscript{2+}] (Fig. 1). The number of channels present showed a clear dependence on the state of activation. The K(Ca) channel was only occasionally observed in patches of resting cells, but the number of channels per patch increased with the days of incubation with PHA. At day 1 of incubation with PHA (18–26 h) approximately 50% of the patches contained one or more K(Ca) channels. Both the percentage of patches containing channels and the apparent number of channels in the patches showed a further increase at day 2. Maximum density of K(Ca) channels appeared to be reached at day 3. After 3–7 days of incubation with PHA virtually every patch contained channels and 10 or more channels per patch were frequently observed. The time course of the increase in channel