Characterization of apamin-sensitive Ca^{2+}-activated potassium channels in human leukaemic T lymphocytes

Christine Hanselmann and Stephan Grissmer*

Department of Applied Physiology, University Ulm, Albert-Einstein-Allee 11, 89081 Ulm, Germany

1. The whole-cell recording mode of the patch-clamp technique was used to study the effect of extracellularly applied ions, toxins and drugs on voltage-independent, apamin-sensitive Ca^{2+}-activated K^+ channels, K(Ca), expressed in the Jurkat human leukaemic T cell line.

2. Extracellular Ba^{2+} and Sr^+ produced a voltage-dependent block. The equilibrium dissociation constant of the Ba^{2+}/K(Ca) channel complex increased e-fold for a 20 mV change of potential. Ba^{2+} block of Jurkat K(Ca) channels is therefore as steep as expected from the movement of a single divalent cation about half-way into the electric field of the membrane from the outside.

3. We determined the ion selectivity as well as the conductance of these channels. Calculated permeability ratios, P_X/P_K, for these K(Ca) channels were 1·0, 0·96, 0·26 and 0·53 for K^+, Rb^+, Cs^+ and NH_4^+, respectively. Conductance ratios, g_X/g_K, for the same ions were 1·0, 1·0, 0·67 and 0·11, respectively. Most strikingly this channel can also carry significant current with Cs^+ as current carrier.

4. Scyllatoxin (ScTX), a thirty-one amino acid peptide toxin, reduced current through these K(Ca) channels with a half-blocking concentration of ~0·3 nM independent of the pH. Other drugs that were able to reduce current through these channels include the classical calcium antagonists diltiazem and verapamil. In contrast, nifedipine, clotrimazole and kaliotoxin (100 nM) were unable to block current through these channels in Jurkat T cells.

The human leukaemic T cell line Jurkat expresses two different types of K^+ channels. One type belongs to the classical voltage-dependent K^+ channel family, K(V). It opens with depolarization, is blocked by charybdotoxin (CTX), noxiustoxin (NTX), kaliotoxin (KTX) and margatoxin (MgTX), but not blocked by apamin, shows C-type inactivation and the gene that encodes this ion channel is Kv1.3 (for review see: Chandy, Gutman & Grissmer, 1993; Chandy & Gutman, 1995; Aiyar *et al.* 1995).

The second type of K^+ channel in Jurkat T cells is activated by an increase in the intracellular calcium concentration ($[Ca^{2+}]_i$). Hille (1992) described two distinct classes of K(Ca) channels. One class, the BK channel, is activated by membrane depolarizations and by an increase in $[Ca^{2+}]_i$ with single-channel conductances of more than 100 pS. The second class, the SK channels, are voltage independent and have conductances smaller than 50 pS. The K(Ca) channels described in this paper belong to the second class. Within this second class, however, again two major groups can be distinguished on the basis of their pharmacology. The first is blocked by CTX, the same toxin that will also block BK channels (reviewed by Latorre, Oberhauser, Labarca & Alvarez, 1989) as well as some K(V) channels, for example, the type *n* channel in human T cells, that is Kv1.3 (Sands, Lewis & Cahalan, 1989; Grissmer *et al.* 1990). The second group is blocked by apamin and ScTX (Hugues, Schmid, Romey, Duval, Frelin & Lazdunski, 1982; Cognard, Traore, Potreau & Raymond, 1984; Blatz & Magleby, 1986; Romey, Rieger, Renaud, Pincon-Raymond & Lazdunski, 1986). In Jurkat T cells the K(Ca) channels are highly sensitive to block by apamin, a peptide component of bee venom, which blocks half the channels at < 1 nM (Grissmer, Lewis & Cahalan, 1992), but are insensitive to block by CTX and NTX. In addition, K(Ca) channel activity in Jurkat T cells depends greatly on $[Ca^{2+}]_i$, suggesting that multiple Ca^{2+} ions must bind to the channel or an associated molecule in order to open the pore. The Ca^{2+} concentration at which half the channels are activated is ~400 nM. Channels do not inactivate during prolonged exposure to high $[Ca^{2+}]_i$ and are activated independently of the applied membrane potential (Grissmer *et al.* 1992). Both Kv1.3 and apamin-sensitive SK channels have been implicated in the modulation of the Ca^{2+} signal of Jurkat T cells after activation by mitogens. The overall goal of this study was to characterize in more detail the properties of small-conductance apamin-sensitive SK

* To whom correspondence should be addressed.

channels. Using Jurkat T cells for this purpose has the advantage that there is little or no contamination by other K(Ca) channels.

In this article, we report biophysical and pharmacological properties of K(Ca) channels in Jurkat T cells. We have used whole-cell recording to determine (a) the sensitivity of K(Ca) channels in Jurkat T cells to a variety of ionic blockers, drugs and peptide toxins, and (b) the ion selectivity of these channels. This characterization should allow a better distinction between different types of K(Ca) channels.

Some of the results have been reported in preliminary communications (Grissmer & Hanselmann, 1996; Hanselmann & Grissmer, 1996).

METHODS

Cells

The experiments were carried out on single cells of a human T-lymphoma cell line, Jurkat E6-1. These cells were obtained from the American Type Culture Collection (Rockville, MD, USA) and maintained continuously in culture medium RPMI 1640 (Gibco) supplemented with 1 mM glutamine and 10% heat-inactivated fetal bovine serum in a humidified, 5% CO_2 incubator at 37 °C. Since the expression of K(Ca) channels in Jurkat T cells did not seem to depend on the passage number, all experiments were performed on cells derived from a single batch of cells kept in culture.

Solutions

All experiments were done at room temperature (21–25 °C). The cells under investigation were normally bathed in K^+ solutions containing (mM): 164·5 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 5 Hepes; adjusted to pH 7·4 with KOH, with an osmolarity of 290–320 mosmol l^{-1}. Rb^+, Cs^+, NH_4^+ and Na^+ solutions contained 164·5 mM of the major cation, plus normal Cl^-, divalents, and pH buffering. The pH 6 K^+ solution was obtained by adjusting the pH to 6·0 with KOH. A simple syringe-driven perfusion system was used to exchange solutions in the recording chamber.

We used two different internal pipette solutions with different Ca^{2+}-buffer capacity to either increase $[Ca^{2+}]_i$ by whole-cell dialysis of a highly buffered Ca-EGTA pipette solution or to use a lightly buffered pipette solution that would allow an increase in $[Ca^{2+}]_i$ by application of ionomycin. Both solutions contained (mM): 135 potassium aspartate, 2·0 $MgCl_2$ and 10 Hepes; adjusted to pH 7·2 with KOH, with an osmolarity of 290–320 mosmol l^{-1}. The solution with highly buffered Ca^{2+} contained (mM): 10 EGTA and 8·7 $CaCl_2$ ($[Ca^{2+}]_{free}$ of 10^{-6} M). The lightly buffered Ca^{2+} solution contained (mM): 1·1 EGTA and 0·1 $CaCl_2$ ($[Ca^{2+}]_{free}$ of 10^{-8} M). $[Ca^{2+}]_{free}$ was calculated assuming a dissociation constant for EGTA and Ca^{2+} at pH 7·2 of 10^{-7} M (Portzehl, Caldwell & Ruegg, 1964).

Ionomycin was purchased from Sigma-Aldrich Chemie (Deisenhofen, Germany). Apamin, ScTX, CTX and KTX were purchased from Latoxan (Rosans, France). NTX was a generous gift from Dr Stephen Brady (Merck, Sharp & Dohme Research Laboratories, Westpoint, PA, USA). Bovine serum albumin (0·1–1 mg ml^{-1}; Fluka Chemie, Neu-Ulm, Germany) was usually added to toxin-containing solutions to prevent binding of the toxins to plastic in the perfusion system and resulted in reproducible dose–response determinations.

Electrophysiology

Experiments were carried out using the whole-cell recording mode of the patch-clamp technique (Hamill, Marty, Neher, Sakmann & Sigworth, 1981). Electrodes were pulled from glass capillaries (Clark Electromedical Instruments, Reading, England) in two stages, coated with Sylgard (Dow Corning, Seneffe, Belgium), and fire polished to resistances measured in the bath of 3–6 MΩ. Membrane currents were recorded with an EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany) interfaced to a Macintosh computer running acquisition and analysis software (Pulse/Pulsefit; HEKA Electronik). All potentials were corrected for the liquid junction potential that develops at the tip of the pipette if the pipette solution is different from that of the bath. The liquid junction potential between normal internal (pipette) and external (bath) solution was −8 mV.

All illustrated results were done at least in triplicate except for the dose–response curve for ScTX block (Fig. 6*B*) where single data points are shown. For the experiments with the toxins as well as the drugs each tested compound was applied for at least 5 min and all illustrated records represent steady-state effects.

All values are means ± S.E.M.

RESULTS

To characterize K(Ca) channels in Jurkat T cells, we elevated $[Ca^{2+}]_i$ by bath application of ionomycin and simultaneously measured conductance changes with the whole-cell recording technique. Figure 1*A* shows ramp currents recorded in response to 400 ms voltage ramps from −160 to 40 mV. Trace 1 was recorded in K^+ solution before the application of ionomycin to the bath solution. The membrane slope conductance at potentials more negative than −60 mV is small and increases around −40 mV as voltage-gated Kv1.3 channels open and conduct inward current below and outward current above 0 mV. Application of 1 μM ionomycin increased the slope conductance at negative potentials (trace 2–4). The time course of this effect is shown in Fig. 1*B*. The numbers in the graph correspond to the current traces shown in panel *A*. Activation of K(Ca) channels in Jurkat T cells can also be achieved by dialysing the cytoplasm during whole-cell recording with pipette solution containing 1 mM free calcium. Using this activation protocol current through K(Ca) channels in Jurkat T cells could be maintained for at least 15 min.

Ionic blockers

Figure 2*A* shows the effect of different concentrations of external Ba^{2+} on current through K(Ca) channels. Current was elicited with the same voltage ramp protocol as described in Fig. 1, also in K^+ solution. Extracellular Ba^{2+} reduced current through K(Ca) channels in a dose-dependent manner and also preferentially at hyperpolarized potentials. Only the highest Ba^{2+} concentrations (10 mM) had an effect on current through voltage-gated Kv1.3 channels as can be seen by the shift of ~10 mV towards more depolarized potentials of the activation voltage (Grissmer & Cahalan, 1989; Grissmer, Nguyen & Cahalan,

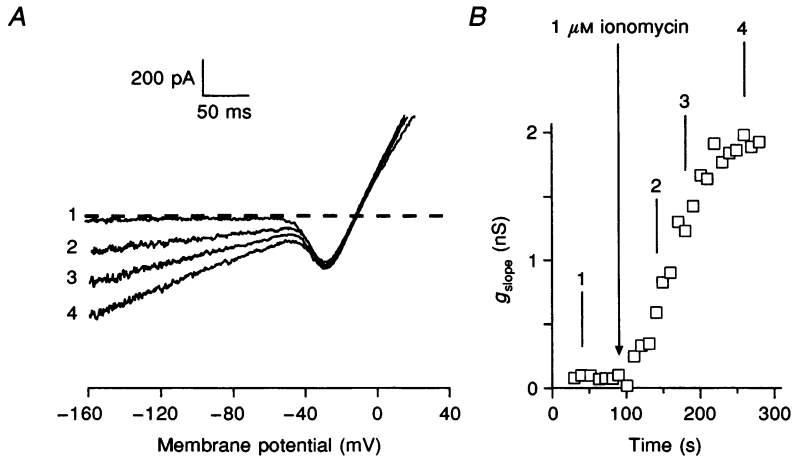

Figure 1. Ionomycin activates a K^+ conductance in Jurkat T cells

Whole-cell recording with pipette solution containing a low Ca^{2+} buffer ($[Ca^{2+}]_{free} = 10^{-8}$ M). *A*, ramp currents were elicited by 400 ms voltage ramps from −160 to 40 mV every 10 s before and after application of a K^+ solution containing 1 μM ionomycin. A progressive increase in $I_{K(Ca)}$ induced by ionomycin is manifest as the increase in slope conductance at potentials below −60 mV. *B*, the slope conductance at potentials below −60 mV, a measure of activation of K(Ca) channels, was plotted as a function of time for the experiment shown in *A*. The slope conductance was determined by linear fits to the ramp currents between −160 and −60 mV.

1993). The effect of Ba^{2+} on current through K(Ca) channels in Jurkat T cells was immediately reversible upon washout (data not shown). To quantify the voltage-dependent Ba^{2+} block of K(Ca) channels in Jurkat T cells we calculated ratios of the ramp current in the presence and absence of Ba^{2+} as shown in Fig. 2*B*. Here, the ratios of the ramp currents at the different Ba^{2+} concentrations were plotted against the membrane potential. The smooth curves through the ratio for the different Ba^{2+} concentrations represent fits to Boltzmann equations. The steepness factor of all three equations was 20 mV per e-fold change. Ba^{2+} block of K(Ca) channels in Jurkat T cells is therefore as steep as expected from the movement of a single divalent cation about halfway into the electric field.

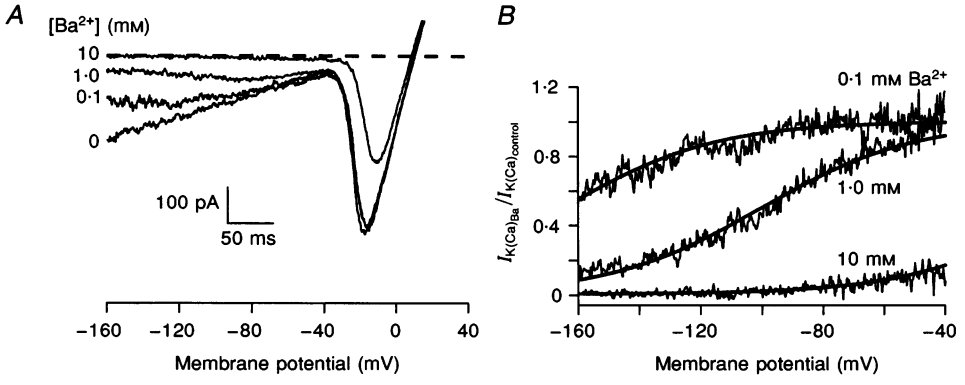

Figure 2. External Ba^{2+} block of K(Ca) channels in Jurkat T cells using voltage ramps

A, K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Ramp currents were elicited by 400 ms voltage ramps from −160 to 40 mV every 10 s before and after application of a K^+ solution containing 0·1, 1 and 10 mM Ba^{2+}. *B*, voltage dependence of the Ba^{2+} block using voltage ramps. Ramp current traces in the presence of external Ba^{2+} were divided by the trace obtained in the absence of Ba^{2+} (from *A*) and plotted against the membrane potential. The smooth curves represent fits (by eye) to a Boltzmann equation:

$$I_{K(Ca)Ba}/I_{K(Ca)} = 1/\{1 + \exp((E_h - E)/k)\},$$

where E_h is the voltage at which half the channels are blocked and k is the steepness of block (mV per e-fold change). The parameters for the fits were, for 0·1 mM Ba^{2+}: $E_h = -165$ mV and $k = 20$ mV; for 1 mM Ba^{2+}: $E_h = -100$ mV and $k = 20$ mV; and for 10 mM Ba^{2+}: $E_h = -35$ mV and $k = 20$ mV.

Table 1. Electrophysiological and pharmacological profile of apamin-sensitive K(Ca) channels in Jurkat T cells ($K(Ca)_{apa}$), and CTX-sensitive K(Ca) channels in human peripheral T lymphocytes ($K(Ca)_{CTX}$)

	Ionic blockers			Peptide toxins[d]					Single channel conductance[a,f]
	Ba^{2+}		$TEA^{+a,d}$	CTX	NTX	KTX	ScTX	Apamin	
	Voltage dependence[b] (δ)	Sensitivity[c] (E_h; mV)	(mM)	(nM)	(nM)	(nM)	(nM)	(nM)	(pS)
$K(Ca)_{apa}$	0·62 ± 0·02	−30	2	>100[a]	>100[a]	>100	0·3	~0·3	4–7[g]
$K(Ca)_{CTX}$	0·74 ± 0·04	−60	40	3	>100	>100	n.d.[e]	>100	11[h], 35[g]

a, data from Grissmer *et al.* 1993; b, δ is the partial electrical distance of the Ba^{2+} binding site across the membrane from the outside. Values are given as means ± S.D.; $n = 3$ for each channel type. The values are significantly different ($P < 0·05$). c, E_h is the potential where half the channels are blocked in 10 mM Ba^{2+}. d, values for the apparent dissociation constant for block. e, n.d., not determined. f, data from Grissmer *et al.* 1992. g, single channel conductance determined in symmetrical 160 mM K^+ solution. h, single channel conductance determined in 4·5 mM $[K^+]_o$ and 160 mM $[K^+]_i$.

Similar experiments with external Ba^{2+} were performed using voltage steps to assure that Ba^{2+} block is fast compared with the ramp (Fig. 3). Channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free calcium. Membrane currents were recorded in K^+ solution (control) and K^+ solution plus 1 or 10 mM Ba^{2+}. Initial experiments yielded large current traces at all potentials tested that decayed rapidly to a steady-state current (data not shown). This current decay originated from current through Kv1.3 since application of 100 nM NTX or 100 nM KTX eliminated this current. Apparently and surprisingly, even at a holding potential of 0 mV in K^+ solution, not all voltage-gated Kv1.3 channels inactivate, giving rise to large decaying tail currents when the

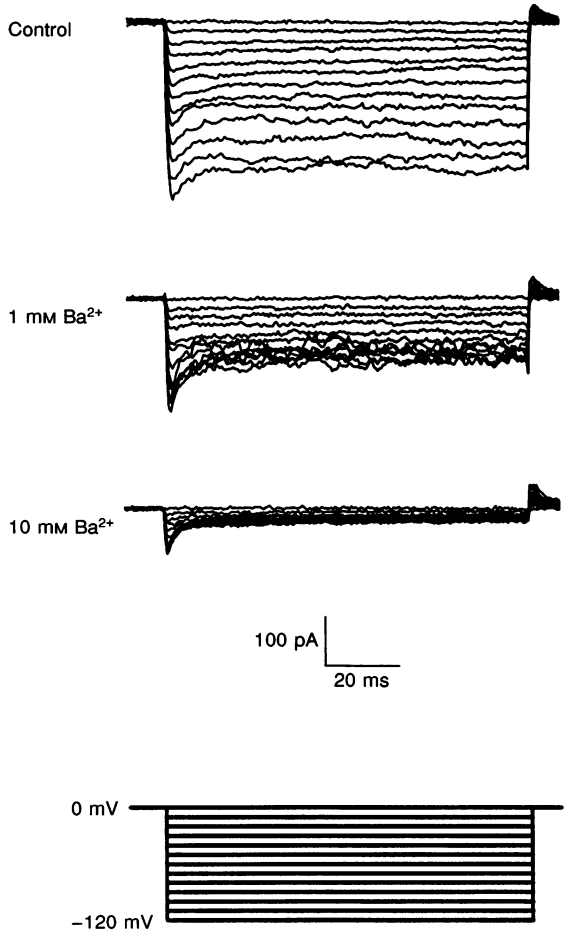

Figure 3. External Ba^{2+} block of K(Ca) channels using voltage steps

K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Membrane currents in K^+ solution (Control) and K^+ solution plus 1 or 10 mM Ba^{2+} were measured in response to a set of 100 ms voltage steps from a holding potential of 0 mV to different test potentials (from −120 to 0 mV). All solutions contained 100 nM NTX to block current through voltage-gated Kv1.3 channels in these cells.

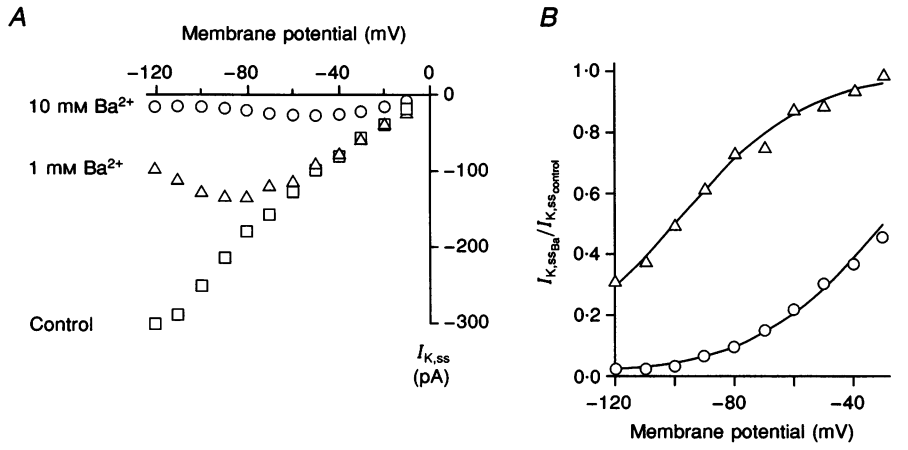

Figure 4. Voltage dependence of the Ba^{2+} block using voltage steps

A, the steady-state current ($I_{K,ss}$) in K^+ solution (Control) and K^+ solution with 1 or 10 mM Ba^{2+} during the 100 ms voltage step shown in Fig. 3 is plotted against the applied membrane potential during the step. *B*, $I_{K,ss}$ obtained in the presence of external Ba^{2+} was divided by $I_{K,ss}$ obtained in the absence of Ba^{2+} (from *A*) and plotted against the membrane potential. The smooth curves represent fits (by eye) to a Boltzmann equation as described in the legend to Fig. 3. The parameters for the fit were almost identical to those shown in Fig. 2, i.e. for 1 mM Ba^{2+}: $E_h = -95$ mV and $k = 20$ mV; and for 10 mM Ba^{2+}: $E_h = -30$ mV and $k = 20$ mV.

potential is stepped to more negative potentials. Therefore, to prevent contamination by current through Kv1.3 channels all solutions for this type of experiment contained 100 nM NTX. To compare the voltage dependence of Ba^{2+} block using this step protocol to the one using voltage ramps we determined the steady-state current in the different solutions and plotted it against the membrane potential during the step (Fig. 4*A*). The steady-state current ($I_{K,ss}$) obtained in the presence of external Ba^{2+} was divided by the steady-state current obtained in the absence of Ba^{2+} and plotted against the membrane potential (Fig. 4*B*). The smooth curves represent fits to a Boltzmann equation as described above. The parameters for the fit were identical to those obtained using ramp currents indicating that Ba^{2+} can

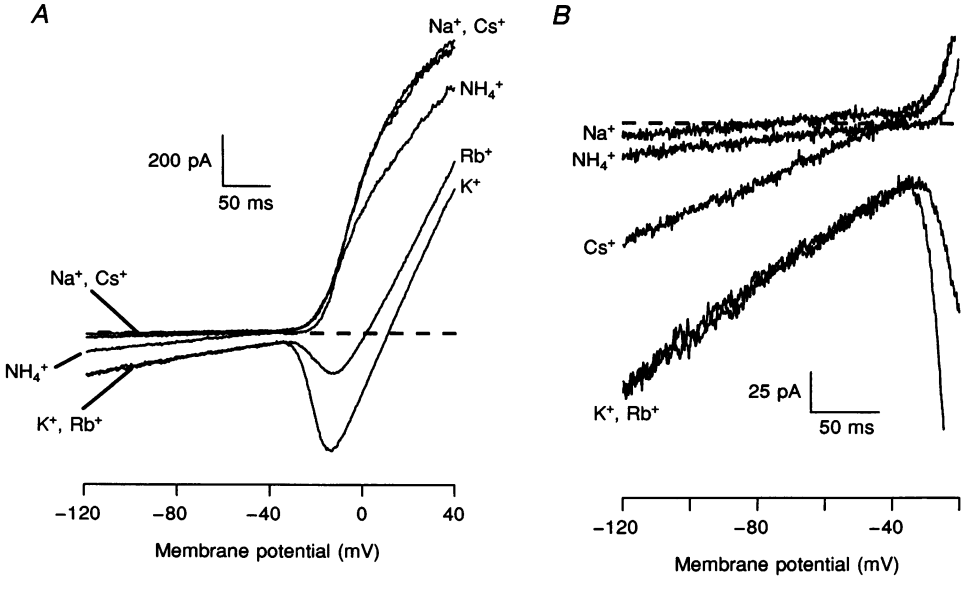

Figure 5. Selectivity sequence of monovalent cations in K(Ca) channels in Jurkat T cells

A, K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Ramp currents were elicited by 400 ms voltage ramps from −120 to 40 mV every 10 s before and during application of a K^+, Rb^+, NH_4^+, Cs^+ and Na^+ solution. The Na^+ solution in this experiment contained 4·5 mM K^+ and E_{rev} for this solution was −85 mV. *B*, same records as in *A* shown at a larger scale and a smaller membrane potential range to enlarge the ramp currents through K(Ca) in the different solutions. All records shown were obtained from a single cell.

move about half-way into the electric field (the partial electrical distance of the Ba^{2+} binding site across the membrane from the outside, $\delta = 0{\cdot}62$) and that Ba^{2+} block is fast compared with the voltage ramp.

The voltage- and concentration-dependent Ba^{2+} block of Jurkat T cells is similar but not identical to the Ba^{2+} block of K(Ca) channels in human peripheral T lymphocytes (Grissmer *et al.* 1993). The voltage dependence of Ba^{2+} block is less steep in K(Ca) channels in Jurkat cells compared with K(Ca) channels in human peripheral T lymphocytes (20 ± 1 *vs.* 17 ± 1 mV; mean ± S.D.; $n = 3$ for each channel type, see also Table 1) suggesting that for K(Ca) channels in Jurkat T cells Ba^{2+} can move only about half-way into the electric field ($\delta = 0{\cdot}62 \pm 0{\cdot}02$; $n = 3$), whereas in human peripheral T lymphocytes Ba^{2+} can reach a site almost three quarters into the membrane field ($\delta = 0{\cdot}74 \pm 0{\cdot}04$; $n = 3$). In addition, the voltage where half the channels are being blocked for $[Ba^{2+}]_o = 10$ mM is −30 mV for Jurkat K(Ca) channels compared with −60 mV for K(Ca) channels in human peripheral T lymphocytes (Table 1). It seems that K(Ca) channels in Jurkat T cells are more sensitive to block by external Ba^{2+} than K(Ca) channels in human peripheral T lymphocytes.

Ion selectivity

To obtain information about the selectivity as well as the permeability of K(Ca) channels in Jurkat T cells we activated the channels with pipette solutions containing 1 μM Ca^{2+}, varied the external cation species, and measured at the same time changes in slope conductance as well as reversal potentials (E_{rev}), during whole-cell recording (Fig. 5). Since the slope conductance of the ramp current in the K^+ solutions was linear at potentials more negative than −40 mV, we determined E_{rev} for current through K(Ca) channels in the K^+ solution by extrapolation of the slope of the ramp current measured below −40 mV. This procedure seems to be justified since the slope of the ramp current in Jurkat T cells was linear for K^+ all the way through the zero-current potential when current through Kv1.3 was blocked by either CTX or 4-AP (see Grissmer *et al.* 1992). In addition, ramp currents through K(Ca) channels in human peripheral T lymphocytes were also linear through the zero-current potential in K^+ and Rb^+ solutions provided that currents through Kv1.3 channels were selectively blocked with NTX (see Grissmer *et al.* 1993). Therefore the extrapolation of ramp currents to determine E_{rev} in K^+ and Rb^+ solutions seemed to be appropriate. Reversal potentials in the other solutions were determined as zero-current potentials. As expected, these reversal potentials were also not different from the extrapolated reversal potential due to the linearity of the ramp currents (Fig. 5). From the changes in reversal potential, under the assumptions that there is no significant anion permeability and that the internal concentration of permeant ion remains invariant, one can calculate P_X/P_K using an expression, based on the Goldman–Hodgkin–Katz equation:

$$\Delta E_{rev} = E_{rev,X} - E_{rev,K} = (RT/zF)\ln\{(P_X[X^+]_o)/(P_K[K^+]_o)\},$$

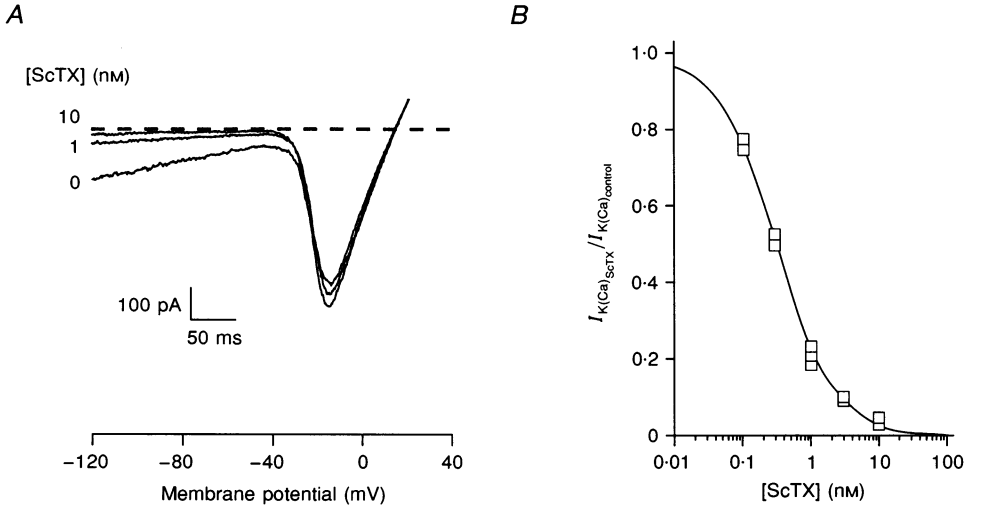

Figure 6. Effect of scyllatoxin (ScTX) on current through K(Ca) channels in Jurkat T cells

K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. *A*, ScTX blocks K(Ca) current without inhibiting current through voltage-gated Kv1.3 channels. After full activation of K(Ca) channels by the pipette solution, ramp currents were elicited by 400 ms voltage ramps from −120 to 40 mV every 10 s in 0·1 mg ml^{-1} bovine serum albumin containing K^+ solution and K^+ solution with 1 and 10 nM ScTX. *B*, dose–response relationship for the block of K(Ca) channels. The line through the points was fitted to a modified Hill equation of the form:

$$I_{K(Ca)_{ScTX}}/I_{K(Ca)_{control}} = 1/[1 + ([ScTX]/K_d)],$$

with K_d for channel block of 0·3 nM. Each cell was tested with at least two concentrations. Results were obtained from five different cells.

Table 2. Permeability and conductance ratios of apamin-sensitive K(Ca) channels in Jurkat T cells ($K(Ca)_{apa}$) and CTX-sensitive K(Ca) channels in human peripheral T lymphocytes ($K(Ca)_{CTX}$)

	P_X/P_K				g_X/g_K			
	K^+	Rb^+	NH_4^+	Cs^+	K^+	Rb^+	NH_4^+	Cs^+
$K(Ca)_{apa}$	1·0	0·96	0·53	0·26	1·0	1·01	0·11	0·67
$K(Ca)_{CTX}$*	1·0	0·96	0·17	0·07	1·0	0·7	1·2	0·1

* Data from Grissmer *et al.* 1993

as described in Hille (1992), where R, T, F and z have their usual meaning. Since in our case $[X^+]_o = [K^+]_o$ and $(RT/zF) \approx 25{\cdot}5$ mV at $T = 23$ °C we can simplify the expression for P_X/P_K to:

$$P_X/P_K = \exp(\Delta E_{rev}/25{\cdot}5 \text{ mV}).$$

E_{rev} changed from −4 mV in K^+ solution to −5, −20 and −38 mV in Rb^+, NH_4^+ and Cs^+ solution, respectively. From the change in E_{rev} for Cs^+, for example, with $\Delta E_{rev} = E_{rev,Cs} - E_{rev,K} = -38 \text{ mV} - (-4 \text{ mV}) = -34$ mV, the ratio P_X/P_K for Cs^+ can be calculated as $P_{Cs}/P_K = \exp(-34 \text{ mV}/25{\cdot}5 \text{ mV}) = \exp(-1{\cdot}33) = 0{\cdot}26$. Similarly, P_X/P_K ratios for Rb^+ and NH_4^+ can be calculated to be 0·96 and 0·53, respectively (Table 2). In addition, we determined E_{rev} in Na^+ Ringer solution (with 4·5 mM K^+) to be −85 mV (Fig. 5*B*). Conductance ratios, g_X/g_K, for these ions were also

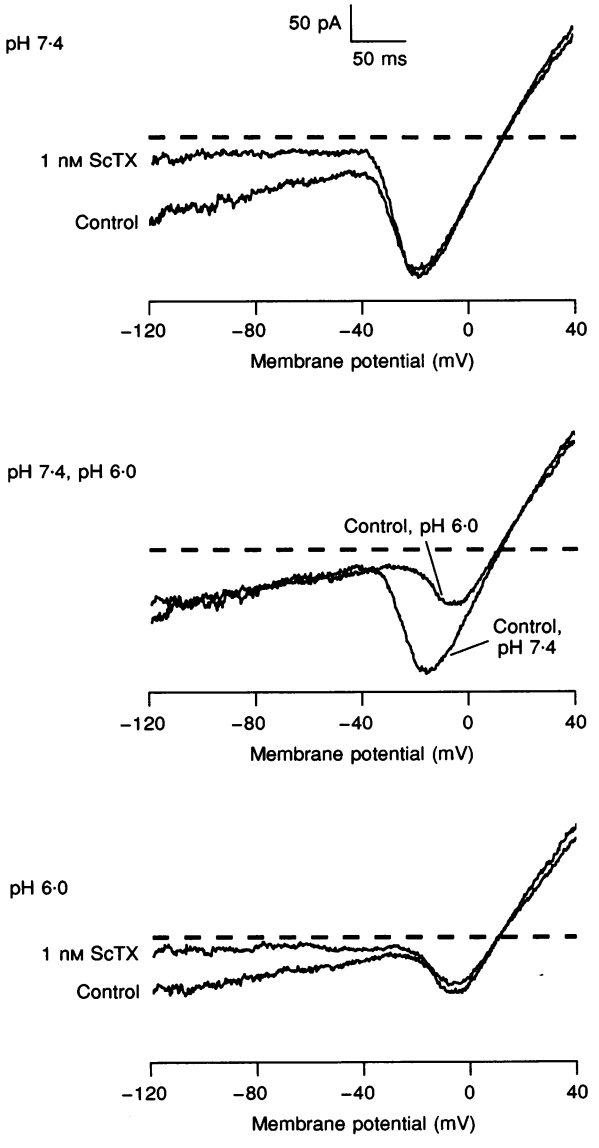

Figure 7. Effect of pH on scyllatoxin (ScTX) block of current through K(Ca) channels in Jurkat T cells

K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Ramp currents were elicited by 400 ms voltage ramps from −120 to 40 mV every 10 s in the different solutions. Upper row, current before and after addition of 1 nM ScTX to the K^+ solution at pH 7·4. Middle row, current obtained in K^+ solution (Control) at pH 7·4 and pH 6·0. Bottom row, current before and after addition of 1 nM ScTX to the K^+ solution at pH 6·0.

determined using the slope of the ramp current at potentials between −100 and −60 mV to be 1·0, 1·01, 0·67 and 0·11 for K^+, Rb^+, Cs^+ and NH_4^+, respectively. In comparison to the CTX-sensitive K(Ca) channels in human peripheral T lymphocytes the most striking property of apamin-sensitive K(Ca) channels in Jurkat T cells is that these channels can also carry significant current with Cs^+ as current carrier (Table 2).

Peptide toxins

Apamin, a component of bee venom, has been shown to irreversibly block current through K(Ca) channels in Jurkat T cells without an effect on current through the voltage-gated K^+ channel Kv1.3 (Grissmer *et al.* 1992). Another peptide toxin, namely ScTX, also blocks K(Ca) channels in Jurkat T cells. ScTX is a thirty-one amino acid polypeptide isolated from the scorpion *Leiurus quinquestriatus hebraeus* and has been shown to inhibit apamin binding to rat brain synaptosomes. ScTX also blocks the adrenaline-induced relaxation of guinea-pig taenia coli, an action similar to that of apamin (Chicchi, Gimenez-Gallego, Ber, Garcia, Winquist & Cascieri, 1988; Auguste *et al.* 1990). Figure 6*A* shows ramp currents in K^+ solution in the absence and presence of 1 or 10 nM ScTX. One can see that this toxin has no effect on current through voltage-gated Kv1.3 channels at these concentrations. ScTX (1 nM) is able to reduce current through K(Ca) channels to about a quarter of the control current. Assuming that one scyllatoxin molecule is able to block one channel, one can calculate a dissociation constant for scyllatoxin to block the channels of about 0·3 nM (Fig. 6*B*). ScTX block was usually reversible upon washout. Other peptide toxins that show homology to ScTX like CTX, KTX and NTX, are unable to block current through K(Ca) channels in Jurkat T cells (data not shown).

Changing the pH of the external K^+ solution does not change the affinity of ScTX block as can be seen in Fig. 7. Reducing pH from 7·4 to 6·0 (middle row), has hardly any

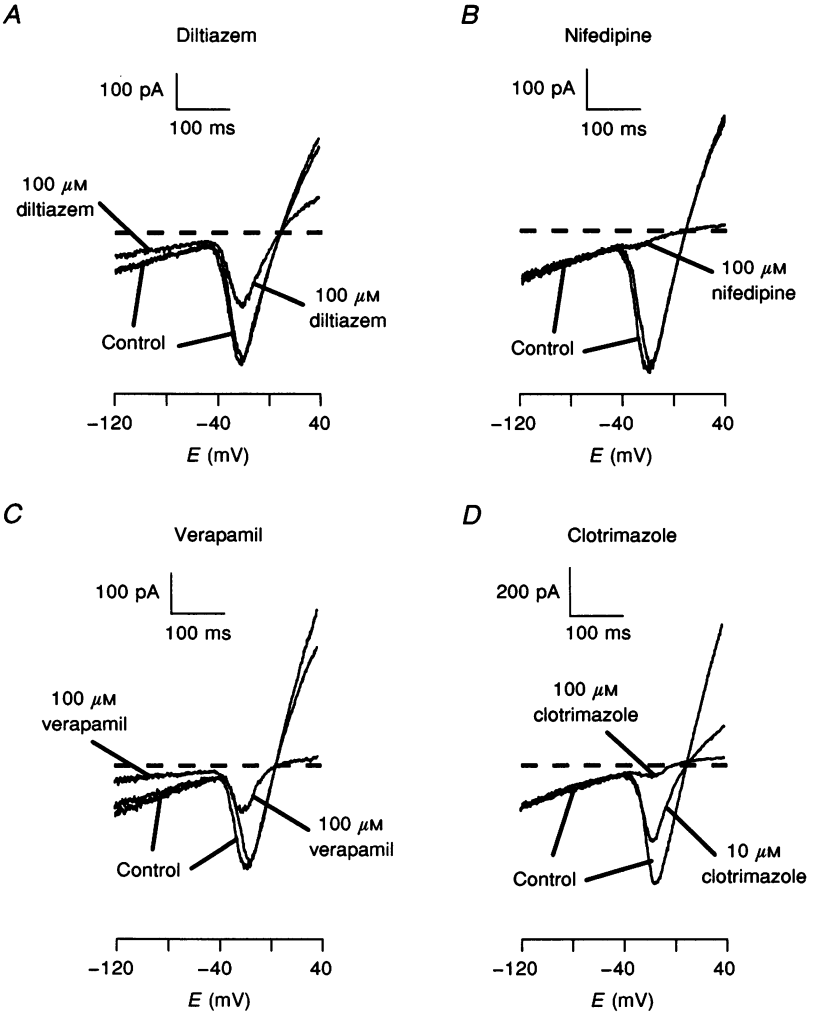

Figure 8. Effect of drugs on current through K(Ca) channels in Jurkat T cells

K(Ca) channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Ramp currents were elicited by 400 ms voltage ramps from −120 to 40 mV every 10 s before and after the application of diltiazem (*A*), nifedipine (*B*), verapamil (*C*), and clotrimazole (*D*).

effect on current through K(Ca) channels, whereas activation of current through voltage-gated Kv1.3 channels is shifted about 20 mV towards more depolarized potentials, an effect usually attributed to neutralization of negative surface charges (Hille, 1992). The ScTX effect at pH 6·0 (bottom row) is nearly identical to the effect at pH 7·4 indicating that the interactive sites on the channel and the toxin do not involve amino acids that can be titrated, that is histidines.

Other drugs

To characterize further the pharmacological profile of K(Ca) channels in Jurkat T cells we tested different drugs for their ability to block current through those channels. Figure 8 shows the results of experiments using the classical calcium channel blockers diltiazem (*A*), nifedipine (*B*) and verapamil (*C*) each tested at 100 μM. Diltiazem and verapamil are both able to reduce current through K(Ca) channels as can be seen by the reduction of slope conductance of the ramp current at negative potentials while having also an effect on current through voltage-gated Kv1.3 channels. The effect of those drugs on both channel types was readily reversible upon washout. In contrast, nifedipine as well as clotrimazole (Fig. 8*D*), a cytochrome P-450 inhibitor that has been reported to block current through K(Ca) channels in red blood cells (Alvarez, Montero & Garcia-Sancho, 1992), had no effect on current through K(Ca) channels in Jurkat T cells but are able to reduce current through voltage-gated Kv1.3 channels in the same cells.

DISCUSSION

We have characterized current through apamin-sensitive K(Ca) channels in Jurkat T cells. These channels are blocked by Ba^{2+} in a voltage-dependent manner, blocked by ScTX independent of the pH of the extracellular solution, and blocked by high concentrations of diltiazem and verapamil. Other peptide toxins that show homology to ScTX such as CTX, KTX and NTX are unable to block current through calcium-activated potassium channels in Jurkat T cells. The results presented in this paper confirm and extend earlier characterizations of K(Ca) channels in Jurkat T cells (Grissmer *et al.* 1992). Taken together these findings should allow a better distinction between different types of K(Ca) channels.

Comparison of apamin-sensitive K(Ca) channels in Jurkat T cells with the CTX-sensitive K(Ca) channels in human peripheral T lymphocytes

Several groups reported the expression of K(Ca) channels in resting and activated T and B lymphocytes from different species (Mahaut-Smith & Schlichter, 1989; Partiseti, Choquet, Diu & Korn, 1992; Leonard, Garcia, Slaughter & Reuben, 1992; Grissmer *et al.* 1993; Verheugen, van Kleef, Oortgiesen & Vijverberg, 1994; Verheugen, Vijverberg, Oortgiesen & Cahalan, 1995). Activation of human T lymphocytes increases the expression of functional K(Ca) channels by a factor of ~25. Since Jurkat is a human T cell line, we compared the properties of the K(Ca) channels in Jurkat T cells and activated human T lymphocytes in more detail as shown in Table 1. It is obvious that the pharmacological profile is quite different between these two channels, the most striking difference being the CTX and apamin/ScTX block, respectively. From this we conclude that the outer vestibule of both channels might be quite different although differences in toxin binding as shown for a voltage-dependent *Shaker* potassium channel (Goldstein & Miller, 1992) could be due to a single amino acid change in the outer vestibule. However, not only the outer vestibule of the two channels differ in at least one amino acid but also those structures that determine ion permeation. In this respect, the major difference between the two channels is the Cs^+ and NH_4^+ permeation. Cs^+ carries current more efficiently through K(Ca) channels of Jurkat T cells whereas NH_4^+ carries current more efficiently through K(Ca) channels of human T lymphocytes (Table 2). This conclusion about differences in structures that determine ion permeation is supported further by the differences in the voltage dependence of the external Ba^{2+} block. The simplest explanation for our results would be to assume that the site that determines ion selectivity that is different in the two channels might also be the reason for the difference in the voltage dependence of Ba^{2+} block.

Toxins

The structure of scorpion toxins has been successfully determined using nuclear magnetic resonance (NMR) and used to estimate the topology of the outer vestibule of voltage-gated K^+ channels (MacKinnon, Heginbotham & Abramson, 1990; Stampe, Kolmakova-Partensky & Miller, 1994; Hidalgo & MacKinnon, 1995; Aiyar *et al.* 1995). All the toxins used (CTX, NTX, KTX, margatoxin (MgTX), and agiotoxin (AgTX)) share similarities with ScTX in so far as ScTX also has three disulphide bonds as well as the central lysine (K20). We therefore assume that ScTX, like the other toxins on voltage-gated channels, acts similarly to block current through K(Ca) channels of Jurkat T cells. Since the effect of ScTX is independent of the pH of the extracellular solution we conclude that interactive sites between the toxin and the channel do not involve histidines. Those residues, if present, could have electrostatically repelled any positively charged toxin residue in its vicinity (compare Aiyar *et al.* 1995).

Role of K(Ca) channels

In non-excitable membranes, K(Ca) channels might help to control the membrane potential when $[Ca^{2+}]_i$ rises and therefore maintain the driving force to sustain Ca^{2+} entry through voltage-independent calcium-release activated Ca^{2+} channels, so-called CRAC channels (Zweifach & Lewis, 1993). This has been suggested for Jurkat T cells where oscillations in $[Ca^{2+}]_i$ induced by mitogenic stimulation could be stopped by blocking CTX-sensitive Kv1.3 as well as apamin-sensitive K(Ca) channels simultaneously (Grissmer *et al.* 1992). These oscillations could not be modulated by selectively blocking just one channel type. The situation is more complex for human peripheral T lymphocytes. Resting

T cells express only few K(Ca) channels and most of those channels are CTX sensitive (Leonard *et al.* 1992; Grissmer *et al.* 1993). They are, however, not the BK channel type but the CTX-sensitive SK channel. After stimulation with mitogens the expression of CTX-sensitive K(Ca) channels increases ~25-fold (Grissmer *et al.* 1993). Since Kv1.3-specific toxins, i.e. NTX and MgTX, inhibit the rise in $[Ca^{2+}]_i$ after stimulation of the T lymphocytes with anti-CD3 (Lin *et al.* 1993), it seemed that Kv1.3 channels alone were responsible for maintaining the electrical driving force to sustain Ca^{2+} entry in resting T lymphocytes (Leonard *et al.* 1992). However, in activated T cells, where an increased number of K(Ca) channels are available, K(Ca) channels may also play a role in regulating $[Ca^{2+}]_i$ after mitogenic stimulation. Recent non-invasive measurements of membrane currents, membrane potential and $[Ca^{2+}]_i$ in intact human T lymphocytes support this idea (Verheugen *et al.* 1995).

Aiyar, J., Withka, J. M., Rizzi, J. P., Singleton, D. H., Andrews, G. C., Lin, W., Boyd, J., Hanson, D. C., Simon, M., Dethlefs, B., Lee, C.-L., Hall, J. E., Gutman, G. A. & Chandy, K. G. (1995). Topology of the pore-region of a K^+ channel revealed by the NMR-derived structures of scorpion toxins. *Neuron* **15**, 1169–1181.

Alvarez, J., Montero, M. & Garcia-Sancho, J. (1992). High affinity inhibition of Ca^{2+}-dependent K^+ channels by cytochrome P-450 inhibitors. *Journal of Biological Chemistry* **267**, 11789–11793.

Auguste, P., Hugues, M., Grave, B., Gesquiere, J. C., Maes, P., Tartar, A., Romey, G., Schweitz, H. & Lazdunski, M. (1990). Leiurotoxin I (Scyllatoxin), a peptide ligand for Ca^{2+}-activated K^+ channels. *Journal of Biological Chemistry* **265**, 4753–4759.

Blatz, A. L. & Magleby, K. L. (1986). Single apamin-blocked Ca-activated K^+ channels of small conductance in cultured rat skeletal muscle. *Nature* **323**, 718–720.

Chandy, K. G. & Gutman, G. A. (1995). Voltage-gated K^+ channel genes. In *Handbook of Receptors and Channels: Ligand and Voltage-Gated Ion Channels*, ed. North, A., pp. 1–71. CRC Press, Boca Raton, FL, USA.

Chandy, K. G., Gutman, G. A. & Grissmer, S. (1993). Physiological role, molecular structure and evolutionary relationships of voltage-gated potassium channels in T lymphocytes. *Seminars in The Neurosciences* **5**, 125–134.

Chicchi, G. G., Gimenez-Gallego, G., Ber, E., Garcia, M. L., Winquist, R. & Cascieri, M. A. (1988). Purification and characterization of a unique, potent inhibitor of apamin binding from *Leiurus quinquestriatus hebraeus* venom. *Journal of Biological Chemistry* **263**, 10192–10197.

Cognard, C., Traore, F., Potreau, D. & Raymond, G. (1984). Effects of apamin on the outward potassium current of isolated frog skeletal muscle fibres. *Pflügers Archiv* **402**, 222–224.

Goldstein, S. A. N. & Miller, C. (1992). A point mutation in a *Shaker* K^+ channel changes its charybdotoxin binding site from low to high affinity. *Biophysical Journal* **62**, 5–7.

Grissmer, S. & Cahalan, M. D. (1989). Divalent ion trapping inside potassium channels of human lymphocytes. *Journal of General Physiology* **93**, 609–630.

Grissmer, S., Dethlefs, B., Wasmuth, J., Goldin, A. L., Gutman, G. A., Cahalan, M. D. & Chandy, K. G. (1990). Expression and chromosomal localization of a lymphocyte K^+ channel gene. *Proceedings of the National Academy of Sciences of the USA* **87**, 9411–9415.

Grissmer, S. & Hanselmann, C. (1996). Voltage-dependent Ba^{2+}-block of apamin-sensitive Ca^{2+}-activated potassium channels in Jurkat T cells. *Biophysical Journal* **70**, A122.

Grissmer, S., Lewis, R. S. & Cahalan, M. D. (1992). Ca^{2+}-activated K^+ channels in human leukemic T cells. *Journal of General Physiology* **99**, 63–84.

Grissmer, S., Nguyen, A. N. & Cahalan, M. D. (1993). Calcium-activated potassium channels in resting and activated human T lymphocytes. *Journal of General Physiology* **102**, 601–630.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Archiv* **391**, 85–100.

Hanselmann, C. & Grissmer, S. (1996). Pharmacology and selectivity of apamin-sensitive Ca^{2+}-activated potassium channels in Jurkat T cells. *Pflügers Archiv* **431**, R84.

Hidalgo, P. & MacKinnon, R. (1995). Revealing the architecture of a K^+ channel pore through mutant cycles with a peptide inhibitor. *Science* **268**, 307–310.

Hille, B. 1992. *Ionic Channels of Excitable Membranes*, 2nd edn. Sinauer Associates, Massachusetts.

Hugues, M., Schmid, H., Romey, G., Duval, D., Frelin, C. & Lazdunski, M. (1982). The Ca^{2+}-dependent slow K^+ conductance in cultured rat muscle cells: characterization with apamin. *EMBO Journal* **1**, 1039–1042.

Latorre, R. A., Oberhauser, A., Labarca, P. & Alvarez, O. (1989). Varieties of calcium-activated potassium channels. *Annual Review of Physiology* **51**, 385–399.

Leonard, R. J., Garcia, M. L., Slaughter, R. S. & Reuben, J. P. (1992). Selective blockers of voltage-gated K^+ channels depolarize human T lymphocytes: mechanism of the antiproliferative effect of charybdotoxin. *Proceedings of the National Academy of Sciences of the USA* **89**, 10094–10098.

Lin, C. S., Boltz, R. C., Blake, J. T., Nguyen, M., Talento, A., Fischer, P. A., Springer, M. S., Sigal, N. H., Slaughter, R. S., Garcia, M. L., Kaczorowski, G. J. & Koo, G. C. (1993). Voltage-gated potassium channels regulate calcium-dependent pathways involved in human T lymphocyte activation. *Journal of Experimental Medicine* **177**, 637–645.

MacKinnon, R., Heginbotham, L. & Abramson, T. (1990). Mapping the receptor site for charybdotoxin, a pore-blocking potassium channel inhibitor. *Neuron* **5**, 767–771.

Mahaut-Smith, M. P. & Schlichter, L. C. (1989). Ca^{2+}-activated K^+ channels in human B lymphocytes and rat thymocytes. *Journal of Physiology* **415**, 69–83.

Partiseti, M., Coquet, D., Diu, A. & Korn, H. (1992). Differential regulation of voltage- and calcium-activated potassium channels in human B lymphocytes. *Journal of Immunology* **148**, 3361–3368.

PORTZEHL, H., CALDWELL, P. C. & RUEGG, J. C. (1964). The dependence of contraction and relaxation of muscle fibres from the crab Maia squinado on the internal concentration of free calcium ions. *Biochimica et Biophysica Acta* **79**, 581–591.

ROMEY, G., RIEGER, F., RENAUD, J. F., PINCON-RAYMOND, M. & LAZDUNSKI, M. (1986). The electrophysiological expression of Ca^{2+} channels and of apamin sensitive Ca^{2+} activated K^+ channels is abolished in skeletal muscle cells from mice with muscular dysgenesis. *Biochemical and Biophysical Research Communications* **136**, 935–940.

SANDS, S. B., LEWIS, R. S. & CAHALAN, M. D. (1989). Charybdotoxin blocks voltage-gated K^+ channels in human and murine T lymphocytes. *Journal of General Physiology* **93**, 1061–1074.

STAMPE, P., KOLMAKOVA-PARTENSKY, L. & MILLER, C. (1994). Intimations of K^+ channel structure from a complete functional map of the molecular surface of charybdotoxin. *Biochemistry* **33**, 443–450.

VERHEUGEN, J. A. H., VAN KLEEF, R. G. D. M., OORTGIESEN, M. & VIJVERBERG, H. P. M. (1994). Characterization of Ca^{2+}-activated K^+ channels in excised patches of human T lymphocytes. *Pflügers Archiv* **426**, 465–471.

VERHEUGEN, J. A. H., VIJVERBERG, H. P. M., OORTGIESEN, M. & CAHALAN, M. D. (1995). Voltage-gated and Ca^{2+}-activated K^+ channels in intact human T lymphocytes. *Journal of General Physiology* **105**, 765–794.

ZWEIFACH, A. & LEWIS, R. S. (1993). Mitogen-regulated Ca^{2+} current of T lymphocytes is activated by depletion of intracellular Ca^{2+} stores. *Proceedings of the National Academy of Sciences of the USA* **90**, 6295–6299.

Acknowledgements

The authors wish to thank Dr Stephen Brady (Merck, Sharp & Dohme Research Laboratories, Westpoint, PA, USA) for his generous gift of NTX. This work was supported by a grant from Pfizer Inc. (US) and from the DFG (Gr848/4-1).

Author's email address

S.Grissmer: stephan.grissmer@medizin.uni-ulm.de

Received 20 March 1996; accepted 23 July 1996.