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SK2 encodes the apamin-sensitive Ca^{2+}-activated K^+ channels in the human leukemic T cell line, Jurkat

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Abstract **T cells express two different types of voltage-independent Ca^{2+}-activated K^+ channels with small (SK) and intermediate (IK) conductance that serve important roles in the activation of T lymphocytes. In contrast to the IK channels from T lymphocytes which are upregulated upon mitogen stimulation, SK channels of Jurkat T cells, a human leukemic T cell line, are constitutively expressed even in the absence of mitogenic stimulation. We have used patch-clamp recordings from transfected or injected mammalian cells to show that the cloned SK2 channel demonstrates the biophysical and pharmacological properties of the majority of K(Ca) channels in Jurkat T cells. The cloned and native channels are voltage-independent, Ca^{2+}-activated, apamin-sensitive, show an equivalent voltage-dependent Ba^{2+} block and possess a similar ion selectivity. In addition, we used the polymerase chain reaction to demonstrate the presence of SK2 mRNA in Jurkat T cells, whereas SK3 transcripts encoding the other cloned apamin-sensitive SK channel were not detected. These data suggest that the voltage-independent apamin-sensitive K(Ca) channel in Jurkat T cells represents the recently cloned SK2 channel.**

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Key words: Jurkat T lymphocyte; Small conductance Ca^{2+}-activated K^+ channel; Apamin; Patch-clamp technique; K(Ca) channel gene; Molecular biology

1. Introduction

Patch-clamp experiments have demonstrated the expression of two major subtypes of K(Ca) channels in human T lymphocytes [1–6]. These channel subtypes are similar with respect to their voltage-independent gating and their Ca^{2+}-sensitivity, but differed in single-channel conductance and pharmacology. Both channel types are implicated in the physiology of the T cells including the regulation of membrane potential, cell volume, calcium signaling and mitogenic activation (for review, [7]).

Resting human peripheral blood (HPB) T lymphocytes express about 20 functional K(Ca)HPB channels per cell, however, expression increases about 25-fold after mitogenic activation [3]. The channels are voltage-independent and open in response to an increase in the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, with an apparent K_d of $\sim$400 nM. The single-channel conductance is between 11 and 35 pS and channels are half-blocked by $\sim$40 mM tetraethylammonium (TEA^+) or by $\sim$4 nM charybdotoxin (CTX), and are resistant to block by apamin up to 100 nM. The biophysical and pharmacological characteristics define these channels as IK channels. A different K(Ca) channel subtype, also found in normal human T cells [2,4], is expressed abundantly in the leukemic T cell line Jurkat [1] and is likely present in rat T and B lymphocytes [8]. These channels may be distinguished from the CTX-sensitive IK channel in HPBs, although the gating mechanism and Ca^{2+}-sensitivity of the two channel types are similar. The channels expressed in Jurkat cells have a smaller single-channel conductance ($\sim$4–7 pS), are more sensitive to TEA^+ (half-blocked at $\sim$2 mM), resistant to CTX (no block at 100 nM) and are very sensitive to block by apamin (half-blocked at $\sim$0.3 nM) and scyllatoxin, ScTX (half-blocked at 0.3 nM).

Recently, a new family of closely related K^+ channel genes encoding small conductance Ca^{2+}-activated K^+ channels (*r*SK1, *r*SK2, *r*SK3, *h*SK1, *h*SK3 = *h*SKCa3) and intermediate conductance Ca^{2+}-activated K^+ channels (*h*IK, *h*IK1, *h*SK4, *h*KCa4) have been cloned and functionally characterized ([9–14]; see Table 1).

Logsdon et al. [13] showed that the SKCa4 channel, when expressed in HEK293 cells, had similar properties to those reported for the native K(Ca) channel in activated HPB T lymphocytes. In addition, they showed increased levels of steady state SKCa4 mRNA in HPB T lymphocytes after mitogenic activation. The transcript was detected by the polymerase chain reaction (PCR) in a variety of tissues including thymus, spleen and many cell lines of hematopoietic origin, and they concluded that the SKCa4 channel represents the K(Ca) channel in HPB T lymphocytes. Jurkat T cells are often used as a model for lymphocyte activation signal cascades. However, the gene encoding the K(Ca) channel in Jurkat T cells has not been identified.

To determine whether the cloned SK2 channel represents the K(Ca) channels in Jurkat T cells, we performed a detailed comparison of the electrophysiological properties of the cloned channel expressed in different mammalian cell lines with the channel in Jurkat cells. The results demonstrated that SK2 channel properties, especially the pharmacological fingerprint, were indistinguishable from those of the Jurkat K(Ca) channel. Furthermore, reverse transcription-PCR (RT-PCR) demonstrated the presence of SK2 mRNA in Jurkat T cells, while SK3 transcripts were not detected. Taken together, these data indicate that the cloned SK2 channel represents the K(Ca) channel in Jurkat T cells and may represent the small conductance K(Ca) channels observed in human T lymphocytes.

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2. Materials and methods

2.1. Cells

tsA201 cells, a HEK293 cell line stably transfected with the SV40 T-antigen [15] (tsA201 = formerly called 293/tsA1609neo; [16]) were grown in MEM containing 10% fetal bovine serum and L-Glutamax I at 37°C and a CO_2 atmosphere of 5%.

2.2. Transfection/injection

tsA201 cells were transiently transfected by the $CaPO_4$ method [17]. DNA for transfection was prepared using Qiagen anion-exchange columns. For injection, in vitro transcribed mRNA was generated with an SK2 bearing plasmid [9] using T7 polymerase in the presence of GpppG cap-nucleotides (T7 Cap-scribe kit, Boehringer Mannheim, Germany). The SK2 mRNA was diluted with a fluorescent FITC dye (0.5% FITC-dextran MW 10 000 in 100 mM KCl) to a final concentration of 0.5–1 mg/ml. The cRNA/FITC solution was filled into injection capillaries (Femtotips, Eppendorf, Germany) and HEK293 cells were injected using an Eppendorf microinjection system (Micromanipulator 5171 and Transjector 5246) previously described [18–20]. 2–6 h later, injected cells were identified by fluorescence and whole-cell currents were investigated.

2.3. Construction of the SK2 expression vector

The SK2 cDNA was subcloned into the eukaryotic expression vector pcDNA3 (Invitrogen) which provides a CMV promoter and an SV40 replication origin. This construct was used for transient transfections together with a CD8 coding plasmid. Anti-CD8 antibodies coupled to paramagnetic beads (Dynal, Hamburg, Germany) were used to identify transfected tsA201 cells [21].

2.4. Electrophysiology

Experiments were carried out using the whole-cell mode of the patch-clamp technique [22] as described earlier [1,3,5,6,19,23]. SK2 channels were activated by whole-cell dialysis with a pipette solution (in mM: 135 K-aspartate, 2 $MgCl_2$, 10 HEPES, 8.7 $CaCl_2$, 10 EGTA, pH 7.2) containing 1 μM free Ca^{2+} and (in mM: 135 K-aspartate, 2 $MgCl_2$, 10 HEPES, 1 $CaCl_2$, 10 EGTA, pH 7.2) containing 10 nM free Ca^{2+}. $[Ca^{2+}]_i$ was calculated assuming a K_d for EGTA and Ca^{2+} at pH 7.2 of 10^{-7} M [24]. Ramp currents were elicited by 400 ms voltage ramps from −160 to 40 mV every 20 s before and after application of a K^+ solution. Measurements were done in mammalian K^+ Ringer solution (in mM: 164.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 5 HEPES, pH 7.4). For Na^+, Rb^+, Cs^+ and NH_4^+ solutions, 164.5 mM NaCl, RbCl, CsCl or NH_4Cl, respectively, was added instead of KCl. The holding potential in all experiments was −80 mV. The EPC-9 amplifier (HEKA elektronik, Lambrecht, Germany) was used to record membrane currents, and was interfaced to a Macintosh computer running acquisition and analysis software (Pulse/PulseFit; HEKA elektronik). Analysis was also performed using IGOR (Wavemetrics, Lake Oswego, OR, USA) software. Peptide toxin block was measured in the presence of 0.1% bovine serum albumin. Apamin was purchased from Latoxan (Rosans, France), ScTX was purchased from Bachem (Heidelberg, Germany). All other chemicals were from Sigma (Deisenhofen, Germany).

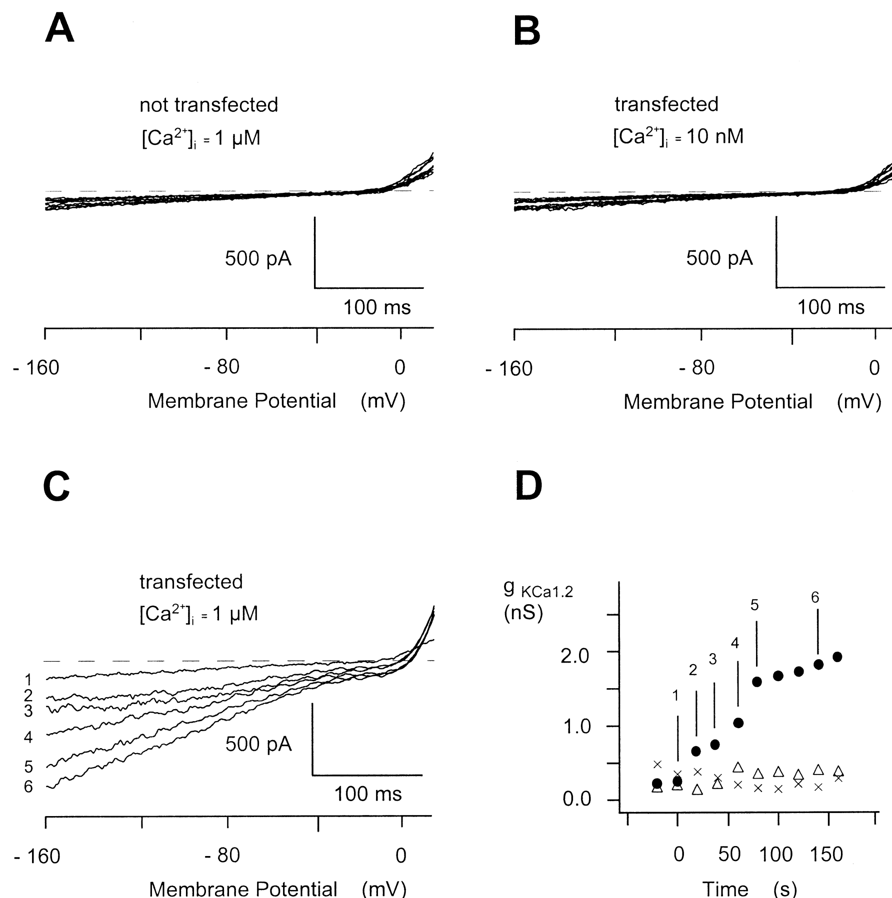

Fig. 1. Expression of SK2 channels in tsA201 cells. Ramp currents were elicited by 400 ms voltage ramps from −160 to 20 mV every 10 s before and after break-in. A: Not transfected cells (pipette solution containing 1 μM free Ca^{2+}). B,C: Activation of SK2 channels expressed in tsA201 cells by whole-cell dialysis with a pipette solution containing 10 nM (B) or 1 μM (C) free Ca^{2+}. C: A progressive increase in current through SK2 channels induced by the pipette Ca^{2+} is manifest as the increase in slope conductance at potentials below −40 mV. D: SK2 conductance was determined for each ramp current by fitting a line to the data points between −160 and −40 mV. These conductance values are plotted as a function of time for the experiments shown in A (crosses), B (open triangles) and C (filled circles). The numbers on the graph correspond to the current traces shown in C (filled circles).

2.5. PCR amplification of K(Ca) mRNA

Total RNA was isolated from 10^7 Jurkat T cells with TRI REAGENT (Sigma, Deisenhofen, Germany). Ready To Go You-Prime First-Strand beads and first-strand cDNA were primed with oligo d(T) and generated with Moloney murine leukemia virus (M-MuLV) reverse transcriptase according to the manufacturer's instructions (Pharmacia, Freiburg, Germany).

First-strand cDNA was used directly as a template for PCR by Taq DNA polymerase (Pharmacia, Freiburg, Germany). The forward and reverse primers were 5′-GGCATGGCAGCAGCAGCGGCACTA-3′, 5′ to the sequence encoding the first transmembrane segment and 5′-CACATGCTTTTCTGCTTTGGTAA-3′ located 3′ to the sequence encoding the sixth transmembrane segment of rat SK2 [9]. As control for human SK3, we used the forward and reverse primers 5′-CCCGCTTTGTCATGAAGACGCTC-3′ and 5′-CCACGCTGACACCCCGG-3′ generating a 769 bp fragment. The PCR was conducted with 1 μM forward and reverse primers and 35 mM $MgCl_2$ for 3 min at 95°C, followed by 30 cycles through a 1 min denaturation step at 94°C, a 2 min annealing step at 55°C and a 1 min extension step at 72°C followed by a final extension for 7 min at 72°C. A 919 bp fragment was amplified. For cycle sequencing, the RT-PCR product served as template to generate overlapping fragments of about 400 bp. The following primer pairs were used: A: 5′-GGCATGGCAGCAGCAGCGGCACTA-3′, 5′-TCCAAGCAGATGAAGAAAATACG-3′, B: 5′-ATGGTGGACAATGGAGCAGATGAC-3′, 5′-ACGAGATACTAAAAACCAAGAGCA-3′, C: 5′-AACTTTTCACCGATGCCTCCTCTA-3′, 5′-CACATGCCTTTTCTGCTTTGGTAA-3′. The PCR products were purified on 5% polyacrylamide gels and sequenced by cycle sequencing (Thermo-sequenase dye terminator cycle sequencing pre-mix kit, Amersham Life Science, Cleveland, USA).

3. Results

3.1. Activation time course

We used tsA201 or HEK293 cells to characterize K(Ca) channels because these cell lines do not express endogenous Ca^{2+}-activated K^+ channels (Fig. 1A). To characterize the expression of K(Ca) channels following transfection, we buffered $[Ca^{2+}]_i$ with different pipette solutions between 10 nM and 1 μM free Ca^{2+}. While 10 nM free Ca^{2+} was not sufficient to activate K(Ca) channels (Fig. 1B), 1 μM free Ca^{2+} activated SK2 channels. Fig. 1C shows ramp currents before and after the transition from the cell-attached to the whole-cell recording mode in K^+-containing extracellular solution with 1 μM free Ca^{2+} in the pipette. The slope conductance, measured as the slope of the ramp current at hyperpolarizing potentials, increased after break-in. The time course for the activation of SK2 channels expressed in tsA201 cells is shown by the plot of the slope conductance (measured between −160 and −40 mV) as a function of time (Fig. 1D). The transition from the cell-attached to the whole-cell recording mode ($t = 0$ s) marked the time at which the Ca^{2+}/EGTA mixture in the pipette ($[Ca^{2+}]_{free} = 1$ μM) started to diffuse into the cell. Within several seconds, the slope conductance began to increase, reaching a plateau value of ~2 nS within ~100 s (Fig. 1D). The time course for the induction of the SK2 channels (filled circles) was similar to the activation time course of the K(Ca) channels obtained in Jurkat cells [1,5,6] under similar conditions as expected from cells of about the same size, so the rate of dialysis should be similar.

Table 1
Nomenclature of voltage-independent Ca^{2+}-activated K^+ channel genes

Name in use	Reference
*h*SK1	[9]
*r*SK1	[11]
*h*SK2	This paper
*r*SK2	[9]
*h*SKCa3	[10]
*r*SK3	[9]
HIK	[14]
*h*IK1	[12]
*h*SK4	[11]
*h*KCa4	[13]

Recently, a new nomenclature was suggested: KCNN1 for SK1, KCNN2 for SK2, KCNN3 for SK3 and KCNN4 for SK4.

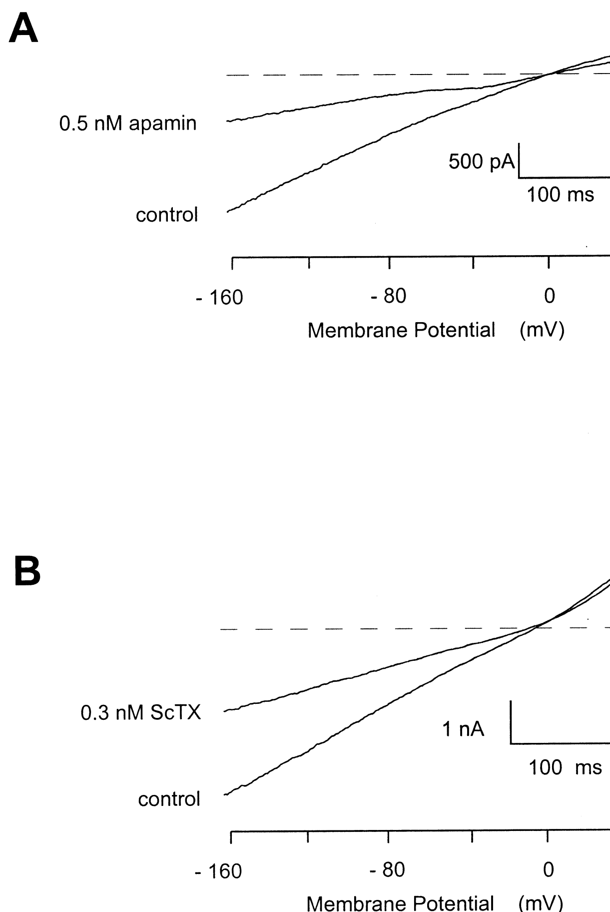

Fig. 2. Effect of peptide toxins on current through SK2 channels expressed in tsA201 cells. SK2 channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Traces were obtained after full activation of the current in K^+ Ringer in the absence and presence of 0.5 nM apamin (A) or 0.3 nM ScTX (B).

3.2. Pharmacology

Two different peptide toxins, apamin, a component of bee venom, as well as ScTX, a peptide isolated from the scorpion *Leiurus quinquestriatus hebraeus*, block current through K(Ca) channels in Jurkat T cells [1,5,6]. The effect of apamin on SK2 channels expressed in *Xenopus* oocytes has been reported [9,12]. We therefore tested whether these two toxins would also block current through SK2 channels expressed in the mammalian cell line tsA201. Apamin, at a concentration of 0.5 nM, blocked about 65% of the current (Fig. 2A) and 0.3 nM ScTX reduced the current about 50% (Fig. 2B). Assuming a 1:1 stoichiometry for apamin block, one can calculate a dissociation constant for apamin to block the channel of about 0.4 nM and for ScTX to block the channel of about

Table 2
Characteristics of K(Ca) channels in Jurkat T cells and SK2 channels expressed in tsA cells

	$K(Ca)_{Jurkat}$	SK2
Peptide toxins[a]		
ScTX	0.3 nM [5]	0.3 nM (this paper)
Apamin	∼0.3 nM [1,5]	0.4 nM (this paper)
CTX	>100 nM [3]	>100 nM (this paper)
Others[a]		
d-Tubocurarine	2 μM [6]	2 μM (this paper), (2.4 μM [9])
Clotrimazol	>100 μM [6]	>100 μM (this paper)
4-AP	>1 mM [6]	>1 mM (this paper)
Ionic blockers		
Ba^{2+}		
Voltage-dependence[b]	$\delta = 0.62 \pm 0.02$ [5]	$\delta = 0.63 \pm 0.04$ (this paper)
Sensitivity[c]	−95 mV (1 mM Ba^{2+}) [5]	−135 mV (1 mM Ba^{2+}) (this paper)
TEA^{+}[a]	2 mM [5]	4 mM (this paper)
Single-channel conductance	4–7 pS[d] [1]	∼10 pS[e] [9]
Ca^{2+}-sensitivity		
K_d[f]	∼450 nM [1]	∼600 nM [9,27]
h[g]	4–5 [1]	∼4.8 [9,27]

[a]Given are values for the apparent dissociation constants for block (K_d). K_d values are derived from the equation $I/I_{max} = 1/[1 + ([drug]/K_d)]$; ($I$) currents in the presence of drug at −160 mV; (I_{max}) currents in the absence of drug at −160 mV; results were obtained from 3–8 different cells. Each cell was tested with at least two concentrations.
[b]δ is the partial electrical distance of the Ba^{2+} binding site across the membrane from the outside. Values are given as mean ± S.D.; $n = 3$.
[c]Potential where half the channels are blocked in 1 mM Ba^{2+}.
[d]Single-channel conductance determined in symmetrical 160 mM K^+ solution.
[e]Single-channel conductance determined in symmetrical 116 mM K^+ solution.
[f]Given are values for the apparent dissociation constant for activation.
[g]h = Hill coefficient.

0.3 nM (Table 2). In addition, we tested CTX and found that 100 nM CTX was unable to reduce the current through these channels (data not shown).

The effect of external Ba^{2+} on current through SK2 channels in tsA201 cells is shown in Fig. 3. Current was elicited with a similar voltage ramp protocol as described in Fig. 1. Extracellular Ba^{2+} reduced current through the SK2 channels in a dose- and voltage-dependent manner with the block increasing with hyperpolarization (Fig. 3A). The effect of Ba^{2+} was rapidly reversible upon washout (data not shown). To quantify the voltage-dependence of Ba^{2+} block, we determined the ratio of the ramp current in the presence and absence of 1 mM Ba^{2+} as a function of the membrane potential. The smooth curve through the ratio represents a fit of a Boltzmann equation to the data with a steepness factor of 20 mV per e-fold change. Ba^{2+} block of SK2 channels was therefore as steep as expected for the binding of a single divalent cation penetrating approximately half-way into the membrane electric field. This value is indistinguishable from the value obtained for the Ba^{2+} block of K(Ca) channels in Jurkat T cells [5].

Several other compounds were examined for their ability to block current though SK2 channels expressed in tsA201 cells. Fig. 4 shows an example of experiments using *d*-tubocurarine. Extracellular application of 4 μM *d*-tubocurarine reduced the slope of the ramp current at potentials below −60 mV more than half, indicating a half-blocking concentration ∼2 μM, similar to values obtained for the *d*-tubocurarine block of the

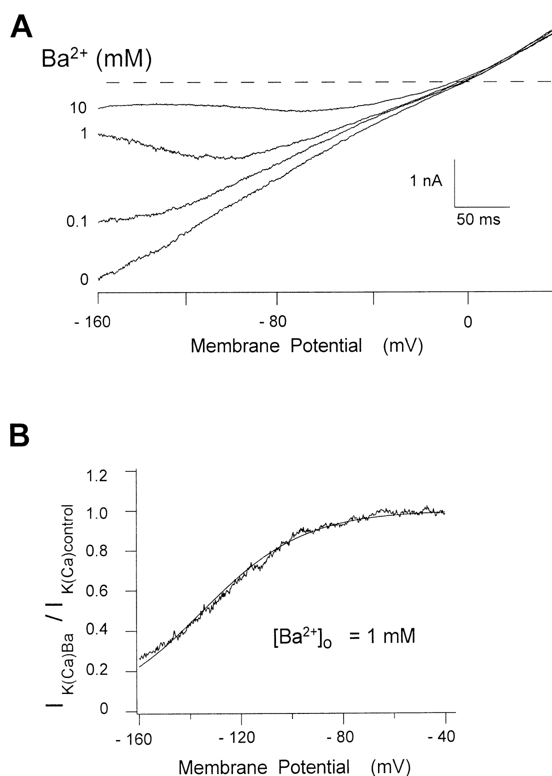

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Fig. 3. External Ba^{2+} block of SK2 channels expressed in HEK293 cells. HEK293 cells were injected with SK2 RNA. SK2 channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. A: Ramp currents were elicited by 400 ms voltage ramps from −160 to 40 mV every 10 s before and after application of a K^+ solution containing 0.1, 1 and 10 mM Ba^{2+}. B: Voltage-dependence of Ba^{2+} block. The ramp current trace in the presence of 1 mM external Ba^{2+} was divided by the trace obtained in the absence of Ba^{2+} (from A) and plotted against the membrane potential. The smooth curve represents a fit (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 fit were $E_h = -135$ mV and $k = 20$ mV.

cloned SK2 channels in *Xenopus* oocytes [9] as well as for the block of current through K(Ca) channels from Jurkat T cells [6].

Extracellular application of 10 mM TEA^+ reduced the slope of the ramp current at potentials below −60 mV more than half, indicating a half-blocking concentration of ∼4 mM (Fig. 4B). In the experiment shown in Fig. 4B, current through another type of K^+ channel may be observed. Under control conditions without TEA in the extracellular K^+ solution, the slope of the ramp current increased at potentials more positive than −40 mV, indicating the activation of a voltage-gated K^+ channel endogenously expressed in this cell. This current was abolished by the application of 2 mM TEA. However, we did observe these TEA-sensitive K^+ channels in some transfected as well as some untransfected cells. From this high TEA-sensitivity, we conclude that these cells endogenously express either the *Shaker*-related K^+ channels, Kv1.1, or the *Shaw*-related K^+ channels, Kv3.1. A similar voltage-dependent K^+ current, endogenously expressed in HEK293 cells, was described by Zhu et al. [25] but was not further characterized. In contrast, Yu and Kerchner [26] described another voltage-dependent endogenous K^+ current in HEK293 cells that was rather insensitive to block by TEA (20 mM blocked about half the current).

The SK2 channels were insensitive to block by 4-amino pyridine (1 mM) and clotrimazol (100 μM), a potent blocker of IK channels (Ishii [12]; data not shown). Thus, the pharmacological profile of SK2 channels expressed in tsA201 cells matched well those of the K(Ca) channel expressed in Jurkat cells, as summarized in Table 2.

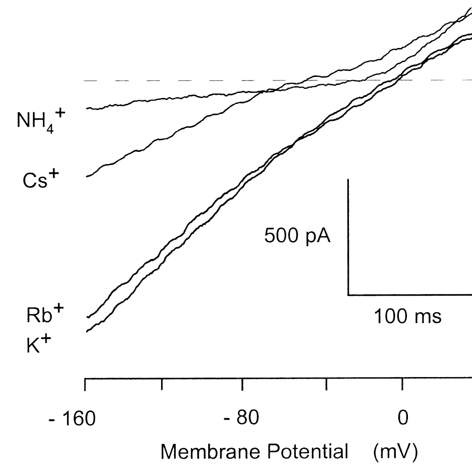

Fig. 5. Selectivity of monovalent cations in SK2 channels expressed in tsA201 cells. SK2 channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Ramp currents were elicited by 400 ms voltage ramps from −160 to 40 mV every 10 s in K^+ solution before and during application of a Rb^+, NH_4^+ and Cs^+ solution. All records shown were obtained from a single cell.

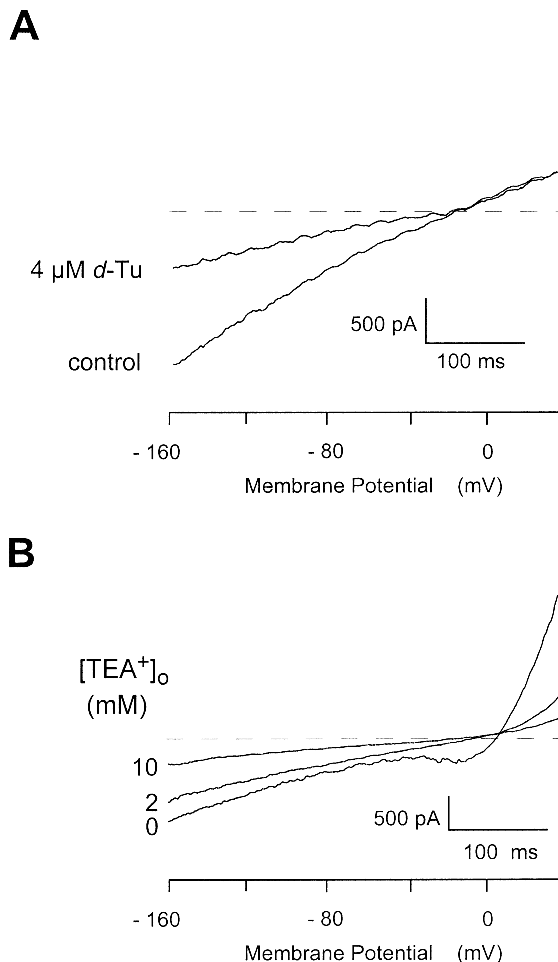

Fig. 4. Effect of externally applied *d*-tubocurarine and TEA^+ on current through SK2 channels expressed in tsA201 cells. SK2 channels were activated by whole-cell dialysis with a pipette solution containing 1 μM free Ca^{2+}. Traces were obtained after full activation of the current in K^+ Ringer in the absence and presence of extracellularly applied 4 μM *d*-tubocurarine (A) and 2 and 10 mM TEA^+ (B).

3.3. Ion selectivity

Ion selectivity and permeability were also investigated. Transfected tsA201 cells were studied with a pipette solution containing 1 μM Ca^{2+}, and the external cation species was varied. Changes in the slope conductance as well as reversal potentials were measured (Fig. 5). Assuming no significant anion permeability and that the internal concentration of permeant ions remains invariant, the permeability ratio P_X/P_K may be calculated from changes in reversal potential (see Table 3). The permeability for Rb^+ is almost as good as for K^+, however, NH_4^+, Cs^+ and Na^+ are less permeable ($K^+ > Rb^+ > NH_4^+ > Cs^+ > Na^+$); the calculated values are shown in Table 3. In addition, we determined conductance ratios, g_X/g_K, using the slopes of the ramp current at potentials between −160 and −40 mV. These values were measured (Table 3) and are quite similar to the permeability and conductance ratios obtained for the K(Ca) channels in Jurkat T cells [5]. Remarkably, both channels pass significant current with Cs^+ as charge carrier.

Table 3
Permeability and conductance ratios of K(Ca) channels in Jurkat T cells and SK2 channels expressed in tsA cells

	P_X/P_K				g_X/g_K			
	K^+	Rb^+	Cs^+	NH_4^+	K^+	Rb^+	Cs^+	NH_4^+
$K(Ca)^a_{Jurkat}$	1.0	0.96	0.26	0.53	1.0	1.01	0.67	0.11
SK2	1.0	0.98	0.32	0.61	1.0	1.02	0.55	0.08

[a]Data from [5].

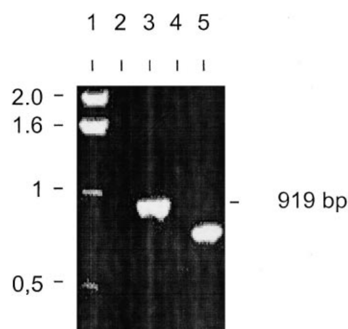

Fig. 6. *h*SK2 mRNA expression in Jurkat T cells as detected by RT-PCR. Lane 1, standard, molecular sizes are indicated in kb; lane 2, control RT-PCR without template using SK2-specific primers; lane 3, RT-PCR with Jurkat T cell mRNA as template using SK2-specific primers; lane 4, RT-PCR with Jurkat T cell mRNA as template using SK3-specific primers; lane 5, PCR with *h*SK3 bearing plasmid as template using SK3-specific primers.

3.4. SK2 mRNA in Jurkat T cells

Total RNA from Jurkat T cells was reverse-transcribed and amplified by the PCR using primers 5′ to the sequence encoding the first membrane spanning domain and 3′ of the region encoding the sixth transmembrane domain of rat SK2. As expected from the cloned rat SK2 sequence, a fragment of about 900 bp was amplified (Fig. 6) and nucleotide sequence analysis of the product showed that the human K(Ca) mRNA in Jurkat T cells was 96% identical at the nucleotide sequence level and 100% identical at the amino acid sequence level to the rat SK2 sequence [9]. The Jurkat T cell cDNA was also used as a template for PCRs using human SK3-specific primers, as SK3 is the only other cloned SK channel with apamin-sensitivity. However, no SK3 PCR product was detected. The PCR conditions were controlled by using *h*SK3-specific primers and an *h*SK3 gene bearing plasmid as a template (Fig. 6, lane 5).

4. Discussion

The two sets of results presented here strongly suggest that the cloned SK2 channel represents the apamin-sensitive K(Ca) channel in Jurkat T cells. First, the biophysical and pharmacological properties of K(Ca) channels in Jurkat T cells are very similar to SK2 channels expressed in HEK293 or tsA201 cells. The time course of activation upon whole-cell dialysis with a calcium-containing solution, ion selectivity and the pharmacological profile of the cloned SK2 channels reflect the previously determined properties of K(Ca) channels in Jurkat T cells. Second, the SK2 mRNA, but not SK3 mRNA, was present in Jurkat cells, as shown by RT-PCR. Based upon these data, we conclude that the SK2 gene encodes the K(Ca) channels in Jurkat T cells. The data also suggest that the endogenous K(Ca) channel in Jurkat T cells is a homomultimeric SK2 channel.

Table 2 gives an overview of the pharmacological properties of the K(Ca) channels in Jurkat cells and SK2 channels expressed in tsA cells. Both channels were blocked with similar potencies by apamin, ScTX and *d*-tubocurarine and neither channel was sensitive to block by CTX, a specific blocker of the K(Ca)HPB channel and the IK1 channel [12]. Our pharmacological characterization of current through SK2 channels confirms and extends earlier measurements reporting K_i values for the block of SK2 channels by apamin of 0.063–0.2 nM when the channels were expressed in *Xenopus* oocytes [9,12]. There are only minor differences in the TEA^+-sensitivity and single-channel conductance between the endogenous Jurkat K(Ca) and the cloned SK2 channels, possibly due to differences in the cell lines used for expression. In this paper, we showed that heterologously expressed SK2 channels have the same voltage-dependent block by extracellular Ba^{2+} as K(Ca) channels in Jurkat T cells. In addition, ion selectivity was very similar between the two channels. Interestingly, both channels showed a similar, high Cs^+ conductance ratio. Both channels show a similar single-channel conductance (4–7 pS compared to 10 pS) and the Ca^{2+} concentration for half-maximal activation yielded values between 450 nM and 630 nM with a Hill coefficient between 4 and 5. Therefore, we conclude that the tetrameric structure of the K^+ channel, the Ca^{2+}-sensitivity conferring structures and the pore region are identical for the K(Ca) channel in Jurkat T cells and the SK2 channel in tsA201 cells, suggesting that the endogenously expressed K(Ca) channel in Jurkat T cells is a homomultimeric protein.

The identification of SK2 as the SK channel in Jurkat cells provides a new avenue to investigate the outstanding questions concerning the physiological roles of these channels in normal and transformed T cells. First, why do leukemic cells such as Jurkat express SK2 channels even under resting conditions and, second, what mechanisms regulate T cell expression of SK channels as a function of activation status. Jurkat T cells are transformed and undergo proliferative growth and cell division during culture. They may represent an early stage in the activation pathway, one that induces expression of K(Ca) channels on their cell surface, similar to activated HPB lymphocytes. Expression of SK instead of IK channels may be a further reflection of the transformed state, compared to mitogen-activated HPB lymphocytes. Alternatively, Jurkat T cells may represent a minor subpopulation in normal peripheral blood T lymphocytes that expresses SK channels. These cells may not be detected under normal conditions, but may have expanded as a consequence of the transforming event.

The aim of this study was to identify the cloned channel corresponding to the apamin-sensitive small conductance Ca^{2+}-activated K^+ channels expressed in human Jurkat T cells. We conclude that SK2 is this channel. In Jurkat T cells, SK channels play a role in the mitogen-induced activation cascade, as demonstrated through a change in the Ca^{2+} signaling triggered by mitogens [1]. Blockade of voltage-gated and Ca^{2+}-activated K^+ channels effectively inhibits antigen driven activation of lymphocytes (for review, see [7]). The mechanism by which voltage-gated and Ca^{2+}-activated K^+ channels may contribute to regulate the Ca^{2+}signal in mitogen-stimulated Jurkat T cells probably involves the modulation of the driving force that governs Ca^{2+} influx through Ca^{2+} channels that are activated by the depletion of intracellular stores [7]. The identification of this channel gene opens up the possibility to search for immune modulatory drugs using this channel as target through mutant cycle analysis strategies. In addition, the gene might be used to link some immune diseases to the dysfunction of this channel.

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