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The Sensitivity of the Human Kv1.3 (hKv1.3) Lymphocyte K^+ Channel to Regulation by PKA and PKC is Partially Lost in HEK 293 Host Cells

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Abstract. cDNA encoding the full-length hKv1.3 lymphocyte channel and a C-terminal truncated (Δ459-523) form that lacks the putative PKA Ser^{468} phosphorylation site were stably transfected in human embryonic kidney (HEK) 293 cells. Immunostaining of the transfected cells revealed a distribution at the plasma membrane that was uniform in the case of the full-length channel whereas clustering was observed in the case of the truncated channel. Some staining within the cell cytoplasm was found in both instances, suggesting an active process of biosynthesis. Analyses of the K^+ current by the patch-clamp technique in the whole cell configuration showed that depolarizing steps to 40 mV from a holding potential (HP) of −80 mV elicited an outward current of 2 to 10 nA. The current threshold was positive to −40 mV and the current amplitude increased in a voltage-dependent manner. The parameters of activation were −5.7 and −9.9 mV (slope factor) and −35 mV (half activation, $V_{0.5}$) in the case of the full-length and truncated channels, respectively. The characteristics of the inactivation were 14.2 and 24.6 mV (slope factor) and −17.3 and −39.0 mV ($V_{0.5}$) for the full-length and truncated channels, respectively. The activation time constant of the full-length channel for potentials ranging from −30 to 40 mV decreased from 18 to 12 msec whereas the inactivation time constant decreased from 6600 msec at −30 mV to 1800 msec at 40 mV. The unit current amplitude measured in cells bathing in 140 mM KCl was 1.3 ± 0.1 pA at 40 mV, the unit conductance, 34.5 pS and the zero current voltage, 0 mV. Both forms of the channels were inhibited by TEA, 4-AP, Ni^{2+} and charybdotoxin. In contrast to the native (Jurkat) lymphocyte Kv1.3 channel that is fully inhibited by PKA and PKC, the addition of TPA resulted in 34.6 ± 7.3% and 38.7 ± 9.4% inhibition of the full-length and the truncated channels, respectively. 8-BrcAMP induced a 39.4 ± 5.4% inhibition of the full-length channel but had no effect (8.6 ± 8.3%) on the truncated channel. Cell dialysis with alkaline phosphatase had no effects, suggesting that the decreased sensitivity of the transfected channels to PKA and PKC was not due to an already phosphorylated channel. Patch extract experiments suggested that the hKv1.3 channel was partially sensitive to PKA and PKC. Cotransfecting the Kvβ1.2 subunit resulted in a decrease in the value of the time constant of inactivation of the full-length channel but did not modify its sensitivity to PKA and PKC. The cotransfected Kvβ2 subunit had no effects. Our results indicate that the hKv1.3 lymphocyte channel retains its electrophysiological characteristics when transfected in the Kvβ-negative HEK 293 cell line but its sensitivity to modulation by PKA and PKC is significantly reduced.

Key words: Kv1.3 K^+ channel — Patch clamp — Transfection — Jurkat lymphocytes — Current recordings — Immunolocalization

Introduction

Voltage-sensitive K^+ channels belong to a family of ion channel proteins ubiquitously distributed among living cells (Jan & Jan, 1989). These proteins are selectively permeable to K^+ and are activated as a result of plasma membrane depolarization (Pongs, 1992). They regulate plasma membrane potential in electrically excitable cells such as nerves and muscle, as well as nonexcitable cells such as lymphocytes (Hille, 1993). K^+ channels consist of an α-subunit that is stoichiometrically associated with

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a cytoplasmic β (Kvβ)-subunit (Catterall, 1993; Heinemann et al., 1994; Rettig et al., 1994; Shi et al., 1994). The α-subunit is an integral plasma membrane protein that consists of six putative transmembrane segments (S1 to S6) linked by short cytoplasmic or extracellular loops, and flanked by cytoplasmic N- and C-terminal sequences (Durell & Guy, 1992; Jan & Jan, 1994). A region (H5 or P) located between the S5 and S6 segments forms the lining of the ion pore (Goulding et al., 1993; Lipkind, Hanck & Fozzard, 1995; Pongs, 1993). The functional K^+ channels assemble as tetramers (MacKinnon, 1991). The Kvβ-subunits belong to gene families that show specificity of interactions with various members of the *Shaker* family of K^+ channels (Nakahira et al., 1996). Kvβ1.1 and Kvβ1.2 subunits have been reported to modulate the kinetic of inactivation of the Kv1.4 and Kv1.5 channels (Leicher et al., 1996; Rettig et al., 1994) and some Kvβ subunits may play a role in the folding of nascent α-subunits of *Shaker* channels (Shi et al., 1996).

Functional K^+ channels are required in the process of T cell activation (Chandy et al., 1984; DeCoursey et al., 1984; Lewis & Cahalan, 1995; Matteson & Deutsch, 1984). Electrophysiological studies of human T lymphocytes have revealed that these cells express mainly the *n* type K^+ channel that is characterized by a slow kinetic of inactivation typical of delayed rectifiers (Cahalan et al., 1985). The channel is activated by membrane depolarization to potentials above −40 mV, displays use-dependent inactivation and is blocked by TEA, 4-AP, quinine and charybdotoxin (Cahalan et al., 1985; Dupuis, Héroux & Payet, 1989; Sands et al., 1989). Cai et al. (1992) have cloned a K^+ channel from a human cDNA genomic library. This channel originally dubbed HGK5 was shown to belong to the *Shaker* family of K^+ channels and renamed Kv1.3, according to the revised nomenclature (Chandy & Gutman, 1993). Expression of the corresponding in vitro hKv1.3 transcript in *Xenopus laevis* oocytes revealed that the protein possessed characteristics that suggested its identity to the *n* type channel found in human T lymphocytes (Cai et al., 1992).

The hKv1.3 lymphocyte K^+ channel possesses a number of putative PKA and PKC consensus phosphorylation sites (Cai et al., 1992; Payet & Dupuis, 1992), suggesting that its activity could be modulated by phosphorylation. In this connection, Cai and Douglass (1993) have shown that the human T lymphocyte Kv1.3 channel could be immunoprecipitated in a phosphorylated form. We have reported that the activity of the Kv1.3 channel of Jurkat T lymphocytes was regulated in a coordinated manner by PKA and by PKC (Payet & Dupuis, 1992). In addition, Holmes, Fadool & Levitan (1996) and Szabo et al. (1996) have recently presented evidence that the activity of the hKv1.3 channel expressed in HEK 293 cells could be modulated by protein tyrosine kinases.

Transfection of K^+ channels in heterologous systems has been used as a means to address questions regarding structure-function relationships, subunits assembly and channel regulation (Chandy & Gutman, 1995). In the present report, we have transfected the cDNA corresponding to the full-length hKv1.3 lymphocyte channel and to a C-terminal truncated (Δ459-523) form that lacks the putative PKA Ser^{468} phosphorylation site (Cai et al., 1992) in the HEK 293 cell line (Graham et al., 1977). The patch clamp technique (Hamill et al., 1981) was used to characterize the properties of the whole and unitary currents of the stably expressed K^+ channels and their sensitivity to PKA and PKC. Our data showed that the electrophysiological characteristics of the full-length and Δ459-523 channels were similar and resembled the properties of the K^+ channel of Jurkat lymphocytes (Dupuis et al., 1989). However, the kinetic of inactivation of the transfected channels at 40 mV was slower (2000 msec) than in Jurkat (250 msec) cells. The sensitivity of the full-length and Δ459-523 channels to modulation by PKC was similar but the channels were less sensitive (approximately 30% inhibition) than the native lymphocyte channel (Payet & Dupuis, 1992). The truncated channel also displayed reduced sensitivity (approximately 30% inhibition) to the effect of PKC and was not affected by PKA. Coexpression of the Kvβ1.2 subunit but not the Kvβ2 subunit decreased the value of the time constant of inactivation of the full-length channel, although it did not modify its sensitivity to PKA and PKC.

Materials and Methods

cDNA corresponding to the human hKv1.3 channel, Kvβ1.2 and Kvβ2 were the generous gifts of Dr. J. Douglass (Vollum Institute, Department of Microbiology and Immunology, Oregon Health Science University, Portland, OR), Dr. M.J. Morales (Department of Pharmacology, Duke University, Durham, NC) and Dr. J. Trimmer (Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY), respectively. A polyclonal antibody directed against the N-terminal portion of the hKv1.3 channel was the generous gift of Dr. M. Sheng (Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA). Eagle's MEM culture medium, antibiotics, PKC, PKA (catalytic subunit) and ATP were purchased from Sigma Chemical (St. Louis, MO). Unless otherwise stated, reagents for molecular biology were obtained from Pharmacia Fine Chemicals (Montreal). The prokaryotic pGEM-3Z amplification vector was from Promega (Montreal) and the eukaryotic pRC/CMV and pREP7 expression vectors were obtained from InVitrogen Corporation (San Diego,

Abbreviations: 4-AP, 4-aminopyridine; DEPC-diethyl pyrocarbonate; EDTA, ethylenediamine tetraacetic acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HEK, human embryonic kidney; Hepes, N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid]; MOPS, 3-[N-morpholino]propane sulfonic acid; PBS, phosphate-buffered saline; PKA, protein kinase A; PKC, protein kinase C; SDS, sodium dodecylsulfate; SSC, sodium chloride-sodium citrate buffer; SSPE, sodium chloride-sodium phosphate-EDTA buffer; TEA, tetraethylammonium; TPA, 12-*O*-tetradecanoyl 13-*O*-acetyl phorbol.

CA). Geneticin (G418), Opti-MEM culture medium, Lipofectamine and synthetic oligonucleotides were purchased from Life Technologies (Gaithersburg, MD). Anti-PKC isozyme-specific monoclonal antibodies were obtained from Life Technologies or from Sigma. Other chemicals were from Sigma or local suppliers.

Cells

HEK 293 cells were obtained from the American Type Culture Collection (Rockville, MD; #CRL-1573) and maintained in Eagle's MEM supplemented with 10% heat-inactivated (56°C, 30 min) FBS containing penicillin (100 units/ml), streptomycin (100 μg/ml) and garamycin (40 ng/ml). The medium was changed weekly. Cell were detached by brisk shaking and passaged by appropriate dilution.

Northern Blot Analysis

RNA was isolated using the TRIzol reagent (Life Technologies). The RNA preparation was visualized by migrating samples in 1% DEPC-treated agarose gels. Samples for Northern blot analyses were prepared as follows. Each sample contained 20 μg of RNA, deionized formamide (10 μl), 10X MOPS (0.2 M MOPS, 50 mM sodium acetate, 10 mM EDTA, pH 8.0) (2μl) and 30% formaldehyde (3.5 μl) (Sambrook, Fritsch & Maniatis, 1989). The samples were heated for 5 min at 65°C and separated on formaldehyde-treated agarose gels in 1X MOPS buffer. The gel was then transferred to a Hybond-N membrane (Amersham, Montreal) using a transfer unit (Tyler Research Instruments, Edmonton, Alberta) under a vacuum equivalent to 50 cm of water. The gel was covered with a solution of NaOH (50 mM) and NaCl (10 mM) for 5 min, blotted and then wetted with a solution of Tris-HCl (0.1 M, pH 7.6) for 5 min. The transfer was completed by maintaining the gel covered with a 20X SSC solution for 2 hr. The membrane was then soaked in a 20X SSC solution for 5 min, air-dried for 30 min and then dried at 80°C for 2 hr.

The membrane was placed in a cylindrical glass container and prehybridized with a (filtered) mixture (10 ml) consisting of 20X SSPE (2.5 ml), 50X Denhart's solution (1 ml), Dextran sulfate (1 g), 10% SDS (1 ml), deionized formamide (5 ml) and salmon sperm DNA (1 mg) (Sambrook, Fritsch & Maniatis, 1989). Hybridization (Tyler Research Instruments, incubator model HI 12000) was done for 4 hr at 42°C. The cDNA probe corresponding to the S1 to S4 segments of the hKv1.3 channel (positions 325 to 523 according to Cai et al., 1992) was labelled with the Multiprime® labeling kit (Pharmacia) following the manufacturer's protocol and purified by gel filtration (Sambrook, Fritsch & Maniatis, 1989). The labeled cDNA probe was placed in boiling water for 10 min, added to the hybridization cocktail and incubation was done for 16 hr at 42°C. The membrane was washed in the following sequence. Two brief washes using a mixture of 2X SSC and 1% SDS followed by a 30 min (42°C) incubation in the same mixture, a 30-min wash with a mixture of 1X SSC and 0.5% SDS and a final brief wash with 0.5X SSC to remove SDS. The membrane was then placed in a cassette for 3 hr and radioactivity revealed with a Phosphor Imager instrument (Molecular Dynamics, Sunnyvale, CA).

PCR Amplification and Ligation

hKv1.3 Channel

Full-length and Δ459-523 K^+ channel sequences were amplified by PCR, in the following manner: Restriction sites were introduced in the sequence of the synthetic oligonucleotides to allow subcloning in the pRC/CMV expression vector. The following oligonucleotides were used to amplify the full-length Kv1.3 channel, 5′-CG**TCTAGA**-TTAAACATCGGTGAATATCTTT-3′(T_m = 57°C) which contained a XbaI restriction site (bold) and 5′-GC**AAGCTT**CGCGAGCTGCC-GCCCGACATG-3′(T_m = 79°C) which contained the HindIII restriction site (bold). PCR amplification of the truncated channel was done with the first oligonucleotide shown above and a 5′-GC**TCTAGA**TTAGCAACTTCCCACGTGCATGTACTG-3′ (T_m = 68°C) oligonucleotide containing the XbaI restriction site (bold). PCR amplification was catalyzed with the *Taq* DNA polymerase on a programmable heath block (Perkin Elmer). The denaturation reaction was performed at 94°C for 1 min, the procedure of annealing was done at various temperatures for 45 sec and the extension reaction at 72°C for 2 min. Twenty-five to thirty cycles of amplification were done. The products of PCR were precipitated (isopropanol), digested simultaneously with HindIII and Xba I, separated on 1% agarose gels and the desired bands were recovered using an electroelution apparatus (IBI, New Haven, CT) or purified with the Geneclean kit (BIO 101, Vista, CA). The homogeneous cDNA were ligated in pRC/CMV and amplified in the XL1 blue *E. coli* strain. The plasmids were recovered using the Qiagen plasmid kit (Qiagen, Chatsworth, CA) and used for transfections.

Kvβ Subunits

The Kvβ2 insert was excised from its Bluescript vector (Shi et al., 1996), digested with XbaI/ClaI and blunt ends generated by treatment with DNA polymerase. The cDNA was ligated at the PvuII site in the pREP7 expression vector. The Kvβ1.2 subunit was directly amplified from its vector (Morales et al., 1995) by PCR using the synthetic oligonucleotide 5′-GC**GGATCC**CAGATACCTCAGAATC-3′ (T_m = 69°C) containing a BamH1 restriction site (bold) and 5′-GC**AAGCTT**GGCACGAGCAGTCTCA-3′ (T_m = 71°C) containing a HindIII restriction site (bold). The conditions of amplification and product purification were the same as described above. The amplified cDNA was ligated in the pREP7 expression vector.

The PCR methodology was used in an attempt to amplify a Kvβ subunit from Jurkat cells. The experimental approach was similar to the *Touchdown PCR* methodology described by Morales et al. (1995). In brief, a synthetic oligonucleotide (5′-GGATCTATAGTCCTTTTT-3′, T_m = 57°C) corresponding to the consensus cDNA sequence of Kvβ subunits C-terminus was used for reverse transcription of total or $polyA^+$ Jurkat RNA and controls (total RNA from 10^7 peripheral white blood cells or total RNA from 1 g of rat brain). The reaction was carried out using the *Superscript* reverse transcriptase (Pharmacia) and *RNA guard* (Pharmacia) and performed for 60 min at 42°C. Attempts at PCR amplification were done with the oligonucleotide described above and a degenerated oligonucleotide, 5′-TCGAATTCAAY-CAGGGMATGGCIATGTAYTGG-3′ (T_m = 71°C) corresponding to positions 101–108 or the degenerated oligonucleotide, 5′-GACCTCGAGCCYTCGTTICKIARRCACCA-3′(T_m = 73°C) corresponding to positions 178–185 of the Kvβ protein sequence (Morales et al., 1995). In these sequences, Y corresponds to C or T, I to inosine, K to G or T and R to A or G. The conditions of reaction of amplification using the *Taq* DNA polymerase were 94°C (denaturation, 1 min), 55°C to 42°C (annealing, 2 cycles each) and 72°C (extension), respectively. Twenty-five to thirty cycles were performed. Products were separated on 1% agarose gels and transferred to a Hybond-N membrane (as described for Northern blot analysis). The membrane was prehybridized (as above) and Southern blot analysis were performed with a [^{32}P]-labeled Kvβ1 probe prepared by Multiprime® labeling.

Transfection Experiments

Transfection experiments were performed with the Lipofectamine reagent using the manufacturer's protocol. In brief, 2 μg of the pRC/CMV constructs (full-length and truncated cDNA) were mixed with 12 μg of Lipofectamine in a volume of 200 μl of Opti-MEM solution and left for 45 min at room temperature. The mixture was added to HEK 293 cells grown to approximately 50% confluency and the cells were left for 8 hr at 37°C. They were washed twice with serum- and antibiotic-free MEM and cultured for 48 hr in complete MEM. Selection was initiated by adding G418 (500 μg/ml, effective concentration). The hKv1.3 and the Kvβ1.2 or Kvβ2 cDNA were cotransfected by the Lipofectamine method and the cells were selected in the same medium containing G418 (500 μg/ml) and hygromycin (150 μg/ml). Colonies of stable transfectants were used after 2 months of selection.

Patch-Clamp Experiments

Patch-clamp studies were performed as described (Payet & Dupuis, 1992). The cells bathed in Gey's balanced salt solution containing (mM), NaCl, 120; $CaCl_2$, 2.12; KCl, 5; $MgCl_2$, 2.2; $MgSO_4$, 0.6; D-glucose, 5.6; Hepes, 39 (pH 7.4). Experiments were done at room temperature. The patch electrodes were filled with (mM); KCl, 120; NaCl, 20; Hepes, 5 (pH 7.3). The pipette resistance (Pyrex glass, Corning 7740) ranged from 4 to 8 megaohms, and the seal resistance varied between 10 and 50 gigaohms. Data were analyzed using in-house computer programs.

Immunostaining of Transfected Cells

These studies were done by using the anti-hKv1.3 antibody directed against the N-terminal region of the channel. HEK 293 transfectants were grown on 22 mm circular coverslips to confluency. The cells were washed twice with PBS for 5 min and treated with 4% paraformaldehyde in PBS for 30 min at room temperature. The fixed cells were permeabilized by treatment (5 min) with 0.5% Triton X-100 in PBS, washed twice (5 min each) with PBS, a solution (5%, w/v) made of powdered skimmed milk in PBS was added and incubation (4°C) was allowed to proceed for 30 min, followed by exposure (60 min, 4°C) to 100 mM glycine in PBS. The cells were washed twice with PBS and then incubated (60 min, room temperature) with the primary antibody (250 ng) in PBS containing 2% (w/v) BSA in 100 μl. Three washings with PBS were done followed by the addition (1 μg) of a fluorescein-conjugated anti-rabbit immunoglobulin antibody raised in the goat. After an incubation of 30 min at room temperature, the cells were washed three times (10 min each) with PBS. Fluorescence quenching was reduced by addition of a mounting solution (10 μl) of Vectashield H-1000 (Vector Laboratories, Burlingame, CA). The coverslips were sealed with nail polish and mounted on a Nikon Diaphot microscope equipped with a Nikon camera. The samples were illuminated with a 100 watt mercury lamp (Nikon) and appropriate optical barrier filters (Nikon) were used.

PKC Activity

Total PKC activity was assayed in Jurkat and HEK 293 cells using a commercial kit (Pierce, Rockford, IL). Purified PKC (Sigma) was used as a reference. The effect of PKC on the activity of the transfected Kv1.3 channels was assessed by using PKC activated by Ca^{2+} and a micellar preparation of dioelin and phosphatidylserine (Kikkawa et al., 1983). Controls were diolein/phosphatidyl micelles lacking PKC.

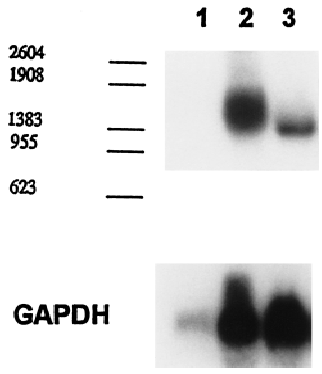

Fig. 1. Northern blot analysis of hKv1.3 channels in transfected HEK 293 cells. Total RNA was sized on a 1% agarose gel, transferred to a nylon membrane and probed with a ^{32}P-labeled cDNA corresponding to nucleotides 329-523 of the hKv1.3 channel (Cai et al., 1992). Results are shown for untransfected HEK 293 cells (lane 1) and cells transfected with cDNA corresponding to the full-length (lane 2) and the truncated (Δ459-523) channel (lane 3). The size of DNA markers is indicated on the left. The membrane was dehybridized and reprobed with a cDNA to GAPDH.

Statistical Analysis

Data were analyzed with the Sigma Stat computer software (Jandel Scientific, San Rafael, CA) using Student's *t*-test for unpaired data.

Results

Expression of the Full-length and the Δ459-523 hKv1.3 Channels

Northern blot analysis of the stable transfectants revealed that they expressed the mRNA corresponding to the full-length and truncated forms of the hKv1.3 channel (Fig. 1). The size of the full-length transcript was 1.6 kb (lane 2) as reported (Cai et al., 1992) and that of the truncated channel (lane 3) was 1.3 kb, as expected. Untransfected HEK 293 cells were unreactive to the cDNA probe (lane 1). Scanning (ScanMan instrument, Logitech, Fremont, CA) and densitometric analysis using the ImageQuant software (Molecular Dynamics, Sunnyvale, CA) were used to normalize the Northern blots to GAPDH signals. Results showed that the signal of untransfected cells corresponded to background values and that the signal ratio of the full-length to truncated hKv1.3 channel was 3:1.

Immunolocalization of the Full-length and Δ459-523 hKv1.3 Channels

The cellular distribution of the K^+ channels in transfected HEK 293 cells was analyzed by indirect immunostain-

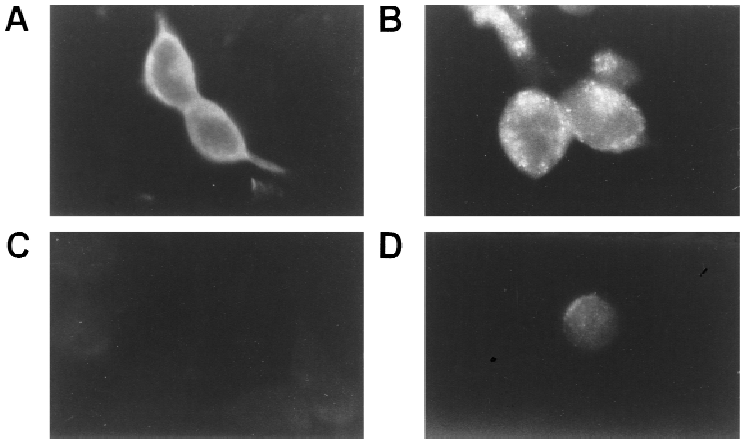

Fig. 2. Indirect immunostaining of HEK 293 cells with a polyclonal antibody directed against the N-terminal portion of the hKv1.3 channel. The cells were fixed with paraformaldehyde, permeabilized (Triton X-100) and incubated with the primary antibody, followed by an anti-rabbit immunoglobulin fluorescein-conjugated secondary antibody. Results are shown for HEK 293 cells transfected with the cDNA corresponding to the full-length hKv1.3 (*A*), the truncated (Δ459-523) channel (*B*) and untransfected cells (*C*). Results obtained in Jurkat E6.1 cells are shown in (*D*).

ing. The full-length hKv1.3 channel was largely distributed at the cell plasma membrane in a uniform manner (Fig. 2*A*). However, the truncated form of the channel appeared as clusters of fluorescence distributed at the cell plasma membrane (Fig. 2*B*). In both cases, staining was also observed within the cells, suggesting an active process of biosynthesis. Untransfected HEK 293 cells did not display positive staining (Fig. 2*C*). The hKv1.3 channel in Jurkat cells appeared as diffused patches of fluorescence that coalesced in one pole of the cells (Fig. 2*D*).

Analyses of the K^+ Current in the Whole Cell Configuration

A depolarizing step to 40 mV from a HP of −80 mV elicited a large outward current of 2 to 10 nA in cells transfected with the full-length and the Δ459-523 hKv1.3 channels. Figure 3*A* illustrates a series of traces of 500 msec duration steps from a HP of −80 mV to a range of test potentials applied in 20 mV increments from −60 to 40 mV. The current threshold was found to be positive to −40 mV in both cases and the current amplitude increased in a voltage-dependent manner as illustrated by the current/voltage relationship (Fig. 3*B*).

The activation curves were obtained by plotting the normalized conductance from current evoked by step potentials from −80 mV to various values. The curve was fitted with a Boltzmann function distribution and the parameters were k (slope factor) = −5.7 mV, $V_{0.5}$ (half activation) = −35.4 mV in the case of the full-length channel and k = −9.9 mV, $V_{0.5}$ = −34.5 mV in the case of the truncated channel (Fig. 3*C*). The inactivation curves were obtained by applying a long duration conditioning pulse (2 sec) to potentials ranging from −100 to 0 mV. A test pulse (300 msec, 40 mV) was then applied and the amplitude of the elicited current was measured. The inactivation curve was plotted for the current amplitude obtained for each value of the conditioning pulse normalized to the maximum current amplitude. The latter was obtained for the more negative conditioning pulse where inactivation was absent. The current was completely inactivated for potentials more positive than 20 mV (Fig. 3*D*). The line was fitted to the experimental points using a Boltzmann function distribution with a slope factor (k) of 14.2 mV and a potential for half inactivation ($V_{0.5}$) of −17.3 mV for the complete hKv1.3 channel. In the case of the truncated K^+ channel, the values were 24.6 mV (k) and −39.7 mV ($V_{0.5}$), respectively.

The kinetic properties of the transfected channels were analyzed by fitting the following equation, $I_t = I_{\max}\{[1 - \exp(-t/\tau_n)]^n \cdot [\exp(-t/\tau_j)]\} + I_l$, where I_t is the current at a fixed potential as a function of time, $I_{\max}$ is the maximum current at a given potential, τ_n and τ_j are the time constants of the activation and inactivation processes, n is the exponent of the activation process and I_l is a time-dependent leak current. The activation time constant (τ_n) for the full-length channel was plotted for various potentials ranging from −40 to 40 mV and decreased from 18 to 12 msec (Fig. 3*E*). The inactivation time constant (τ_j)-voltage relationship showed a decrease from 6600 msec at −30 mV to 1800 msec at 40 mV (Fig. 3*F*). Similar values were determined in the case of the truncated hKv1.3 channel (Fig. 3*E* and *F*).

Analysis of the K^+ Unitary Current of the Full-length hKv1.3 Channel

Current traces obtained in the cell-attached mode are shown in Fig. 4. A depolarization step to 40 mV (500 msec duration) was applied from a HP of −80 mV. The channel usually opened at the beginning of the pulse and appeared to go in an inactivated state as openings became less frequent as a function of time (Fig. 4*A*). An amplitude histogram showed that the current amplitude

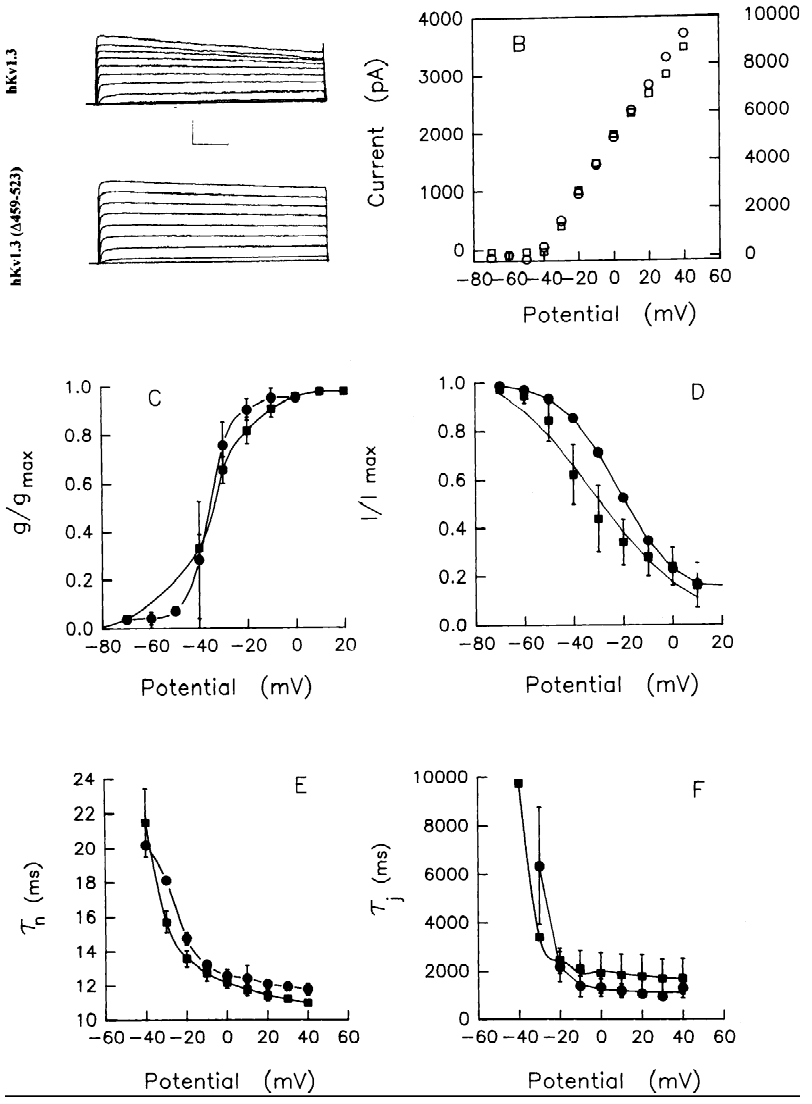

Fig. 3. K^+ current in transfected HEK 293 cells. (*A*) Representative tracings of 500 msec duration of the current obtained by a depolarizing step from a HP of −80 mV to a range of test potentials applied in 20 mV increments from −60 to 40 mV. Data representative of 50 similar experiments are shown in the case of cells expressing the full-length (hKv1.3) or the truncated (hKv1.3 Δ459-523) channel. The horizontal scale corresponds to 60 msec and the vertical scale, to 1250 (full-length) and 2500 (Δ459-523) pA, respectively. (*B*) Corresponding current-voltage relationships for the full-length (○) and the Δ459-523 (□) channels. (*C*) Activation curves of the K^+ current for the full-length (●) and the Δ459-523 (■) channels. (*D*) Inactivation curves of the K^+ current for the full-length (●) and the Δ459-523 (■) channels. The curves were obtained by applying a 2-sec conditioning pulse to various potentials. A test pulse (300 msec, 40 mV) was applied for each potential and the recorded current amplitude was normalized to the maximum current amplitude. (*E*) Variation of the time constant of activation for the full-length (●) and the Δ459-523 (■) channels for various potentials. (*F*) Variation of the time constant of inactivation for the full-length (●) and the Δ459-523 (■) channels for various potentials.

was 1.32 ± 0.1 pA at 40 mV under symmetrical KCl (140 mM) (Fig. 4*C*). In the majority of the patches, several channels were found to be active (Fig. 4*B*), suggesting a relatively high density of expressed channels in agreement with the high current intensity recorded in the whole cell configuration (Fig. 3*A*).

The unit current-voltage relationship was obtained under conditions where the concentration of KCl in the pipette medium was 140 mM. The unit conductance was 34.5 pS and zero current voltage was 0 mV (Fig. 4*D*). When the concentration of K^+ in the pipette medium was decreased to 5 mM, the zero current voltage shifted toward negative potentials and was approximately −80 mV (Fig. 4*D*). Under these conditions, the unit conductance was 18 pS.

Pharmacological Properties of the hKv1.3 Channels

The pharmacological properties of the native hKv1.3 channel have been previously determined in peripheral T lymphocytes (Sands, Lewis & Cahalan, 1989) and in the Jurkat T cell line (Dupuis et al., 1989). We tested whether the full-length and the Δ459-523 K^+ channels retained their pharmacological properties when expressed in HEK 293 cells. It was found that TEA blocked the K^+ current of both channels in a dose-related manner (Fig. 5). The full-length hKv1.3 channel and its truncated form were also inhibited by 4-AP, charybdotoxin and Ni^{2+} (Fig. 5).

Effects of PKA and PKC on the Activity of the Transfected Channels

Previous studies have reported that the activity of the hKv1.3 channel can be regulated by protein kinases (Bastin, Payet & Dupuis, 1990; Payet & Dupuis, 1992; Holmes et al., 1996; Szabo et al., 1996). We tested whether PKA and PKC would modify the properties of the currents recorded in the whole cell configuration. In one set of experiments, TPA (16 nM) was added and

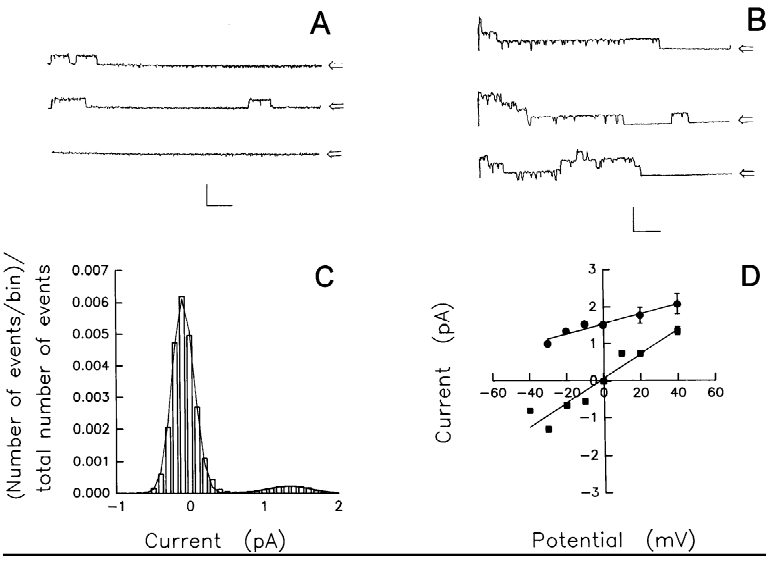

Fig. 4. Tracings of the K^+ current recorded in the cell-attached mode for the expressed full-length hKv1.3 channel. Activity of a single (*A*) and multiple (*B*) channels. The arrows represent the close state of the channel. The scale corresponds to 60 msec (horizontal) and 2 (*A*) and 2.5 (*B*) pA, respectively. (*C*) Amplitude histogram recorded at 40 mV under a symmetrical KCl (140 mM) concentration. (*D*) Unit current-voltage relationships determined under conditions of symmetrical (■) (140 mM in the patch pipette) or asymmetrical (●) (5 mM in the patch pipette) KCl concentrations.

the current recorded. Data showed a 35.0 ± 7.3% (n = 10) inhibition of the current amplitude in the case of the full-length channel and 39.4 ± 9.4% (n = 10) inhibition in the case of the truncated channel (Fig. 6*A*). In one other set of experiments, the cell permeant 8-BrcAMP derivative (1 mM) decreased the amplitude of the current by 38.7 ± 5.4% (full-length channel, n = 7) but had no effect (8.6 ± 8.3%, n = 10) on the truncated (Δ459-523) channel (Fig. 6*A*).

Work from our laboratories has shown that the activation of PKA and PKC fully inhibits the Kv1.3 channel of Jurkat lymphocytes (Payet & Dupuis, 1992). We tested whether the reduced sensitivity of the transfected channels to modulation could be due to a decreased accessibility of the putative phosphorylation sites to these protein kinases. The question was addressed by experiments done in the inside-out patch clamp configuration. Unit current traces were recorded at a membrane potential of 40 mV from a HP of −80 mV. The addition of activated (Kikkawa et al., 1983) PKC resulted in a decrease of the activity of the channel that translated into a fewer number of channel openings by sweep stimulation (*data not shown*). In 9 separate experiments, moderate to full inhibition was observed in 6 cases. The results were quantitated by measuring the probability of finding the K^+ channel in its open state during one stimulation (sweep) of 500 msec. As most of the patches contained several channels, the probability was expressed as NP_o where N is the number of active channels and P_o is the opening probability. In the case of experiments where full inhibition was observed, exposing the patch to a mixture consisting of the PKC inhibitor staurosporine and alkaline phosphatase increased the activity of the channel as illustrated in Fig. 6*B*. In this example, the slope of the cumulative NP_o was constant (0.59) during the control time period, indicating that the mean NP_o did not change. Adding activated PKC resulted in a rapid decrease of the slope (0.10) until it reached a null value, suggesting that the activity of the channels decreased until it became silent. When a mixture of staurosporine and alkaline phosphatase was added, the value of the slope increased (0.19), suggesting a partial recovery of channel activity. Similar results were obtained by adding the catalytic subunit of PKA instead of PKC. In the example shown in Fig. 6*C*, the initial value of the slope was 0.33. It rapidly decreased to a value of 0.17 after the addition of PKA (2 units) and fell to a value of 0.07. The slope increased to 0.13 after the addition of alkaline phosphatase, in agreement with a partial recovery of channel activity.

Investigation of the State of Phosphorylation of the Transfected hKv1.3 Channel

The partial loss of sensitivity of the transfected channels to the action of PKC and PKA suggested that the transfected channels could be already in a phosphorylated state. This possibility was investigated by dialyzing the patch pipette with alkaline phosphatase (10 units/ml). Results showed that this treatment did not affect the amplitude of the K^+ current that was 94 ± 9.5% (average recorded at 10 min ± SD, n = 3) of the initial current (normalized at 100%). A corollary would be that HEK 293 cells displayed a high level of protein phosphatase activity that would maintain the population of hKv1.3 channels in an unphosphorylated state and rapidly inhibit the effect of protein kinase modification of the channel. This possibility was found unlikely by dialyzing the cells with the protein phosphatase inhibitors microcystin (20 nM) (Honkanen et al., 1990) or by adding okadaic acid (1 μM) (Ozaki et al., 1987) to the bath. These experimental protocols did not change the sensitivity of the channel to TPA or 8Br-cAMP (n = 6, *not shown*).

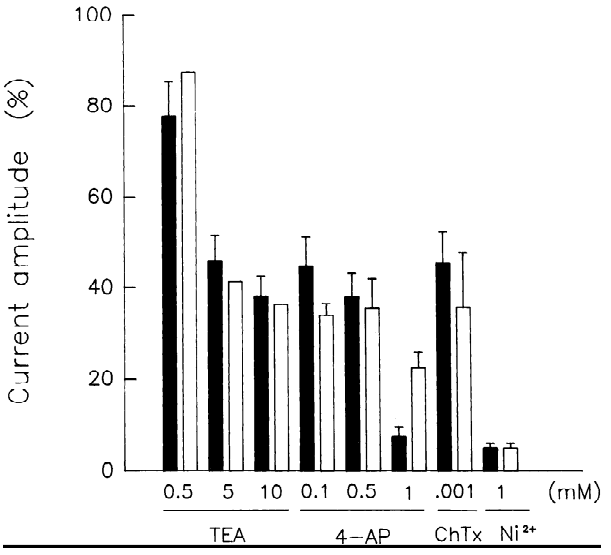

Fig. 5. Sensitivity of the transfected hKv1.3 channels to K^+ channel inhibitors. Data show the concentrations of inhibitors used in the case of the full-length (filled columns) and the Δ459-523 (empty columns) expressed channels.

Expression of PKC in HEK 293 Cells

The decreased sensitivity of the transfected hKv1.3 channel to inhibition by PKC could be due to a reduced level of expression of PKC isozymes in HEK 293 cells. This possibility was found to be unlikely based on the results of Western blot experiments which revealed that HEK 293 cells expressed the α, βI, βII, δ, ϵ and ζ PKC isozymes that are also present in Jurkat cells (Tsutsumi et al., 1993 and our own unpublished observations). Furthermore, total PKC activity in HEK 293 cells (0.30 $mU/10^7$ cells) was not different than Jurkat cells (0.21 $mU/10^7$ cells).

Effect of the Kvβ1.2 or Kvβ2 Subunits on the hKv1.3 Channel

K^+ channel-associated Kvβ subunits can modulate the kinetic of inactivation (Rettig et al., 1994). In addition, the Kvβ2 subunit has been shown to help in the folding and expression of the Kv1.2 channel (Shi et al., 1996). However, it is not known whether Kvβ proteins can modulate the regulation of K^+ channels. The Kvβ-negative HEK 293 cell line (Uebele et al., 1996) provided an opportunity to test this question. HEK 293 cells were cotransfected with the cDNA corresponding to the full-length hKv1.3 channel and the Kvβ1.2 or Kvβ2 subunit. Northern blot analysis showed that the cDNA were coexpressed in HEK 293 cells. The size of the messages were 1.6 (hKv1.3), 3.6 (Kvβ1.2) and 4.2 kb (Kvβ2), as reported (Cai et al., 1992; Heinemann et al., 1994; Rettig et al., 1994; Morales et al., 1995; Shi et al., 1996). The presence of the Kvβ1.2 subunit resulted in a decrease of the time constant of inactivation of the hKv1.3 channel from 1460 msec to 1022 msec (Fig. 7*A*). Cotransfection

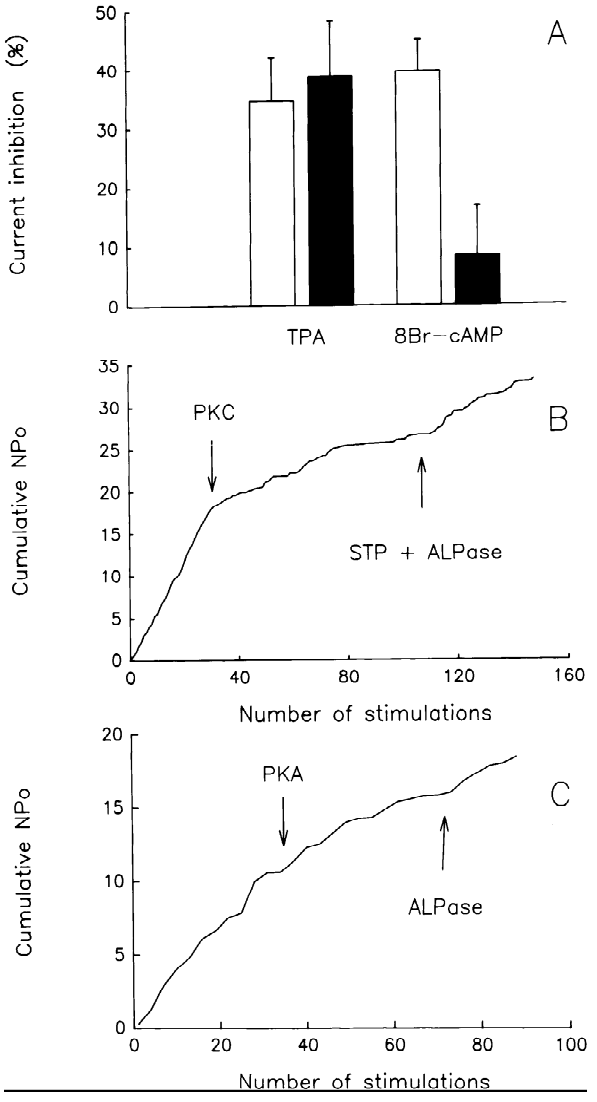

Fig. 6. (*A*) Effects of PKA and PKC on the activity of the transfected K^+ channels. Channel activity was recorded in the whole-cell configuration. The patched cells were exposed to TPA (16 nM) or 8Br-cAMP (1 mM). Results are shown for the full-length (empty columns) and the Δ459-523 (filled columns) channels and are the average of 8 experiments ± SD (indicated by the vertical bars). (*B*) and (*C*). Unit K^+ current in excized patches of HEK 293 cells transfected with the full-length hKv1.3 channel. Tracings were recorded at a membrane potential of 40 mV from a HP of −80 mV. Results are shown before and after the addition of (*B*) PKC (10 μg/ml), followed by the addition of a mixture of staurosporine (200 nM) and alcaline phosphatase (ALPase, 10 units/ml) or (*C*) the addition of the catalytic subunit of PKA (2 units), followed by the addition of alcaline phosphatase (ALPase, 10 units/ml). Data are expressed as cumulative NP_o, where N is the number of active channels under one patch and P_o the probability of channel opening, as a function of the number of stimulations.

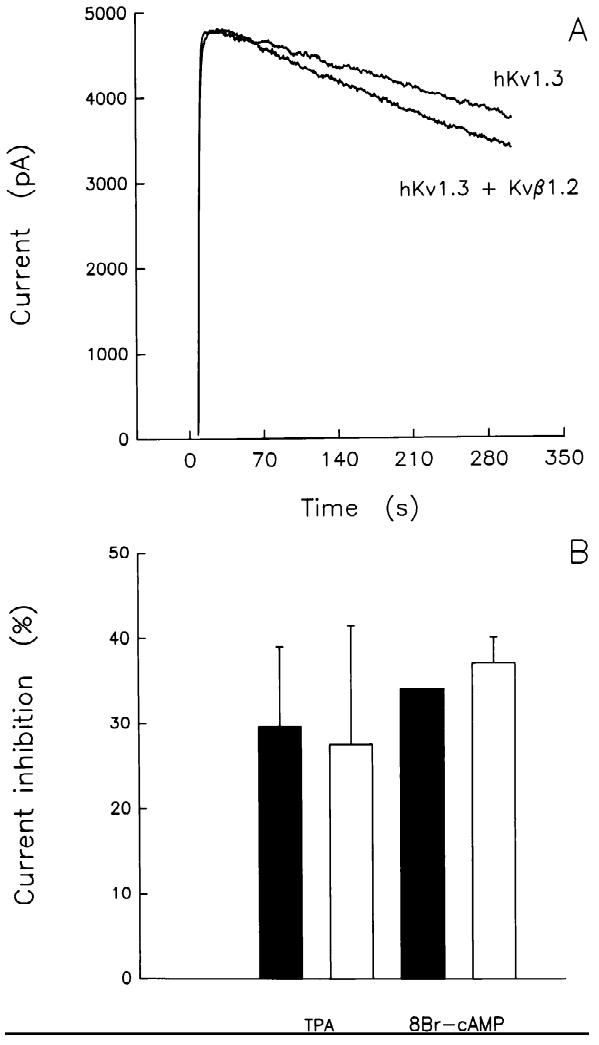

Fig. 7. (*A*) Time course of the current in cells transfected with the cDNA corresponding to the hKv1.3 channel and in cells cotransfected with the cDNA of the hKv1.3 channel and the Kvβ1.2 subunit, as indicated. (*B*) Effects of PKA and PKC on the activity of the K^+ current in HEK 293 cells cotransfected with the cDNA of the hKv1.3 channel and the Kvβ1.2 subunit (filled columns) or the cDNA of the hKv1.3 channel and the Kvβ2 subunit (empty columns). The cells were exposed to TPA (16 nM) or 8Br-cAMP (1 mM). The results are the average of 8 experiments ± SD (indicated by the vertical bars). The current was recorded in the whole-cell configuration.

of the Kvβ2 subunit had no effects. The effect of activating PKC were tested by exposing the cotransfected cells to TPA (16 nM) and that resulted in an inhibition of the current of 27.0 ± 15.4% (hKv1.3/Kvβ1.2, $n = 8$) and 29.5 ± 13.4% (hKv1.3/Kvβ2, $n = 8$), respectively (Fig. 7*B*). The addition of 8-BrcAMP (1 mM) produced an inhibition of 37.0 ± 4.2% (hKv1.3/Kvβ1.2, $n = 8$) and 35.0% (hKv1.3/Kvβ2, $n = 8$) (Fig. 7*B*). These data were not significantly different ($P = 0.31$) from those obtained in the case of HEK 293 cells transfected with the full-length hKv1.3 cDNA only. Furthermore, there were no statistical differences between the cells cotransfected with hKv1.3/Kvβ1.2 and hKv1.3/Kvβ2 ($P = 0.59$).

Discussion

Expression of the Transfected K^+ Channels

Peripheral (Lewis & Cahalan, 1995) and Jurkat T cells (Dupuis et al., 1989; Bastin et al., 1990) express the *n*-type K^+ channel whose electrical properties are consistent with the cloned *Shaker* hKv1.3 channel (Cai et al., 1992). The channel possesses putative phosphorylation sites for PKA (Cai & Douglass, 1993), PKC (Payet & Dupuis, 1992) and protein tyrosine kinases (Holmes et al., 1996; Szabo et al., 1996) that are dispersed throughout the cytoplasmic domains. We have used the HEK 293 host cell system (Stern et al., 1994) to stably transfect cDNA corresponding to the full-length and a truncated form (Δ459-523) of hKv1.3 that lacks the putative Ser^{468} PKA phosphorylation site (Cai et al., 1992). Northern blot analyses revealed a robust expression of the corresponding messages (Fig. 1). Immunostaining showed that the channels were expressed at the cell membrane and in the cytoplasm which suggested an active process of biosynthesis (Fig. 2). The truncated channel appeared to form clusters in HEK 293 cells to an extent higher than in the case of the full-length protein (Fig. 2*B* compared to 2*A*). However, we did not find any significant differences in the number of expressed channels under the patched membranes. In this connection, it has been reported that the Kv2.1 channel displays a higher level of clustering when transfected in COS-1 cells (Shi et al., 1994). These findings do not appear to be an artefact due to cell hosts since channel clustering in skeletal muscle has also been reported (Almers, Stanfield & Stuhmer, 1983). In the present report, the full-length and Δ459-523 channels were expressed at a higher level in HEK 293 cells than in Jurkat cells (Fig. 2*A* and *B* compared to *C*), in agreement with the electrophysiological recordings which displayed a large outward current (Fig. 3*A*).

K^+ Current in Cells Expressing the Full-length and Δ459-523 Channels

Global Current

The full-length hKv1.3 channel retained some of its electrical properties reported in peripheral T (Cahalan et al., 1985; Bregestovski, Redhozubov & Alexeev, 1986) and Jurkat (Dupuis et al., 1989) cells as well as in the case of the channel expressed in *Xenopus laevis* oocytes (Cai et al., 1992). These included current-voltage relationships

(Fig. 3*B*), activation (Fig. 3*C*) and inactivation (Fig. 3*D*) curves and sensitivity to blockers such as TEA, 4-AP, charybdotoxin and Ni^{2+} (Fig. 5). However, a striking difference was observed with respect to its kinetic. The inactivation was significantly decreased with a time constant of 1800 msec at a membrane potential of 40 mV (Fig. 3*F*) that contrasted with 200 to 300 msec in the case of the native channel in Jurkat cells (Grissmer & Cahalan, 1989; Dupuis et al., 1989). These results resemble those reported by Cai et al. (1992) in the case of the channel expressed in *X. laevis* oocytes where a time constant of inactivation of 8000 msec was found at 40 mV and by Tu, Santarelli & Deutsch (1995) who found a time constant of 966 msec at 50 mV. In contrast, Tu et al. (1995) have reported that the inactivation kinetics of the K^+ currents of Kv1.3 are indistinguishable for endogenous channels in native T lymphocytes and for heterologously expressed channels in the murine CTLL-2 cell line.

These observations raise the possibility of post-transcriptional modifications that could be different in lymphocytes compared to oocytes or to HEK 293 cells, or that a protein coassembling subunit is absent in host cells. This question remains unanswered. One additional possibility is the formation of heteromultimeric channels in transfected cells. In this respect, it has been shown that mKv1.1 can assemble with mKv1.3 to form functional heteromultimeric channels (Hopkins, Demas & Tempel, 1994). In these instances, electrophysiological studies have revealed that the heteromultimeric channels possess an inactivation time constant value of 1843 msec which is intermediate between the values of the mKv1.1 (2337 msec) and the mKv1.3 (694 msec) channels (Hopkins et al., 1994). Heteromultimers of mKv1.1 and mKv1.2 have been found to occur in neurons (Wang et al., 1993). Furthermore, it has recently been reported that Kv1.2 and Kv3.1 (Shahidullah et al., 1995), Kv8.1 and Kv2.1 and, Kv2.2 and Kv3.4 (Hugnot et al., 1996) can form heteromultimeric channels. We cannot discount the possibility of heteromultimer formation between the transfected hKv1.3 and the HEK 293 native K^+ channel(s). However, we feel this possibility to be unlikely based on global K^+ current recordings in untransfected HEK 293 cells which were consistently in the pA range. These observations contrast with recordings in the nA range (Fig. 3*A*) in transfected cells which suggest that if heteromultimeric channel formation occurs, it would be diluted by the overwhelming level of expression of the hKv1.3 channel.

Deletion of most (positions 459-523) of the cytoplasmic C-terminal portion of the channel had only small effects on its electrophysiological properties. The values for half-maximal activation were identical whereas the value of the slope factor was increased by a factor of two with respect to the full-length channel. Small differences were also noted in the case of the parameters of the steady-state inactivation. The values of the slope factor and half-time of inactivation were nearly twice those determined in the case of the full-length channel. The inactivation time constant was similar, suggesting that the C-terminal portion of the hKv1.3 channel was not involved in channel inactivation. In this connection, it has been reported (Hoshi, Zagotta & Aldrich, 1991) that C-type inactivation was affected by a single amino acid residue (Val^{463}) in the S6 transmembrane segment of the channel but not by the cytoplasmic domain. Furthermore, our data suggest that the C-terminal portion of the hKv1.3 channel is not essential for its expression and function as reported in the case of the *Shaker* hKv1.5 channel where it was shown that the C-terminal portion of the channel could be extended without affecting its activity (Philipson et al., 1993).

Effects of PKA and PKC on the Activity of the Transfected Channels

The regulation of transfected K^+ channels by phosphorylation has received less attention than their voltage-dependent properties. For example, modulation of channel activity by PKA has been reported in the case of an IRK1 inwardly rectifying channel expressed in COS cells (Wischmeyer & Karschin, 1996) and the Kv2.1 channel expressed in *X. laevis* oocytes (Wilson et al., 1994). With respect to modulation by PKC, this has been reported for the hKv1.3 channel expressed in HEK 293 cells (Critz et al., 1993; Bowlby & Levitan, 1995) and the I_{sk} channel expressed in *X. laevis* oocytes (Busch et al., 1992; Aiyar, Grissmer & Chandy, 1993). Dual modulation by PKA and PKC has also been reported for the I_{sk} channel expressed in frog oocytes (Varnum et al., 1993) and for the native hKv1.3 channel in Jurkat cells (Payet & Dupuis, 1992). Protein tyrosine kinase-dependent phosphorylation inhibits the native hKv1.3 channel in Jurkat T cells (Szabo et al., 1996) and the channel expressed in HEK 293 cells (Holmes et al., 1996).

Data presented here show that the full-length hKv1.3 channel expressed in HEK 293 cells was less sensitive to modulation by PKA and PKC. The current of the full-length and truncated channels was inhibited by approximately 30% when the cells were exposed to TPA (Fig. 6*A*). The observations that the current in cells expressing the truncated Δ459-523 was not sensitive to the cell-permeant 8Br-cAMP analogue (Fig. 6*B*) supported the notion that the reduced sensitivity of the channels to TPA was not due to the conditions of assay. Our observations suggested that the HEK 293 host cells did not provide the environment required for full modulation of hKv1.3 channel activity. In this connection, Zong et al. (1995) have reported that the L-type Ca^{2+} channel

reconstituted in HEK 293 cells did not display the same extent of sensitivity to modulation by cAMP-dependent phosphorylation as the native channel in myocytes. We have investigated a number of possibilities that could account for the decreased sensitivity of the transfected hKv1.3 channel to regulation by protein kinases.

PCR-introduced Mutations in the Transfected hKv1.3 Channels

The *Taq* polymerase is known to introduce unwanted mutations in the amplified PCR product (Tindall & Kunkel, 1988) and these could have modified the sensitivity of the hKv1.3 channel to regulation. This possibility was rejected on the basis of sequencing results of the cDNA corresponding to the full-length channel that showed identity to the published sequence (Cai & Douglass, 1992). Similarly, the sequence of the Δ459-523 cDNA was as expected.

Covalent Modification of the Transfected hKv1.3 Channel

The Mr of the hKv1.3 channel predicted from molecular cloning experiments is 58 kDa (Cai et al., 1992). However, transfection of the hKv1.3 channel in HEK 293 cells results in the expression of a protein of Mr 58-75 kDa (Holmes et al., 1996) and the Kv1.3 channel immunoprecipitated from Jurkat lymphocytes has a Mr of 65 kDa (Cai & Douglass, 1993). These observations suggest that the channel undergoes post-translational modifications such as phosphorylation and/or glycosylation (Holmes et al., 1996) although modification by glycosylation appears to be unlikely (Cai & Douglass, 1993). In the present study, HEK 293 cells expressed a hKv1.3 protein as a single band of Mr 55-65 kDa (*not shown*).

Ionic channels are subject to a basal level of phosphorylation in the resting state (Busch et al., 1992; Covarrubias et al., 1994; Drain, Dubin & Aldrich, 1994; Levitan, 1994). This has been functionally shown to be the case of the hKv1.3 lymphocyte channel (Payet & Dupuis, 1992; Cai & Douglass, 1993). If one assumes that the hKv1.3 channel transfected in HEK 293 cells is already in a phosphorylated state, it may no longer be susceptible to the action of protein kinases. Such a possibility has been described for the L-type Ca^{2+} channels expressed in frog oocytes where channel activity can be modulated by dephosphorylation but not by cAMP-dependent phosphorylation (Singer-Lahat et al., 1994). However, we feel this possibility does not account for our observations. Transfected cells dialyzed with a medium containing alkaline phosphatase (10 units/ml) did not show changes in the amplitude of the current. One additional possibility was that HEK 293 cells displayed a high level of protein phosphatase activity that would largely maintain the population of hKv1.3 channels in an unphosphorylated state and rapidly inhibit the effect of protein kinase modification of the channel. This possibility was found unlikely by dialyzing the cells with the protein phosphatase inhibitors microcystin (Honkanen et al., 1990) or by adding okadaic acid (Ozaki et al., 1987) to the bath. These experimental protocols did not change the sensitivity of the channel to TPA or 8Br-cAMP.

Expression of PKC in HEK 293 Cells

The decreased sensitivity of the transfected hKv1.3 channel to inhibition by PKC could be due to a reduced level of expression of PKC isozymes in HEK 293 cells. This possibility was found to be unlikely based on the results of Western blot experiments which revealed that HEK 293 cells expressed the same PKC isoforms that are present in Jurkat cells (Tsutsumi et al., 1993). Furthermore, total PKC activity in HEK 293 cells was not different than in Jurkat cells.

Resistance of the Transfected hKv1.3 Channel to Phosphorylation

The possibility exists that the hKv1.3 channel expressed in HEK 293 cells adopts a conformation that, without grossly affecting its electrical properties, may result in a decreased accessibility to PKA and to PKC. We investigated this possibility by performing a series of experiments in the inside-out configuration and measuring the probability of opening of the channel. The addition of exogenous PKC triggered a reduction of the probability of opening that was reversible by the addition of staurosporine and alkaline phosphatase (Fig. 6*B*). Similarly, the probability of channel opening was reduced by treatment of the excized patches with the catalytic subunit of PKA (Fig. 6*C*). These results were similar to those obtained here in the whole cell configuration, suggesting that endogenous PKA and PKC were not defective in the HEK 293 cell host and arguing in favor of the interpretation that the HEK 293 host cells did not provide the environment for full regulation of the hKv1.3 channel by PKA and PKC.

Unidentified Cellular Components Essential for Regulation of the hKv1.3 Channel

Cai and Douglass (1993) have reported that the lymphocyte hKv1.3 channel co-immunoprecipitates with a 40 kDa protein than can exist in a phosphorylated form. This observation suggests the possibility that the native hKv1.3 channel is associated with specific protein factor(s) that attunes the channel to regulation by phosphorylation. If this were the case, modulation of the expressed channel may not be observed due to the absence

of the required factor(s) or the overexpression of the channel may result in depletion of some regulatory factor(s). Data presented here do not allow us to distinguish between these possibilities. However, it has been pointed out (Holmes et al., 1996) that the hKv1.3 channel embodies a proline-rich PXXP motif (^{43}RYEPLPPSLP), suggesting the possibility that some SH3 domain-containing protein(s) may associate with the channel and modify its activity.

We investigated the possibility that one of the recently cloned Kvβ subunits which can be phosphorylated by PKA and PKC (England et al., 1995*a,b*) could influence the regulation of the transfected channel. HEK 293 cells do not express Kvβ subunits (Uebele et al., 1996), providing a suitable cell system to investigate the role of these proteins. HEK 293 cells were cotransfected with the full-length hKv1.3 channel and the Kvβ1.2 (Morales et al., 1995) or the Kvβ2 (Shi et al., 1996) subunits. These two Kvβ subunits interact with the Kv1 subfamily of K^+ channels as shown by immunoprecipitation experiments (Nakahira et al., 1996). Results showed that the coexpressed Kvβ1.2 or the Kvβ2 subunit had no effect on the susceptibility of the hKv1.3 channel to PKA or PKC (Fig. 7*B*). However, coexpression of the Kvβ1.2 subunit slightly decreased the time constant of inactivation (Fig. 7*A*), in agreement with its suggested regulatory role (Heinemann et al., 1994; Rettig et al., 1994; England et al., 1995*a,b;* Sasaki et al., 1995; Stephens et al., 1996). The coexpression of the Kvβ2 subunit did not modify the electrical behavior of the channel or its sensitivity to PKA and PKC modulations (*not shown*). Our data do not exclude the possibility that Jurkat cells express a hKv1.3-associated Kvβ subunit. However, we could not demonstrate the presence of Kvβ-positive PCR products using the *Touchdown PCR* methodology successfully used by Morales et al. (1995). These negative results did not appear to be due to our methodological approach since a positive signal of 4.2 kb (Rettig et al., 1995) was obtained in an extract of rat brain. We cannot formerly exclude the possibility that Jurkat cells express a Kvβ subunit, especially in light of the recent results of Autieri et al. (1997) who have reported that Kvβ1.1 and Kvβ2.1 subunits are inducible in a cloned murine T-helper cell line in response to interleukin-2.

Final Comments

Our data showed that the hKv1.3 lymphocyte channel expressed in the HEK 293 host cell line largely displayed electrical properties that were similar to those found in T cells. However, the expressed channel was only partially sensitive to regulation by protein kinases as previously reported in the case of the L-type cardiac Ca^{2+} channel (Zong et al., 1995). These observations could be a common feature of ionic channels expressed in heterologous cells, raising the intriguing possibility of the requirement of specific protein factor(s) and/or cellular environment which may not be provided by some host cell lines.

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