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Pharmacological Characterization of Five Cloned Voltage-Gated K^+ Channels, Types Kv1.1, 1.2, 1.3, 1.5, and 3.1, Stably Expressed in Mammalian Cell Lines

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SUMMARY

We have analyzed the biophysical and pharmacological properties of five cloned K^+ (Kv) channels (Kv1.1, Kv1.2, Kv1.3, Kv1.5, and Kv3.1) stably expressed in mammalian cell lines. Kv1.1 is biophysically similar to a K^+ channel in C6 glioma cells and astrocytes, Kv1.3 and Kv3.1 have electrophysiological properties identical to those of the types *n* and *l* K^+ channels in T cells, respectively, and Kv1.5 closely resembles a rapidly activating delayed rectifier in the heart. Each of these native channels may be formed from the homomultimeric association of the corresponding Kv subunits, and pharmacological compounds that selectively modulate them may be useful for the treatment of neurological, immune, and cardiac disorders. The cell lines described in this report could be used to identify such drugs and we have therefore embarked on a pharmacological characterization of the five cloned channels. The compounds tested in this study include 4-aminopyridine, capsaicin, charybdotoxin, cromakalim, dendrotoxin, diltiazem, D-sotalol, flecainide, kaliotoxin, mast cell degranulating peptide, nifedipine, noxiustoxin, resiniferatoxin, and tetraethylammonium.

Drugs that alter ion flux through K^+ channels are used in the treatment of hypertension, cardiac arrhythmias, type II diabetes mellitus, and male-pattern baldness (1), and new applications are being investigated. The recent isolation of at least 24 genes encoding distinct types of mammalian K^+ channels, including those belonging to the voltage-gated, calcium-activated, and inward rectifier K^+ channel families, has for the first time allowed a rational approach to the design of specific channel-modulating drugs.

The *Xenopus* oocyte expression system has been successfully used to analyze the electrophysiological properties of cloned K^+ channels and to delineate the structural domains underlying fast or N-type inactivation, toxin binding, and ion permeation (for review see Ref. 2). However, electrophysiological and pharmacological measurements made by the two-electrode recording technique in whole oocytes can be quantitatively different from those made with the patch-clamp technique in native mammalian cells or in isolated patches from oocytes. In particular, channels studied in whole oocytes are less sensitive to block by certain pharmacological agents and inactivate more slowly than channels examined in isolated patches. The presence beneath the vitelline membrane of a huge reservoir of yolk with the potential to interact with lipophilic pharmacological compounds may contribute to the reduced potency of channel blockers. In contrast, cloned Kv channels stably expressed in mammalian cell lines (for review, see Ref. 3) and studied by the patch-clamp method are likely to have biophysical properties more closely resembling those of native channels. Another advantage of such mammalian expression systems is that large numbers of the transfected cells can be generated by standard tissue culture techniques, which would facilitate their use in high-throughput drug-screening assays.

Here we make a detailed comparison of the pharmacological and electrical properties of five cloned homomultimeric K^+

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ABBREVIATIONS: Kv channels, voltage-gated K^+ channels; 4-AP, 4-aminopyridine; CTX, charybdotoxin; DTX, dendrotoxin; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; HEPES, *N*-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid; KTX, kaliotoxin; MCDP, mast cell degranulating peptide; NTX, noxiustoxin; TEA, tetraethylammonium; MEL, mouse erythroleukemia; mKv, mouse Kv; hKv, human Kv; rKv, rat Kv; kb, kilobase(s).

channels (mKv1.1, rKv1.2, mKv1.3, hKv1.5, mKv3.1), each stably expressed in a mammalian cell line, with those of native mammalian channels. Such an analysis sets the stage for future mutational experiments to delineate molecular structures that correlate with drug block, and provides reagents for high-throughput screens to identify potential therapeutic agents that selectively modulate each of these channels.

Materials and Methods

Construction of the expression plasmids. A 2.0-kb *Bam*HI/*Bgl*II fragment containing the entire coding sequence of the mKv1.3 gene was removed from the plasmid pMK3T (4) and ligated into the *Bam*HI site of pcDNA/Neo (Invitrogen). After transfection of *Escherichia coli* MC1061/PS cells, the resultant transformants were screened for orientation of the insert by restriction enzyme analysis. One clone, pcDNA1/Neo/mKv1.3T, containing the K^+ channel sequences in the correct orientation with respect to the human cytomegalovirus immediate-early promoter, was digested with *Xmn*I and *Bam*HI to remove unwanted 3′ sequences; the carboxyl-terminal portion of the K^+ channel was repaired by ligation to the following oligonucleotides: 5′-TATTCACTGATGTCTAATATATG-3′ and 5′-GATCCATATATTAGACATCAGTGAATA-3′. Transformants from this ligation were screened by restriction enzyme analysis; one clone, pcDNA1/Neo/mKv1.3T3.4, was confirmed to be correct by dideoxy nucleotide sequence analysis.

A 1.8-kb fragment containing the mKv3.1b sequence (one of the two alternatively spliced variants of this gene) was excised from the pBSKS plus Kv3.1b plasmid (5) by digestion with *Xho*I and *Xba*I and was directionally ligated into the same restriction enzyme sites of pcDNA1/Neo. The products of the ligation reaction were transfected as described above and the resultant colonies were screened by restriction enzyme analysis for sequence content and orientation. One clone demonstrating the appropriate restriction enzyme pattern, pcDNA1/Neo/Kv3.1b-3, was sequenced and shown to be correct.

A 1.8-kb fragment encoding the mKv1.1 gene was isolated from pBSTA Kv1.1 (6) by digestion with *Bgl*II and was ligated into the *Bam*HI site of pcDNA1/Neo. One clone, pcDNA1/Neo/Kv1.1–1, containing this gene in the correct orientation was isolated and sequenced.

Generation of cell lines stably expressing mKv1.1, mKv1.3, and mKv3.1. NIH/3T3 and L929 cells were used because of the apparent absence of Kv channels endogeneously expressed in these cells. The cells were grown to 40–50% confluence in 60-mm dishes and transfected with 5 μg of CsCl-banded DNA using the Lipofectin reagent, as described by the supplier (Bethesda Research Laboratories, Bethesda, MD). After transfection, the cells were incubated for 3 days in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 units/ml penicillin-G sodium, and 100 μg/ml streptomycin sulfate. The cells were trypsinized, seeded into 100-mm dishes, and then selected with 300 μg/ml G418. Isolated clones were then recovered and subjected to patch-clamp analysis. Cell clones demonstrating functional K^+ channels were used for further analysis.

Cell lines stably expressing rKv1.2 and hKv1.5. B82 mouse fibroblasts stably transfected with rKv1.2 (NGK1) were obtained from Dr. Higashida, Neuroinformation Research Institute, Kanazawa University School of Medicine (Kanazawa, Japan). MEL cells stably transfected with hKv1.5 (HPCN1) were obtained from Dr. E. Conley, Department of Biochemistry, University of Leicester (Leicester, UK).

Solutions. Cells were bathed in normal Ringer solution containing (in mM) 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, and 5 HEPES, adjusted to pH 7.4 with NaOH (290–320 mOsM). For whole-cell recording, the internal (pipette) solution contained (in mM) 134 KF, 2 $MgCl_2$, 10 HEPES, 1 $CaCl_2$, and 10 EGTA, adjusted to pH 7.2 with KOH (290–320 mOsM). A simple syringe-driven perfusion system was used to exchange the bath solution. 4-AP was purchased from Aldrich Chemical Co. (Milwaukee, WI). Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), cromakalim [(±)-(*trans*)-6-cyano-3,4-dihydro-2,2-dimethyl-4-(2-oxopyrrolidin-1-yl)-2*H*-1-benzopyran-3-ol], diltiazem, MCDP, nifedipine, and resiniferatoxin were purchased from Sigma Chemical Co. (St. Louis, MO). CTX was purchased from Peptides International (Louisville, KY) and DTX and KTX from Latoxan (distributed by Accurate Chemical and Scientific Corp., Westbury, NY). NTX was a generous gift from Dr. Robert Slaughter (Merck Institute, Rahway, NJ). TEA was purchased from Eastman Kodak Co. (Rochester, NY). D-Sotalol and flecainide were kindly provided by Pfizer Ltd. (Sandwich, UK).

Electrophysiology. Experiments were carried out in the whole-cell configuration of the patch-clamp technique (7). All membrane currents were recorded at room temperature (22–26°) with a List EPC-7 patch-clamp amplifier (Adam and List Associates, Great Neck, NY), with 80% series-resistance compensation if the current exceeded 2 nA. Capacitative currents were removed by analog subtraction and leak currents were subtracted using the *P*/8 procedure. The command input of the patch-clamp amplifier was controlled by a PDP 11/73 computer via a digital-to-analog converter (Indec Systems, Sunnyvale, CA). The holding potential in all experiments was −80 mV. For drug screening, the voltage was usually stepped from −80 to 40 mV for 200 msec every 30 sec, before, during, and after drug application.

Results and Discussion

Biophysical Characterization of the Cell Lines Expressing mKv1.1, rKv1.2, mKv1.3, hKv1.5, and mKv3.1

Activation and inactivation. K^+ currents were recorded from cell lines expressing each Kv gene (Fig. 1). Currents were elicited with 200-msec depolarizing voltage steps from −50 to +50 mV in 10-mV increments (Fig. 1A). Upon depolarization, all channels opened with a sigmoidal time course, reached a peak within a few tenths of a millisecond, and then slowly inactivated; the only exception was rKv1.2, which showed a much slower time course of activation.

The voltage dependence of each ion channel type was determined by calculating normalized peak conductance values from the peak current amplitudes at different potentials and fitting a Boltzmann function to the data (Fig. 2A). The fit gives a value for $V_{½}$, the voltage at which half of the channels are activated, and k, a value for the steepness of the voltage dependence (Table 1). The $V_{½}$ values for mKv1.1 and mKv1.3 were about −30 mV, whereas hKv1.5, rKv1.2, and mKv3.1 activated at more depolarized potentials, with $V_{½}$ values of −14, 27, and 16 mV, respectively.

Deactivation. Deactivation rates (channel closing upon repolarization) were determined by first opening the channels with a 15-msec conditioning pulse to 40 mV and then forcing the channels to close by repolarization to different potentials. (Fig. 1B). The closing rate of these K^+ channels usually increased at more negative potentials and was quantified by fitting single-exponential functions to the decay of the K^+ current during repolarization. This procedure yielded tail-current time constants at different deactivation potentials (Fig. 2B). Tail-current time constants measured at −60 mV ranged from about 15 to 40 msec for all of the channels studied, except for mKv3.1 channels, which closed about 10 times more rapidly, with a time constant of ~2 msec (Table 1).

Cumulative inactivation. Inactivation of certain K^+ channels accumulates during repetitive depolarizing pulses delivered at 1 Hz because recovery during the interpulse interval is incomplete. This property, visualized as a reduction in current amplitude with each pulse, is termed cumulative or "use-dependent" inactivation. Fig. 1C shows that only mKv1.3 exhibited use-dependent inactivation. Interestingly, rKv1.2 currents

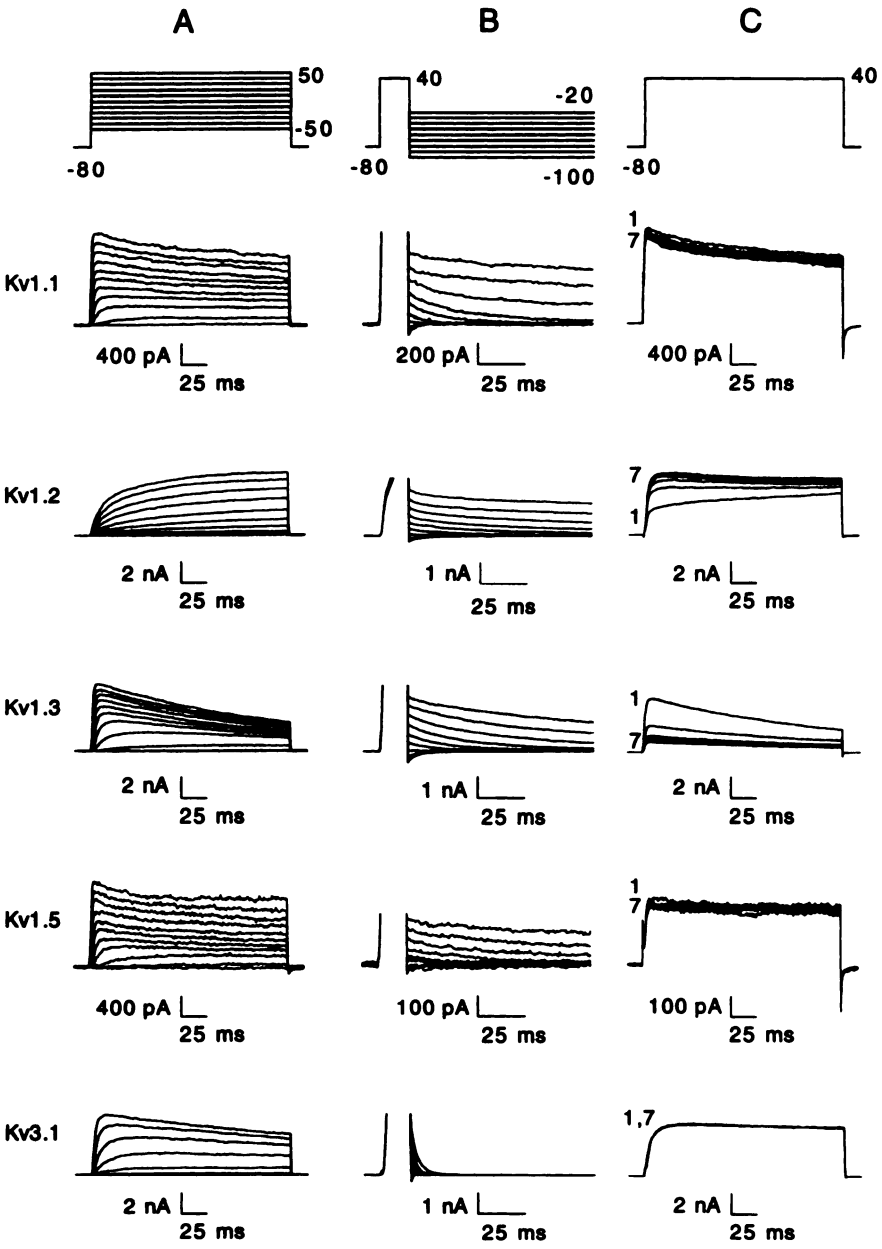

Fig. 1. Original K^+ currents of different cell lines stably transfected with Kv1.1, Kv1.2, Kv1.3, Kv1.5, or Kv3.1. A, Families of K^+ currents elicited by voltage steps from −50 to 50 mV in 10-mV increments, applied from a holding potential of −80 mV. B, Families of K^+ tail currents elicited by voltage steps from −100 to −20 mV after a 15-msec depolarizing prepulse to 40 mV, applied from a holding potential of −80 mV. C, Cumulative (use-dependent) inactivation of K^+ currents. Currents were elicited by a train of seven depolarizing voltage steps to 40 mV once every second, from a holding potential of −80 mV.

became larger with repetitive pulses. In chromaffin cells, Artalejo *et al.* (8) described a similar feature for Ca^{2+} current, i.e., repetitive depolarizations increased the whole-cell current amplitude. Those authors concluded that in those cells two types of Ca^{2+} channels exist and only one type, responsible for the "facilitation" current but not the "standard" current, could be induced by repetitive pulses. The strongest argument for this conclusion was that the facilitation current was pharmacologically distinct from the standard current (8). In the case of rKv1.2, the larger current seen after repetitive pulses exhibits the same pharmacological profile as the base-line rKv1.2 current observed with the first pulse. We therefore favor the idea that the increase in current amplitude with repetitive pulses for the cell line expressing rKv1.2 is due to the existence of distinct closed states of the channel. After depolarization the channel must go through those closed states to get to the open state. One or more of the closed states are relatively long lived, compared with the first closed state. In this case a second repolarization might not be long enough to bring the channels back to the original first closed state before the train of pulses. This hypothesis would predict a speeding up of activation, i.e., faster openings of the channels in response to depolarizations, with repetitive pulses. This can actually be seen in Fig. 1C.

The relationship between the normalized peak current and the depolarizing pulse number is shown in Fig. 2C. After a train of repetitive pulses, the normalized peak current rapidly diminished for mKv1.3, stayed the same for mKv1.1, hKv1.5, and mKv3.1, and increased (by a factor of 1.5) for rKv1.2 (Fig. 2C).

Relating Cloned Channels to Native Channels Present in Mammalian Cells

To compare cloned channels with native channels in mammalian cells, it is important to keep in mind that heteromultimeric channels might be able to form within a cell and the resulting channels could have dramatically different channel functions, compared with both parent channels. The finding that functional properties of a cloned channel are identical to

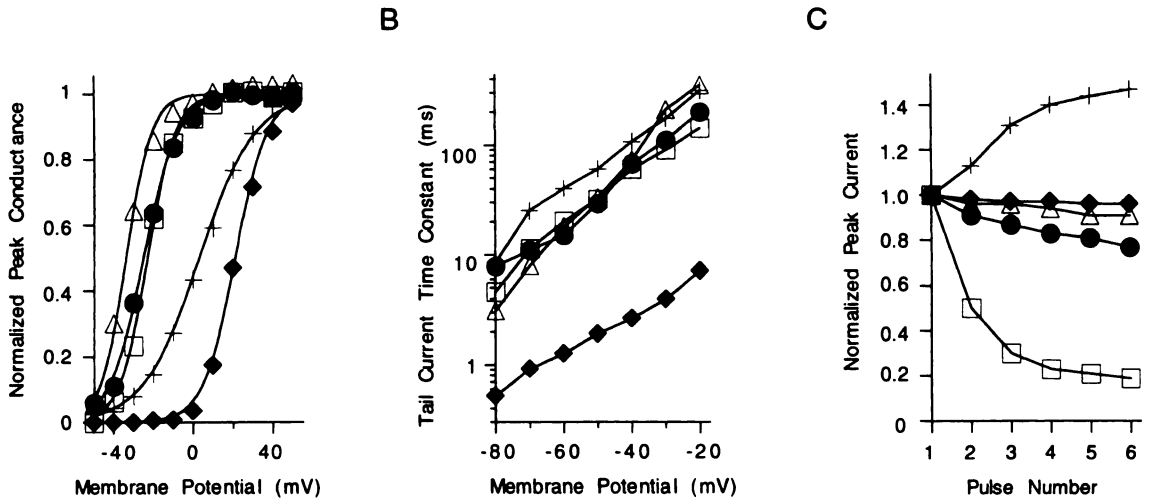

Fig. 2. Comparison of voltage dependence, deactivation kinetics, and time course of use-dependent inactivation in cell lines stably transfected with Kv1.1 (△), Kv1.2 (+), Kv1.3 (□), Kv1.5 (●), or Kv3.1 (♦). A, Normalized peak K^+ conductance-voltage relation for the K^+ currents shown in Fig. 1A. The *lines* through the data points represent a least-squares fit of a Boltzmann function. The values for the fit are summarized in Table 1. B, K^+ tail current decay time constants, τ_t, fitted with single-exponential functions from the current records shown in Fig. 1B and plotted versus the applied membrane potential during the decay. C, Cumulative (use-dependent) inactivation of K^+ currents during repetitive depolarizations. Peak K^+ currents during the train of the depolarizing voltage steps shown in Fig. 1C are plotted.

TABLE 1

Biophysical properties of K^+ currents in different cell lines stably transfected with Kv1.1, Kv1.2, Kv1.3, Kv1.5, Kv3.1

Values are given as mean ± standard error of the number of cells in parentheses.

	mKv1.1 (L929)	rKv1.2 (B82)	mKv1.3 (L929)	hKv1.5 (MEL)	mKv3.1b (L929)
Activation					
$V_{1/2}$ (mV)	−32 ± 2 (2)	27 ± 6 (8)	−26 ± 8 (3)	−14 ± 3 (6)	16 ± 1 (3)
k	8.5 ± 0.5 (2)	13 ± 2 (8)	7 ± 1 (3)	12 ± 1 (6)	8.7 ± 0.4 (3)
Deactivation, τ_t at −60 mV (msec)	14 ± 5 (2)	23 ± 7 (5)	39 ± 6 (3)	23 ± 4 (6)	1.4 ± 0.2 (3)
Cumulative inactivation	No	No[a]	Yes	No	No
Single-channel conductance (pS)[b]	10	18	14	8	27
Current amplitude (at +40 mV) (pA)	1108 ± 376 (11)	3377 ± 920 (10)	3657 ± 1207 (10)	1211 ± 288 (10)	3836 ± 1116 (10)
Cell capacitance (pF)	22 ± 3 (11)	17 ± 3 (10)	15 ± 3 (10)	11 ± 2 (10)	16 ± 2 (10)
Channels/cell	923 ± 313 (11)	1563 ± 426 (10)	2177 ± 219 (10)	1261 ± 300 (10)	1184 ± 344 (10)

[a] Current amplitude increased with repetitive pulses.
[b] Determined by the slope of the ramp current during channel openings in outside-out recording mode, ramping the voltage from −80 mV to 80 mV within 450 msec.

those of a native channel might therefore indicate the formation of homomultimers and justify the use of the cloned channels to characterize or modulate channel function, with the goal of modulating the native channel and thereby modulating cell behavior. Table 1 summarizes the biophysical properties of the K^+ currents of the five cell lines stably expressing mKv1.1, rKv1.2, mKv1.3, hKv1.5, and mKv3.1. The activation, inactivation, and deactivation kinetics and single-channel conductance of the mKv1.1 channel closely resembled those of a K^+ current present in C6 glioma cells and in type 1 and type 2 astrocytes (9) (Table 1). Because Kv1.1 mRNA is detected in C6 glioma cells (9), it is likely that this particular glioma K^+ channel is formed from the homomultimeric association of Kv1.1 subunits. The mKv1.3 and mKv3.1 channels were biophysically indistinguishable from the types *n* and *l* K^+ channels in lymphocytes (for review, see Ref. 10) (Table 1), and their transcripts are expressed in these cells (4, 5, 11–13), indicating that these channels are homomultimers of Kv1.3 and Kv3.1, respectively. Similar considerations suggest that the hKv1.5 channel encodes a rapidly activating delayed rectifier in the heart (14) (Table 1).

Expression Levels of the Five Cloned K^+ Channels in the Different Cell Lines

Each cell line expressed an average of about 1000 channels/cell, as can be seen in Table 1. The actual current amplitude, measured as the peak current amplitude elicited by depolarizing pulses from a holding potential of −80 to 40 mV, varied from ~1100 pA for Kv1.1 to ~3800 pA for Kv3.1. Taking into account the different single-channel current amplitudes listed in Table 1, the expression levels in the different cell lines varied from ~900 channels/cell for the Kv1.1-expressing cell line to ~2200 channels/cell for the Kv1.3-expressing cell line, indicating no major difference in the expression levels of the different cloned K^+ channels.

Pharmacological Characterization of the Five Cloned K^+ Channels

Because tetrameric associations of each of the Kv subunits could form native channels in neuronal, cardiac, or immune cells, drugs directed against the channels may have therapeutic value for disorders affecting these systems. Cell lines, each stably expressing a particular Kv gene, can be used to screen

compounds for blockade of these K^+ channels. In this study, we have examined the effects of several known pharmacological agents. Fig. 3 shows the effect of CTX, DTX, nifedipine, and resiniferatoxin on each of the five cloned K^+ channels. The K^+ currents were elicited by depolarizing the membrane from −80 to 40 mV for 200 msec; the pulses were repeated every 30 sec, before and after application of the toxins or drugs. Our pharmacological profile of the various K^+ channels stably expressed in different cell lines confirms and extends the initial characterization by Stühmer *et al.* (15) of K^+ channels expressed in *Xenopus* oocytes.

"Classical" K^+ channel blockers 4-AP and external TEA. The K^+ channel blockers 4-AP and TEA blocked all five Kv channels (Table 2). The half-blocking concentrations for 4-AP ranged between ~200 and ~600 μM for all clones tested except mKv3.1, which was ~10 times more sensitive to block by 4-AP, with a half-blocking concentration of ~30 μM. This result with the cell line expressing mKv3.1b is different from our earlier studies performed with oocyte patches (5), which yielded a K_d value of 4-AP for mKv3.1b of 180 μM. In addition, in the experiments by Stühmer *et al.* (15) with whole oocytes, ~1 mM 4-AP produced 50% reduction of current through rKv1.1 (RCK1), rKv1.2 (RCK5), and rKv1.3 (RCK3), whereas in our experiments 4-AP seemed to be more potent (compare Table II of Ref. 15 with Table 2 of this paper). In the case of hKv1.5 we found that the half-blocking 4-AP dose was 290 μM; in contrast, similar studies performed with oocytes yielded a K_d of ~50 μM (14). The reasons for these discrepancies remain unclear. The block by 4-AP of all of the different K^+ channels expressed in the cell lines seemed to follow a general rule; 4-AP increased the apparent rate of inactivation, suggesting open channel block. In addition, 4-AP seemed to be trapped in the closed channel (see Ref. 16).

External TEA interacts with a tyrosine at the carboxyl-terminal end of the P-region (17). As predicted, the two Kv channels with tyrosines at this position, mKv1.1 and mKv3.1, were half-blocked by ~0.3 mM external TEA. Surprisingly, Ramaswami *et al.* (18) reported that hKv1.1 was only moderately sensitive to external TEA (K_d of 20 mM), unlike its mouse and rat homologues. Current through mKv1.3, which has a histidine in the homologous position, was half-blocked by 10 mM external TEA both in L929 and A4 fibroblasts and in

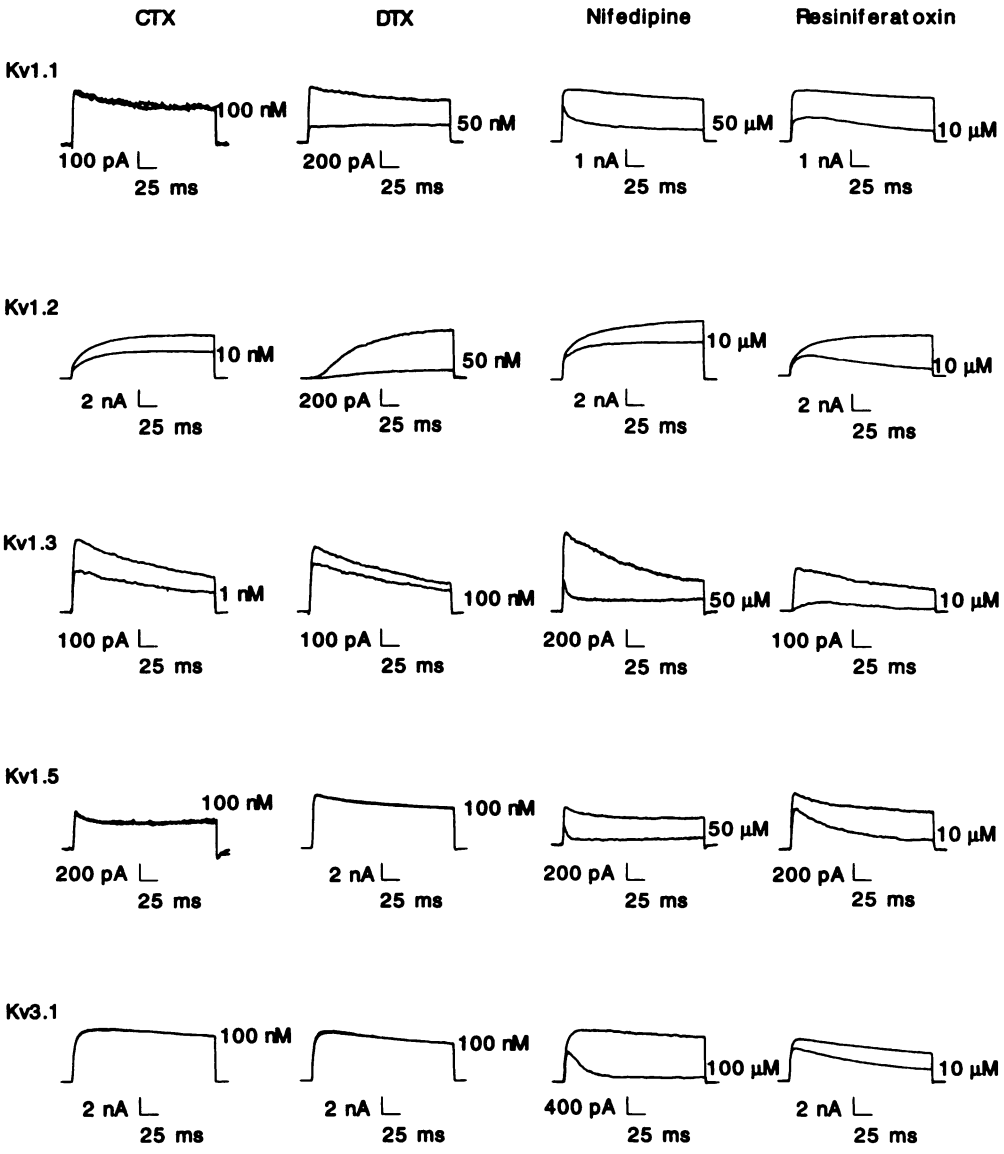

Fig. 3. Pharmacological characterization of K^+ currents of different cell lines stably transfected with Kv1.1, Kv1.2, Kv1.3, Kv1.5, or Kv3.1. Currents were elicited by depolarization of the membrane from −80 to 40 mV for 200 msec every 30 sec, before and during the application of CTX, DTX, nifedipine, and resiniferatoxin.

TABLE 2

Pharmacological characterization of K^+ currents in different cell lines stably transfected with Kv1.1, Kv1.2, Kv1.3, Kv1.5, or Kv3.1

Each drug was tested with at least two different concentrations. Values for the apparent dissociation constant, K_d of blocking peak K^+ currents were calculated by fitting a modified Hill equation to the data points (not shown), where p is proportional to $K_d/(K_d + [drug])$; p is the ratio of the peak K^+ current amplitude in the presence and the absence of the drug.

	K_d				
	Kv1.1	Kv1.2	Kv1.3	Kv1.5	Kv3.1
4-AP	290 μM	590 μM	195 μM	270 μM	29 μM[a]
Capsaicin	29 μM	45 μM	26 μM	23 μM	158 μM
Resiniferatoxin	9 μM	31 μM[a]	3 μM[a]	26 μM[a]	46 μM
D-Sotalol	>1 mM	>1 mM	>1 mM	>1 mM	>1 mM
Flecainide	209 μM	217 μM	53 μM	101 μM	108 μM
Nifedipine	96 μM	18 μM	5 μM	81 μM	131 μM
Diltiazem	144 μM	187 μM	27 μM	115 μM	97 μM
Cromakalim	0.7 mM	>2 mM	>3 mM	>1 mM	0.237 mM
MCDP	0.49 μM	0.44 μM	>2 μM	>10 μM	>2 μM
DTX	20 nM[a]	17 nM	250 nM	>1 μM	>1 μM
CTX	>1 μM	14 nM	2.6 nM	>100 nM	>1 μM
NTX	>25 nM	2 nM	1 nM	>25 nM	>25 nM
KTX	41 nM	>1 μM	0.65 nM	>1 μM	>1 μM
TEA	0.3 mM	560 nM	10 mM	330 mM	0.2 mM

[a] Drug effect not reversible.

isolated *Xenopus* oocyte patches (Ref. 4 and this paper). Stühmer *et al.* (15), however, using the *Xenopus* oocyte two-electrode voltage-clamp method, reported that 50 mM external TEA was required to produce the equivalent block of rKv1.3. Later, Douglass *et al.* (11) reported a K_d of ~13 mM for rKv1.3 in oocytes, akin to our results. The reason for these inconsistencies is not clear. The histidine in Kv1.3 is involved not only in TEA binding but also in slow C-type inactivation; external TEA slows down the apparent rate of inactivation of Kv1.3, suggesting that a channel blocked by TEA cannot inactivate (19), and the titration of the histidine appears to change the rate of inactivation (20). The two external TEA-resistant channels, rKv1.2 and hKv1.5, have a valine and an arginine, respectively, in place of the tyrosine. Our data for rKv1.2, mKv1.3, hKv1.5, and mKv3.1 are consistent with previous reports (4, 5, 15, 21).

CTX. CTX, a peptide component of scorpion *Leiurus quinquestriatus* venom, originally was reported to specifically block current through Ca^{2+}-activated K^+ channels in skeletal muscle (22). In recent years, however, it has become clear that this toxin can also block voltage-gated K^+ channels (10, 15). Fig. 3 and Table 2 demonstrate that CTX blocked current through rKv1.2 and mKv1.3 channels, but it had no effect on currents through mKv1.1, hKv1.5, and mKv3.1b channels. Although the CTX effects we observed with rKv1.2 and mKv1.3 channels agree with published results (4, 23), the CTX resistance of mKv1.1 is at odds with experiments by Stühmer *et al.* (15), who reported that 22 nM CTX inhibited 50% of peak current through rKv1.1 (RCK1). Differences in the purity of CTX might explain this discrepancy. We used recombinant CTX, whereas Stühmer *et al.* (15) used a preparation containing a contaminant that elutes at a higher salt concentration than does CTX in ion exchange chromatography (24, 25). This contaminant blocks the *Shaker* K^+ channel (24, 25) and is likely to be the material that also blocks Kv1.1.

Replacing a phenylalanine at the mouth of the CTX-sensitive *Shaker* channel with the smaller glycine (~60 Å^3) made the channel sensitive to recombinant CTX (26). This suggests that the *Shaker* channel is resistant to CTX because of a narrow entry to its outer vestibule, rather than a lack of CTX binding sites (26). Other *Shaker*-related K^+ channels we investigated appear to share this feature. Kv1.1 and Kv1.5 have a phenylalanine (~190 Å^3) at this position and were resistant to block by CTX, whereas Kv1.3 has a glycine (~60 Å^3) at this position and was very sensitive to block by CTX. Kv1.2 has the intermediate-sized glutamine (~144 Å^3) at this position, which appears to allow CTX to reach its binding site but hinders its access significantly, causing a weaker CTX block than that of Kv1.3.

NTX. NTX is a 39-amino acid polypeptide isolated from the Mexican scorpion *Centruroides noxius*. The toxin shares significant sequence identity with CTX, especially at the carboxyl terminus (27). NTX, like CTX, blocks Ca^{2+}-activated K^+ channels from skeletal muscle incorporated into lipid bilayers (27). In addition, NTX, unlike CTX, can block voltage-dependent K^+ channels in squid axons (28). NTX has not yet been tested on cloned voltage-gated K^+ channels, with the exception of Kv1.3 (10). Our results presented here for the cell lines expressing the different K^+ channel gene products support and extend our earlier results showing Kv1.3 to be highly NTX sensitive (K_d of ~2 nM). The only other K^+ channel that was highly sensitive to NTX was Kv1.2 (K_d of ~2 nM).

KTX. KTX is a peptide isolated from the venom of the scorpion *Androctonus mauretanicus mauretanicus* (29). This toxin shares 44% sequence identity with CTX and 52% sequence identity with NTX (29). Originally, it was described as a "specific" blocker of the Ca^{2+}-activated K^+ current in *Helix pomatia* nerve cells, because it did not appear to block voltage-gated K^+ currents or L-type Ca^{2+} currents in the same preparation (29). Later it was found, however, that KTX could compete for the binding of radiolabeled DTX to rat brain synaptosomal membranes (30). In accordance with these later findings are our results on the block of current through voltage-gated K^+ channels by KTX. The half-blocking concentration of KTX for Kv1.1 was ~40 nM. Kv1.3 was almost 2 orders of magnitude more sensitive to block by KTX than was Kv1.1, with a half-blocking concentration of ~0.65 nM. The other three K^+ channels tested (Kv1.2, Kv1.5, and Kv3.1) were not sensitive to block by KTX.

DTX. DTX, a peptide component of venom from the snake

Dendroaspis angusticeps, blocked current through mKv1.1 and rKv1.2 channels with high affinity (half-blocking concentration of ~20 nM) and through mKv1.3 channels with lower affinity (half-blocking concentration of ~250 nM) but had no effect on current through hKv1.5 and mKv3.1b at 100 nM (Fig. 3; Table 2). These concentrations are in rough agreement with values reported by others (15, 23, 31). The high affinity block of rKv1.2 supports a recent report that the DTX-binding protein in bovine brain is Kv1.2 (32).

MCDP. MCDP, a 22-amino acid peptide isolated from the honey bee (*Apis mellifera*), affects both fast-inactivating (A-type) and slow-inactivating (delayed rectifier) K^+ channels (15). Both mKv1.1 and rKv1.2 were half-blocked by ~450 nM MCDP; three Kv channels (mKv1.3, hKv1.5, and mKv3.1) were resistant. Our K_d values for mKv1.1 are about 10-fold lower than those of Stühmer *et al.* (15). Again, the discrepancy might be attributed to differences in the purity of MCDP. Our sample of MCDP was purchased from Sigma Chemcial Co. (St. Louis, MO), whereas the MCDP used by Stühmer and colleagues was received from Drs. F. Dreyer and E. Habermann (Universität Giessen, Germany).

Classical calcium channel blockers nifedipine and diltiazem. Nifedipine is used for the treatment of cardiac disorders and blocks voltage-gated calcium channels at nanomolar concentrations. We found that nifedipine also blocks types *n* and *l* voltage-gated K^+ channels in lymphocytes, but only at micromolar levels (10). In Fig. 3 and Table 2, we show that K^+ current through all five Kv channels was half-inhibited by nifedipine at concentrations ranging from 5 μM (for mKv1.3) to 131 μM (for mKv3.1b). Because of the complex nature of the block, i.e., changes in kinetics and use-dependent drug action similar to the action of 4-AP (see above), the half-blocking dose depends on the method of measurement; therefore, quantitative comparisons of the five Kv channels may be somewhat arbitrary. Diltiazem also inhibited all five Kv channels at micromolar concentrations.

Antiarrhythmic drugs flecainide and D-sotalol. Flecainide is a class Ic antiarrhythmic drug that blocks delayed rectifiers but not rapidly inactivating I_{to} K^+ channels in the heart (33, 34). Because transcripts of Kv1.1, Kv1.2, and Kv1.5 have been detected in the heart and Kv1.5 most likely encodes a rapidly activating cardiac delayed rectifier (14) (Table 2 and discussion above), we determined whether any of these channels were the potential targets for flecainide. All five channels were blocked, with half-blocking concentrations ranging from 53 μM (for mKv1.3) to 217 μM (for rKv1.2). These concentrations are significantly higher than the therapeutic dose, indicating that, in the heart, homomultimers of Kv1.1-Kv1.3, Kv1.5, or Kv3.1 are probably not the therapeutic targets of flecainide.

D-Sotalol, a class III antiarrhythmic drug, blocks delayed rectifier and I_{to} K^+ channels in the heart (33, 34). All five Kv channels were resistant to D-sotalol, suggesting that these channels are not the targets for this drug (Table 2). Yamagishi *et al.* (21) also reported that D-sotalol had little effect on rKv1.2 and rKv1.4 currents. Collectively, these results suggest that the target is not composed of homomultimers of any of the five Kv channels tested. Yamagishi *et al.* (21) suggested that associated structural proteins might, however, modify the tetramers so that the channels become susceptible to inhibition by D-sotalol. Those structural proteins could be, for example, a β subunit similar to those described for voltage-gated Na^+ or Ca^{2+} channels (35). Alternatively, heteromultimers containing one or more of these cloned subunits may be blocked by D-sotalol at therapeutic doses.

Other class III antiarrhythmic drugs have been reported to block Kv channels at concentrations higher than the therapeutic dose; tedisamil blocks mKv1.1 at 100 μM (36) and clofilium blocks hKv1.3 at 60 μM (13) and hKv1.5 at 50 μM (14).

Capsaicin. Derived from capsicum fruit, capsaicin is frequently used to define nociceptive sensory neurons (37). Its use provides a good experimental model for deep hyperalgesia (38). Capsaicin blocks voltage-gated K^+ currents in rabbit Schwann cells (39), dorsal root ganglion cells (37), T cells (type *l* K^+ currents) (40), and vertebrate axons (41). In Schwann cells, the block is voltage dependent, becoming maximal with prepulse potentials more positive than −30 mV (39). Capsaicin also induces inactivation of these channels. In our experiments, all five Kv channels were blocked by capsaicin, with half-blocking concentrations varying from 23 μM (for hKv1.5) to 158 μM (for mKv3.1b).

Resiniferatoxin. Resiniferatoxin, a diterpene ester isolated from the plant *Euphorbia poisonii* and an ultrapotent analogue of capsaicin, binds with high affinity to rat dorsal root ganglion and spinal cord (42, 43). We tested resiniferatoxin on the five Kv channels and found that the toxin blocked all five Kv channels, with half-blocking concentrations ranging from 3 μM (for Kv1.3) to 46 μM (for Kv3.1).

Cromakalim. This drug activates ATP-sensitive K^+ channels at nanomolar concentrations (44). Our studies show that cromakalim can block mKv3.1 and mKv1.1 channels but only at high concentrations, namely 237 and 700 μM, respectively.

Conclusions

In recent years, potassium channels have emerged as potential targets for therapeutic action in a variety of tissues, including pancreas, heart, and smooth muscle (for review, see Ref. 45). In addition, we and others have shown that blocking of voltage-gated K^+ channels in human T lymphocytes inhibits cell activation, suggesting the potential use of channel blockers as immunosuppressants (for review, see Ref. 10). Regulation of the membrane potential through modulation of K^+ channels is probably the common mechanism that alters cell behavior. K^+ channel-modulating drugs are also used as class III antiarrhythmic agents and as vasodilators (45). Rapid advances in our understanding of the molecular structure of K^+ channels have made it possible to develop highly potent and selective channel blockers as well as openers. The cell lines described in this paper could be used in screening assays to identify novel immunosuppressants (Kv1.3 or Kv3.1), class III antiarrhythmic agents (Kv1.1, Kv1.2, or Kv1.5), or glial cell modulators (Kv1.1) or for the biochemical characterization of channel proteins.

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