The voltage-sensitive currents observed following hKv1.5 α subunit expression in HEK 293 and mouse L-cells differ in the kinetics and voltage dependence of activation and slow inactivation. Molecular cloning, immunopurification, and Western blot analysis demonstrated that an endogenous L-cell Kvβ2.1 subunit assembled with transfected hKv1.5 protein. In contrast, both mRNA and protein analysis failed to detect a β subunit in the HEK 293 cells, suggesting that functional differences observed between these two systems are due to endogenous L-cell Kvβ2.1 expression. In the absence of Kvβ2.1, midpoints for activation and inactivation of hKv1.5 in HEK 293 cells were -0.2 ± 2.0 and -9.6 ± 1.8 mV, respectively. In the presence of Kvβ2.1 these values were -14.1 ± 1.8 and -22.1 ± 3.7 mV, respectively. The β subunit also caused a 1.5-fold increase in the extent of slow inactivation at 50 mV, thus completely reconstituting the L-cell current phenotype in the HEK 293 cells. These results indicate that 1) the Kvβ2.1 subunit can alter Kv1.5 α subunit function, 2) β subunits are not required for α subunit expression, and 3) endogenous β subunits are expressed in heterologous expression systems used to study K+ channel function.

Voltage-gated K+ channels represent a diverse group of membrane proteins in terms of both function and structure. These channels establish the resting membrane potential and modulate the frequency and duration of action potentials in nerve and muscle (1–3). In addition, K+ channels are involved in processes not usually associated with electrically excitable membranes such as T-lymphocyte activation, cell volume regulation, and pancreatic beta cell function (4–7). Multiple membrane proteins in terms of both function and structure. These channels establish the resting membrane potential and modulate the frequency and duration of action potentials in nerve and muscle (1–3). In addition, K+ channels are involved in processes not usually associated with electrically excitable membranes such as T-lymphocyte activation, cell volume regulation, and pancreatic beta cell function (4–7). Multiple most recently Kvβ2.1 has been shown to increase the N-type inactivation of K+1.4 2.3-fold (26). The Kvβ1.1, 1.2, and 2.1 subunits were originally designated β1, 3, and 2, respectively. The Kvβ1.1–1.3 proteins arise by alternative splicing from the same gene, whereas Kvβ2.1 is derived from a distinct gene (24, 26). The nomenclature used here reflects a subfamily classification based on genomic structure as previously proposed (24). An issue that must be resolved with all heterologous expression systems is whether they contain endogenous, function-altering β subunits.

The Kv1.5 delayed rectifier has been cloned from heart, insulinoma tissue, gastrointestinal smooth muscle, and skeletal muscle from rat, mouse, canine, and human species (10, 11, 27–30). The present study was undertaken to determine why the human Kv1.5 channel has different properties when expressed in the HEK 293 system compared with L-cells. Specifically, in L-cells, the voltage sensitivity is shifted 10 mV in the negative direction and slow inactivation is increased. The data presented here indicate that the L-cells express a Kvβ2.1 subunit isoform that assembles with the transfected Kv1.5 α subunit.

The HEK 293 cells lack a β subunit based on both mRNA and protein analysis. Coexpression of the mouse L-cell β subunit with the Kv1.5 channel in the HEK 293 cells reconstitutes the L-cell current phenotype. These results indicate that the functional differences observed between the L-cell and HEK 293 expression systems are due to the presence of an endogenous L-cell β subunit and represent the first description of functional effects of Kvβ2.1 on delayed rectifier function.
Experimental Procedures

Materials—Trans 35S-label ([35S]methionine (1100 Ci/mmol) was purchased from ICN (Irvine, CA). Enzymes and buffers were from New England Biolabs (Beverly, MA), Boehringer Mannheim, and Promega (Madison, WI). Protein A cross-linked to Sepharose 4B-CL and reagent grade chemicals were obtained from Sigma. The Sequenase 2.0 kit was purchased from U. S. Biochemical Corp. The polyacrylamide gel for electrophoresis, including lipofectamine, were obtained from Life Technologies, Inc. K+BAPTA was obtained from Molecular Probes (Eugene, OR). The origins of other materials are specified below.

Northern Blot Analysis—Total RNA was isolated by the guanidinium thiocyanate method (31), and 10 μg was electrophoretically separated on a formaldehyde denaturing agarose gel and transferred overnight to a Nytran-Plus membrane (Schleicher & Schuell) by capillary action. Ethidium bromide (40 μg/ml) was added to the samples prior to electrophoresis, enabling visual confirmation of RNA integrity. The filter for the random primer synthesized, [α-32P]ATP-labeled probe was a polymerase chain reaction-generated rat brain cDNA fragment of Kv1.1 (nucleotides 6 to 1567). The filters were hybridized over-night at 65°C in 10% dextran, 0.1% SDS, 0.1 M NaCl, 40 mM NaH2PO4, 1 mM EDTA, pH 7.4), 5% BFP (1 g/ml bovine serum albumin, 1 g/liter polyvinylpyrrolidone 40, 1 g/liter Ficoll, 0.001% sodium azide), 0.1 mg/ml sonicated salmon sperm DNA, 0.2 mg/ml yeast tRNA, and 5% SDS supplemented with 10cpm/ml of the probe. The filter was then washed three times at 65°C for 30 min with 3× SSC.

L-cell cDNA Library Construction, Screening, and Sequence Analysis—The ZAPII cDNA library was constructed from oligo(dT) purified mouse fibroblast L-cell mRNA using a cDNA synthesis system, as described by the manufacturer. No size fractionation of the cDNA was performed. Polymerase chain reaction-generated cDNA fragments corresponding to nucleotides +435 to +1089 of rat Kv1.2 (rKv1.2) served as template for the random primer-generated radiolabeled probe that was used to screen ~3.5×1010 unamplified recombinants. Two clones, 1.5 and 1.0 kilobases in length, were plaque-purified and recovered by in vivo excision into plBluescript (SK–). Nucleotide sequence was determined from both strands of the full-length 1.5-kilobase cDNA by the dideoxynucleotide chain termination method using double stranded templates and appropriate dideoxynucleotide primers.

Antibody Production—A polymerase chain reaction-generated cDNA fragment corresponding to nucleotides +435 to +1089 of rKv1.2 was subcloned into pGEM7 at the EcoRI site. A DNA fragment corresponding to nucleotides +577 to +1089 (amino acids 193–363) was digested from this construct, subcloned into the Ncol and Xhol sites of the pGEX-2T vector (Pharmacia), and transformed into Escherichia coli strain UT481 as described previously (32). This construct utilized the stop codons of the pGEX-2T vector. The glutathione S-transferase-Kv1.2 fusion protein (rKv1.2) was prepared and immunized into two New Zealand White rabbits as reported (32). The ability of anti-Kv1.2 antiserum to specifically detect Kv1.2 was confirmed by Western blot analysis of in vitro translated full-length rKv1.2 and the Kv1.2 and the conserved COOH-terminal domain of Kv1.2 (amino acids 74–401) (data not shown). Neither of the anti-Kv1.2 antiserum was able to immunopurify in vitro translated or endogenous Kv1.2 subunits or detect Kv1.2 in L-cells or heart cytoskeletons by immunohistochemistry. Polyclonal antibodies directed against the amino-terminal 112 amino acids (anti-Kv1.2 (N-term)) and the first extracellular loop of Kv1.5 (anti-hKv1.5 (S1-S2)) have been described (32).

Metabolic Labeling and Immunopurification of hKv1.5 from Transfected L-cells—Two cell lines of stably transfected L-cells were homogenized with a glass/glass Dounce homogenizer (33), the debris was removed by centrifugation at 1000 × g at 4°C for 15 min, and the membranes were sedimented from the supernatant at 17,000 × g for 1 h. The final pellet was resuspended in 150 μl of sterile phosphate-buffered saline and stored at −80°C. Of this membrane preparation, 70 μl were solubilized in 1 ml of Triton X-100 extraction buffer. The membrane suspension was fractionated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with antibody according to standard protocols as described previously (32, 33). The primary Kv1.2 antiserum was diluted 1:200, whereas the secondary antibody, horse- radish peroxidase-conjugated goat anti-rabbit IgG (Sigma), was diluted 1:10,000. Detection was by the ECL kit from Amersham Corp. according to manufacturer’s directions. Exposure was for 1–10 s.

Electrical Recording—Recordings were made with a DAGON 3900 patch clamp amplifier (Dagan Corp., Minneapolis, MN) using the whole cell configuration of the patch clamp technique. Currents were recorded at room temperature (21–23°C) and were sampled at 1–5 kHz after analog-to-digital conversion at 0.5–2 kS/s. Data acquisition and analysis were controlled by pClamp software (Axon Instruments, Foster City, CA). To ensure voltage clamp quality, electrode resistance was kept below 3 MΩ; the average resistance was 2.4 ± 0.4 MΩ (mean ± S.E., n = 18). Junction potentials were zeroed with the electrode in the standard bath solution. Gigaseal seal formation was achieved by suc- tioning the pipette tip (=1.4 GΩ) to the cell. Establishing the whole cell configuration, the capacitive transients elicited by symmetrical 10-mV voltage clamp steps from −80 mV were recorded at 50 kHz for calculation of cell capacitance, access resistance, and input impedance. The average access resistance was 5.1 ± 0.5 MΩ (n = 18), and after analog compensation the residual access resistance was 1.0 ± 0.3 MΩ. Cells expressing current in excess of 5 nA were discarded.
concentration of ~145 mmol/liter. The bath solution contained in milli/ liter: 130 NaCl, 4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES, and 10 glucose and was adjusted to pH 7.35 with NaOH.

Pulse Protocols and Analysis—The holding potential was ~80 mV unless indicated otherwise, and the cycle time for the protocols was 10 s or slower. The standard protocol to obtain current-voltage relationships and activation curves consisted of 250-ms pulses that were imposed in 10-mV increments between ~80 and 60 mV with additional interpolated pulses to yield 5-mV increments between ~35 and 25 mV (activation range of hKv1.5). The steady-state current-voltage relationships were obtained by measuring the current at the end of the 250-ms depolarizations. Between ~80 and ~40 mV, only passive linear leak was observed; least squares fits to the data were used to obtain the 

The voltage dependence of channel opening and inactivation (activation and inactivation curves) were fitted with a Boltzmann equation \( y = \frac{1}{1 + \exp^{-\frac{E - E_0}{k}}}, \) in which \( k \) represents the slope factor and \( E_0 \) represents the voltage at which 50% of the channels are open or inactivated, respectively. Because inactivation was incomplete, data were normalized after subtraction of the noninactivating fraction at the test potential. The time course of tail currents and slow inactivation were fitted with a sum of exponentials. Activation kinetics were fitted with a single exponential to the latter 50% of activation to obtain the dominant 

The results are expressed as the means ± S.E. Analysis of variance with appropriate post hoc comparisons were used to compare the differences in mean values; \( p < 0.05 \) was considered significant. Specific values are presented in the text or figure legends. All pooled data were collected from at least three separate transfections.

**RESULTS AND DISCUSSION**

hKv1.5 Displays Expression System-dependent Characteristics—When expressed in mouse L-cells or CHO cells, hKv1.5 has a midpoint of activation of ~19 ± 3.0 to ~14 ± 4 mV and an activation time constant of 3.4 ± 0.6 ms at 0 mV (12, 14). Expression in HEK 293 cells differs in 0.2 ± 2.0 mV for the activation midpoint and 11.8 ± 4.6 to 16.5 ± 1.2 ms for the activation time constant at 0 mV (10). In addition, the degree of slow inactivation is less in the HEK 293 cells relative to the L-cells. It was hypothesized that perhaps the HEK 293 cells have different populations of glyco- sylating enzymes, kinases, or other post-translational modifying enzymes that produce cell-specific mature forms of hKv1.5 at the plasma membrane. Alternatively, differences in the plasma membrane or cytoskeletal composition could account for the observed functional differences. Finally, another possible explanation for the differences in hKv1.5 expression is that function-altering \( \beta \) subunits are differentially expressed in these cell lines. HEK 293 cells sometimes express endogenous, delayed rectifier type currents (see “Experimental Procedures”), whereas the L-cells always lack voltage-dependent outward currents and in fact have a resting potential near zero (19). Because the HEK 293 cells contain an occasional endogenous \( \alpha \) subunit, it was hypothesized that these cells might also contain an endogenous \( \beta \) subunit.

Identification of Endogenous \( \nu \beta \) Subunits in Heterologous Expression Systems—In order to determine if endogenous \( \beta \) subunits were present in commonly used expression systems, total RNA from Xenopus oocytes, L-cells, and Madin-Darby canine kidney, LLC-PK, CHO, and HEK 293 cells was subjected to low stringency Northern analysis as illustrated in Fig. 1. The probe contained the complete Kvβ1.1 coding sequence, which was predicted to hybridize with all known mammalian \( \beta \) subunit isoforms under the conditions used. A 2.5-3.0-kilobase \( \beta \) subunit mRNA species was detected in CHO cells and L-cells at levels similar to that in rat brain, whereas \( \beta \) subunit mRNA was not detected in Xenopus oocytes or Madin-Darby canine kidney, LLC-PK, or HEK 293 cell lines. However, the difference in nucleotide sequence between Xenopus and mammals may have prevented hybridization. Neither dexamethasone nor the presence of the \( \alpha \) subunit significantly altered the level of L-cell \( \beta \) subunit mRNA (data not shown).

The presence of \( \beta \) subunit message in CHO and L-cells suggested that previously unrecognized endogenous \( \beta \) subunits may be responsible for the functional differences described above. In order to identify the \( \beta \) subunit isoform present in L-cells, a cDNA library was constructed and screened as described under “Experimental Procedures.” Two clones (1.5 and 1.0 kilobases) were purified to homogeneity and excised into pBluecript. The longer clone was sequenced in both directions, yielding an open reading frame of 1101 nucleotides. An in-frame 5’ stop codon was found at ~30, thus ensuring that the full coding region was obtained. Translation of the coding region predicted a 367-amino acid 41-kDa protein identical to rat Kvβ2.1 with 96.4% nucleotide identity in the coding region (19). The nucleotide and amino acid sequence of the mouse Kvβ2.1 (mKvβ2.1) is depicted in Fig. 2. The sequence between the arrows represents the region used to generate the Kvβ2.1-specific antisera. The shaded residues represent amino acids within this region that differ between the Kvβ2.1 isoform and the Kvβ1.1, 1.2, and 1.3 proteins. Despite 81% sequence identity in this region between all known \( \beta \) subunits, the antisera raised was specific for the Kvβ2.1 subunit as determined by Western analysis as described under “Experimental Procedures.”

Analysis of the Kvβ1.5 Subunit Composition in the L-cell and HEK 293 Expression Systems—Analysis of 200 base pairs of the 5’-untranslated region detected two upstream start codons, each followed by an in frame stop codon (Fig. 2). Because such upstream start sites can inhibit protein synthesis (40), it was necessary to determine that the Kvβ2.1 clone was translated. It was also necessary to demonstrate a direct interaction between the Kvβ1.5 and Kvβ2.1 subunits in order to conclude that the endogenous subunit is responsible for the observed differences between expression systems. Immunopurifications were performed from metabolically labeled L-cells expressing the hKv1.5 \( \alpha \) subunit to address these concerns.

L-cells transfected with either hKv1.5 or a sham vector were labeled with \(^{[35S]}\)methionine, solubilized, and immunopurified with anti-hKv1.5 antisera as described under “Experimental Procedures” (Fig. 3, lanes 1 and 2, respectively). Comparison of the two lanes indicates that two prominent bands with electrophoretic mobilities corresponding to molecular weights of ap-
Approximately 40,000 and 75,000 are present exclusively in the Kv1.5 transfected cells. Similar results were obtained when the channel proteins were immunopurified with either anti-Kv1.5 (S1-S2 or NH2-terminal) antisera. The larger protein represents the Kv1.5 α subunit, whereas the smaller band of 40 kDa has the predicted mass of the cloned mKvβ2.1 subunit protein. Additionally, in vitro translated \[^{35}S\]methionine-labeled mKvβ2.1 comigrated with the immunopurified lower mass protein (data not shown). The Western blot analysis illustrated in lane 3 of Fig. 3 demonstrates that the 40-kDa protein copurifying with Kv1.5 is mKvβ2.1. The Kv1.5 channel was immunopurified from nonlabeled hKv1.5- or sham-transfected L-cell membranes (Fig. 3, lanes 3 and 4, respectively), fractionated by SDS gel electrophoresis, transferred to nitrocellulose, and incubated with the anti-Kvβ2.1 antiserum. The 40-kDa band was specifically detected by anti-Kvβ2.1 antibodies in the material purified from the hKv1.5-expressing cells, demonstrating that this band represents the mKvβ2.1 protein, as opposed to a proteolytic fragment of the α subunit.

The absence of detectable β subunit mRNA in HEK 293 cells does not guarantee a complete absence of β subunits. Another family of subunits could exist that does not cross-hybridize, even at low stringency, with the cDNA probe used in the Northern analysis. Therefore, the hKv1.5 protein was immunopurified from transfected and radiolabeled HEK 293 cells, looking specifically for a copurifying protein of 30–50 kDa. As shown in Fig. 3 (lanes 5 and 6), when HEK 293 cells transfected with hKv1.5 were immunopurified (lane 5), a unique doublet, probably representing the immature and glycosylated forms of hKv1.5, appeared at 66 and 75 kDa. These bands were absent when nontransfected cells were used as the starting material (lane 6). Low molecular weight bands representing putative β subunits are noticeably absent from lane 5, demonstrating that...
β subunits capable of associating with hKv1.5 are absent from the HEK 293 cells. Because functional channels are obtained after transfection of the α subunit alone, β subunits are not required for the synthesis of functional channels. It is also unlikely that the β subunit plays a role in regulating cell surface channel density because the current densities are similar between the L-cell and HEK 293 expression systems (10, 14).

Fig. 4. Functional analysis of Kv1.5 and Kvβ2.1 coexpression in HEK 293 cells. A and B show the expression of hKv1.5 in HEK 293 cells without and with the mKvβ2.1 subunit, respectively. Outward currents were elicited by a series of 250-ms step depolarizations from a holding potential of −80 mV to potentials between −30 and +50 mV in 10-mV increments. Note that the 0-mV stimulus is indicated in both panels. C shows the voltage activation curve, in the presence and the absence of the mKvβ2.1 subunit. D illustrates the voltage dependence of the activation time constant plus minus mKvβ2.1. E illustrates the voltage dependence of inactivation with and without the mKvβ2.1 subunit. Pulse protocols are as described previously (14). F summarizes effects of the mKvβ2.1 subunit on hKv1.5 slow inactivation. The data are plotted as the percentage of inactivation (relative to peak current) at the indicated pulse duration. The asterisks represent significant differences (p < 0.01). In A, B, C, and E, representative data from a single paired experiment are shown, i.e. the curves in C and E were obtained from the same cells represented in A and B. For D and F, data were derived from five or six separate cells from three independent transfections. The dashed lines in C and D represent data obtained from hKv1.5 expression in mouse L-cells (14).

Like many delayed rectifiers, hKv1.5 displays partial and slow inactivation, presumably of the C-type (41). Often, hKv1.5 was coexpressed with Kvβ2.1, substantially more current was activated at the lowest depolarizations as indicated by the arrows in A and B. Although slow inactivation was limited in both cases, the current declined more when hKv1.5 was coexpressed with mKvβ2.1, especially at the strongest depolarizations.

To directly quantify the effects of mKvβ2.1 coexpression, we analyzed the voltage-dependence of activation from the amplitude of the decaying tail currents. Fig. 4C shows that coexpression with mKvβ2.1 results in a negative displacement of the activation curve. The sigmoidal voltage-dependence was fitted with a single Boltzmann equation resulting in half-activation voltages of −0.2 ± 2.0 mV (n = 8) and −14.1 ± 1.8 mV (n = 9; p < 0.01) without and with mKvβ2.1, respectively. The slope factors were not significantly different (6.2 ± 0.6 mV without and 5.6 ± 0.4 mV with mKvβ2.1; p > 0.1). Because the steady-state voltage dependence and the kinetics of activation are determined by the same underlying rate constants, we determined whether a similar shift existed in the activation kinetics. For comparison with previous results, the dominant time constant of activation was determined (see “Experimental Procedures”).

Fig. 4 shows typical recordings from HEK 293 cells transiently transfected with hKv1.5 alone or with mKvβ2.1, (A and B, respectively). Tracings for depolarization from −80 mV to potentials between −30 mV and +50 mV were superimposed for comparison. No voltage-activated current was observed between −100 and −40 mV, but a progressively larger outward current was recorded with depolarization above −30 mV. In both A and B of Fig. 4, the activation of the current proceeded with a sigmoidal time course, and the rate of activation increased with depolarization. Upon repolarization to −30 mV, outward tail currents were recorded. These hKv1.5 currents were in qualitative agreement with observations in various expression systems (10–14). However, when hKv1.5 was coexpressed with Kvβ2.1, substantially more current was activated at the lowest depolarizations as indicated by the arrows in A and B. Although slow inactivation was limited in both cases, the current declined more when hKv1.5 was coexpressed with mKvβ2.1, especially at the strongest depolarizations. 

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Fig. 4D shows that coexpression of mKvβ2.1 resulted in a faster activation rate between −10 and +60 mV, resulting in a parallel shift of the same magnitude as for the activation curve of C. The results obtained in the absence of mKvβ2.1 correspond closely to those of Fedida et al. who expressed hKv1.5 in HEK 293 cells (10). The dashed lines in C and D of Fig. 4 illustrate the corresponding results previously obtained in L-cells under identical conditions (14). In both cases, the results for coexpression of hKv1.5 with mKvβ2.1 correspond closely to hKv1.5 expressed in L-cells.

Like many delayed rectifiers, hKv1.5 displays partial and slow inactivation, presumably of the C-type (41). Often, hKv1.5
expressed in oocytes displays less extensive inactivation than in the L-cells. Therefore we compared both the voltage dependence of slow inactivation and the degree of slow inactivation at 250 ms, 1 s, and 5 s during depolarizations to 50 mV. Fig. 4E shows the results from the cells shown in A and B. Coexpression with mKvβ2.1 resulted in a hyperpolarizing displacement of the normalized inactivation curve with average values $E_h = -9.6 \pm 1.8$ mV (n = 6) and $-22.1 \pm 3.7$ mV (n = 5; p < 0.01) without and with mKvβ2.1, respectively. Slope factors were not significantly different (5.2 ± 0.4 and 5.1 ± 0.2, p > 0.1). Because the voltage dependence of C-type inactivation appears to be linked to that of activation (41), these results are consistent with the effect of Kvβ2.1 on activation. Indeed, when the difference in the midpoints of activation and inactivation were determined for each cell individually, we observed a similar displacement between both curves of 9.8 ± 1.3 mV (n = 6) and 8.9 ± 2.8 mV (n = 5) for hKv1.5 alone or with mKvβ2.1, respectively (p > 0.1). This displacement is consistent with the 8.6-mV displacement observed in L-cells (14). The presence of the mKvβ2.1 subunit also appeared to enhance the degree of slow inactivation as illustrated in Fig. 4 (A and B). Fig. 4F shows that the average amount of inactivation (with respect to the peak current) at 250 ms, 1 s, and 5 s was greater when hKv1.5 was coexpressed with mKvβ2.1. The percentage of inactivation in the HEK 293 cells expressing both hKv1.5 and mKvβ2.1 compares well with the results for hKv1.5 in L-cells (69% at 60 mV) (14). It should be noted that the comparison is somewhat complicated by the high temperature sensitivity of this process (14). Nevertheless, the results from the HEK 293 cells reported here were obtained at the same temperature. The mKvβ2.1 subunit had no effect on ion selectivity based on the finding that the reversal potential was unchanged in the presence of mKvβ2.1: −82.3 ± 1.4 mV (n = 4) with β and −83.7 ± 0.9 (n = 8) without β. No effect of mKvβ2.1 on channel surface density was detected, making it unlikely this subunit plays a regulatory role in channel biosynthesis, although such a role may be apparent only in the native cell.

Comparison of KvL.5 Function in Commonly Used Expression Systems—The functional characteristics of hKv1.5 expressed in mouse L-cells, HEK 293, and CHO cells and Xenopus oocytes are summarized and compared in Table I. Comparison of the L-cell and HEK 293 data indicate that the primary effects of the Kvβ2.1 subunit are to alter the voltage sensitivity of activation as demonstrated by the leftward shift in the midpoint of the activation and inactivation curves and the voltage dependence of the activation time constants. The activation midpoint did not change in L-cells expressing mutants of hKv1.5 lacking the conserved TDV motif and up to 57 COOH-terminal amino acids, indicating that this region is not involved in the Kvβ2.1 modulation (15). Another notable effect of Kvβ2.1 is that slow inactivation is increased approximately 2-fold. Although the Kvβ1.2 subunit has a marked effect on hKv1.5 deactivation (23), Kvβ2.1 did not alter the kinetics of the tail currents at −30 mV. As Table I indicates, the properties of hKv1.5 expression in CHO cells suggest that the endogenous β subunit in this system is the Kvβ2.1 isoform. No β subunit mRNA was detected in the Xenopus oocytes, as shown by the Northern analysis of Fig. 1. The midpoint of activation in the oocyte system is closer to that found in HEK 293 cells without Kvβ2.1 coexpression. The degree of slow inactivation also appears to be reduced in the oocyte relative to CHO and L-cells (13), although this parameter is very temperature-dependent and difficult to compare between laboratories. Although further experiments are required to determine if endogenous β subunits are present in oocytes, the present data suggest that they are not.

Pharmacological studies on the Kv1.5 channel have been performed by several laboratories using the L-cell, CHO, and HEK 293 expression systems. Comparison of data obtained with these systems suggests that no pharmacological effect can be assigned to the Kvβ2.1 subunit. For example, verapamil and terfenadine modification of Kv1.5 currents are similar in HEK 293 and L-cells (42-46).

CONCLUSIONS

The data presented here are the first to indicate that Kvβ2.1 modifies delayed rectifier potassium channel function. Immunopurification of hKv1.5 from HEK 293 cells indicated that these cells do not contain a β subunit that assembles with hKv1.5. Because these cells are capable of expressing hKv1.5 current when transfected with only the α subunit, the β subunit is not required for expression. Coexpression of Kvβ2.1 with hKv1.5 in HEK 293 cells resulted in a 10-mV leftward shift in the activation curve and an almost 2-fold increase in the degree of slow inactivation. The resulting HEK 293 cell current closely mimics that recorded from hKv1.5 transfected CHO and L-cell lines (which contain endogenous β subunits). In addition to demonstrating a functional effect of Kvβ2.1 on Kv1.5, these data illustrate the need to test for and identify the β subunits present in heterologous expression systems.

The Kv1.5 K⁺ channel is expressed most abundantly in cardiac myocytes and vascular smooth muscle (32). Because the Kvβ2.1 subunit has also been cloned from cardiac tissue (23), the potential exists for Kv1.5 and Kvβ2.1 coassembly in heart. Such in vivo assembly would result in an earlier activation of the rectifying potassium current, perhaps serving as a regulatory mechanism controlling action potential duration. The Kvβ2.1 subunit has several potential phosphorylation sites that could regulate assembly and/or interaction with the α subunit, allowing for a rapid response system controlling heart rate. A search for potential pathophysiological conditions resulting from altered Kvβ2.1 expression must wait until the cell-specific expression and in vivo α subunit association is confirmed.

Acknowledgments—We thank Dr. Lou Philipson for the generous gift of the anti-hKv1.5 (S1-S2) antiserum, Paul Bennett for review of the manuscript, and Holly Shear, Debbie Mays, and Ian Hopkirk for excellent technical support.

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**Table I**

| Functional parameter | L-cells a | CHO cells b | HEK 293 cells c | HEK 293 cells + Kvβ2.1 d | Xenopus oocytes e |
|----------------------|-----------|-------------|-----------------|------------------------|-----------------|
| Activation midpoint (mV) | $-14 \pm 4$ | $-19 \pm 3$ | $-0.2 \pm 2.0$ (n = 8) | $-14.6 \pm 1.8$ (n = 9) | $-6 \pm 0.6$ |
| Inactivation midpoint (mV) | $-25 \pm 4$ | $-33 \pm 3$ | $-9.6 \pm 1.8$ (n = 6) | $-22.1 \pm 3.7$ (n = 5) | $-25.3 \pm 0.4$ |
| Activation rate constant at 0 ms (ms) | $101.1 \pm 1.9$ | $3.4$ | $16.3 \pm 1.2$ (n = 5) | $8.9 \pm 0.6$ (n = 5) | $8.3 \pm 0.4$ |
| % inactivation (+50 mV) at 5 s | $69 \pm 3$ | | $37.9 \pm 1.6$ (n = 6) | $51.7 \pm 3.3$ (n = 5) | |
