Functional expression of a GFP-tagged Kv1.5 α-subunit in mouse ventricle

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POTASSIUM-SELECTIVE ION CHANNELS are more diverse than other types of channels in cardiac myocytes, and these channels play important roles in setting resting membrane potentials, shaping the waveforms of action potentials, as well as in regulating refractoriness and automaticity (3, 5). Depolarization-activated K⁺ currents, for example, regulate the amplitudes and durations of action potentials in cardiac cells, and several distinct types of voltage-gated K⁺ currents that subserve these functions have been identified (2, 3, 5, 16, 23, 33, 34). The various K⁺ currents underlie distinct phases of action potential repolarization in cardiac cells, and differences in the densities and/or properties of these currents underlie observed variations in action potential waveforms recorded in different species and/or in different regions of the heart in the same species (2, 5, 11, 16, 33). A number of voltage-gated, K⁺ channels, pore-forming (α), and auxiliary (mink/Mirp, β, KChIP, and KChAP) subunits have now been cloned from heart cDNA libraries (12, 34), and a variety of experimental approaches have been exploited to probe the molecular basis of functional K⁺ channel diversity in the mammalian heart. Considerable progress has been made recently in identifying the voltage-gated, K⁺ channel, pore-forming α-subunits that underlie the various types of transient outward (Iₒ) and delayed rectifier K⁺ currents (I_K) in cardiac cells (6, 8, 12–14, 17, 18, 22, 25, 26, 45, 47, 50, 51).

Changes in functional voltage-gated K⁺ current densities and properties occur in conjunction with myocardial damage or disease (9, 31, 32, 41, 43), and these changes result in action potential prolongation, often leading to the generation of life-threatening arrhythmias (48). It has been suggested that a promising therapeutic approach would be to increase the functional expression of the channels that are altered exploiting gene-targeting approaches (21, 36). Very little is presently known, however, about the factors that limit the functional cell surface expression or determine the detailed properties of voltage-gated K⁺ channels in cardiac (or other) cells. The experiments here were undertaken to determine directly whether “extra” K⁺ channels can be incorporated into the plasma membranes of cardiac myocytes and, if so, to define the properties of the currents and explore the functional consequences of (K⁺ channel) overexpression. Two lines of transgenic mice expressing a green fluorescent protein (GFP)-tagged Kv1.5 α-subunit driven by the cardiac-specific α-myosin heavy chain (MHC) promoter (35, 39) were generated. Unexpectedly, expression of the transgene in both lines is heterogeneous, and Kv1.5-GFP is detected in only 50% of ventricular myocytes.
higher in Kv1.5-GFP-expressing ventricular myocytes and that the waveforms of the outward K⁺ currents in these cells are distinct from those recorded in wild-type cells (52). In addition, the expression of the Kv1.5-GFP leads to potential shortening. These results clearly attest to the feasibility of increasing functional cell surface K⁺ channel expression in the myocardium. In addition, although in the mouse, this increase in K⁺ channels is not manifested in electrocardiographic changes, the kinetic properties of the currents are not identical to the component of wild-type mouse ventricular delayed rectifier K⁺ current (Iₖ,slow) encoded by Kv1.5 (25–27), suggesting differences in association with accessory subunits and/or posttranslational processing between the “overexpressed” Kv1.5 channels and the endogenous Kv1.5 channels.

METHODS

Construction of GFP-tagged Kv1.5 β-subunit. The complete (mouse) Kv1.5 β-coding sequence was amplified by high-fidelity polymerase chain reaction (PCR) using Accutaq DNA polymerase (Sigma; St. Louis, MO). The primer pair used for this purpose was synthesized such that a SmaI restriction site was introduced at both 5’ and 3’ ends of the amplified product. The primer sequences were the following: forward, 5’-ATACC CGGGTGAGTTGGTGTGTAGCAACCGGTT and reverse, 5’-AGCAGATAAGCAAGCTTGACCCGGGACGGTACGCTATGGTTCGGCTAGT-TGT. The 1,850-bp PCR product was then digested with SmaI and subcloned into the SmaI site in pEGFP-N1 (Clontech) to yield pKv1.5-GFP with the downstream GFP-coding sequence in frame with Kv1.5. Sequence analysis confirmed the intended reading frame at the Kv1.5-GFP junction and revealed that no sequence changes had been introduced by PCR amplification. Proceeding from the NH₂ to COOH terminus, the resultant fusion protein consists of full-length Kv1.5, a linker sequence of eight amino acids and GFP. The predicted molecular mass of the resulting Kv1.5-GFP fusion protein is 96 kDa. Wild-type Kv1.5 (without a GFP tag) in pBK-CMV (pKv1.5) was also used in expression studies to compare the properties of the Kv1.5-GFP with wild-type Kv1.5.

Cell lines and transient transfections. HEK-293 cells, a human embryonic kidney cell line, were obtained from the American Type Culture Collection (ATCC) to yield pKv1.5-GFP with the downstream GFP-coding sequence in frame with Kv1.5. Sequence analysis confirmed the intended reading frame at the Kv1.5-GFP junction and revealed that no sequence changes had been introduced by PCR amplification. Proceeding from the NH₂ to COOH terminus, the resultant fusion protein consists of full-length Kv1.5, a linker sequence of eight amino acids and GFP. The predicted molecular mass of the resulting Kv1.5-GFP fusion protein is 96 kDa. Wild-type Kv1.5 (without a GFP tag) in pBK-CMV (pKv1.5) was also used in expression studies to compare the properties of the Kv1.5-GFP with wild-type Kv1.5.

Generation of transgenic mice expressing Kv1.5-GFP. After the pKv1.5-GFP was cleaved at NotI and the α-MHC expression vector (provided by Dr. Jeffery Robbins, University of Cincinnati, Cincinnati, OH) was digested at HindIII, the sticky ends were filled in with Klenow polymerase using deoxynucleotide triphosphates; both plasmids were subsequently digested with SalI. A 2.6-kb fragment containing the Kv1.5-GFP coding sequence was gel purified and subcloned into the purified α-MHC vector by blunt and cohesive end ligation. Digestion of the resultant pMHC-Kv1.5-GFP with NotI generated a 9-kb fragment, which contains the α-MHC promoter, the Kv1.5-GFP coding sequence, and the human growth hormone (hGH) polyadenylation signal sequence. After electrophoresis through a 1.5% agarose gel (1% low-melting agarose plus 0.5% regular agarose), this (9 kb) fragment was isolated, and an equal volume of 0.3 M NaCl-Tris-EDTA (TE, pH 7.4) was added. After being melted at 70°C for 30 min, the mixture was extracted with phenol-chloroform-isomyl alcohol (25:24:1), and the DNA was ethanol precipitated. The DNA was then suspended in 1 ml 0.5 M NaCl-TE, purified over a Prepac column, and ethanol precipitated. The recovered DNA was resuspended in 100 μl of injection buffer (10 mM Tris buffer containing 10 mM NaCl and 0.1 mM EDTA at pH 7.4), dialyzed against a 0.1-μm Millipore filter, and diluted to a final concentration of 1 ng/μl for injection into fertilized C57BL6 mouse blastocysts. After the (~200) injections were completed, the oocytes were transplanted into pseudopregnant ICR strain adult mice. A total of 36 offspring were obtained, and all were screened for integration of the transgene by PCR analysis of (tail) genomic DNA using probes directed against the GFP coding sequence; three founders were identified. At 8 wk, animals positive for the transgene were bred to wild-type C57BL6 adult mice. In two of the (3) founders transgene expression was transmitted to the F1 progeny, and two lines of Kv1.5-GFP-expressing transgenic mice were established.

Expression of Kv1.5-GFP in transgenic mice. For RT-PCR analysis, mRNA was prepared from the ventricles and brains of adult Kv1.5-GFP-expressing transgenic and nontransgenic (control) littermates using the Micro-FasTrack mRNA isolation kit (Invitrogen). cDNA was synthesized in a 20-μl reaction mixture containing (in mmol/l) 50 Tris-HCl (pH 8.3), 5 MgCl₂, 75 KCl, 4 sodium pyrophosphate, 5 dNTPs, and 10 dithiothreitol (DTT), as well as 0.5 μg of oligo(dT)₂₅, 0.1 μg of mRNA, 10 units of RNase inhibitor, and 5 units of avian myeloblastosis virus reverse transcriptase (Sigma). After a 1-h incubation period at 42°C, the reaction was terminated by heating at 95°C for 2 min. Approximately 2 μl of the resulting reaction mixture were used for PCR amplification. PCR was carried out in a 25-μl reaction mixture containing (in mmol/l) 10 Tris-HCl (pH 9.0), 50 KCl, 2 MgCl₂, 1 DTT, and 0.2 dNTPs, as well as 0.5 μmol/l of each primer (see below) and 1 unit of Taq DNA polymerase (Sigma). The reaction proceeded for 30 cycles as follows: 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min. After fractionation by SDS-PAGE and fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, membrane proteins were prepared using a protocol developed for the preparation of rat cardiac membrane proteins (49). After fractionation by SDS-PAGE and transfer to polyvinylidene difluoride membranes (Amerham), samples were probed with a polyclonal anti-Kv1.5 antibody (1:250, Transduction or UBI), a monoclonal anti-Kv2.1 antibody (1:200, UBI), followed by an alkaline phosphatase-conjugated goat anti-rabbit (or anti-mouse for anti-GFP antibody) secondary antibody. Bound antibodies were detected using the CDP* (Tropix) chemiluminescent alkaline phosphatase substrate.
**Electrophysiological recordings.** Whole cell, voltage-clamp recordings from GFP-positive HEK-293 cells were obtained at room temperature within 48 h of transfection. The recording pipettes contained (in mM) 115 KCl, 15 KOH, 10 EGTA, 10 HEPES, and 5 glucose (pH 7.2; 300–310 mosM). The bath solution contained (in mM) 140 NaCl, 4 KCl, 2 MgCl₂, 1 CaCl₂, 10 HEPES, and 5 glucose (pH 7.4; 300–310 mosM). Experiments were performed using an Axopatch 1B patch-clamp amplifier (Axon Instruments) interfaced to a Gateway 350-MHz Pentium computer interfaced to the recording equipment with a Digidata 1200 analog/digital interface and the pCLAMP 7 software package (Axon Instruments). Recording electrodes were fabricated from soda lime glass (Kimble), coated with Sylgard (Dow Corning), and fire-polished; tip resistances were 1.5–2.5 MΩ. Series resistances were in the range of 3–4 MΩ and were compensated electronically by 80–90%; voltage errors resulting from the uncompensated series resistance were always <5 mV and were not corrected. Outward K⁺ currents in transiently transfected HEK-293 cells were evoked during 140-ms depolarizing voltage steps to test potentials between –40 and +60 mV from a holding potential (HP) of –70 mV; data were filtered at 5 kHz before storage.

Left ventricular (LV) myocytes were isolated from wild-type and Kv1.5-GFP-expressing adult (6–8 wk) C57BL6 mice, and whole cell, voltage-clamp recordings were obtained within 48 h of cell isolation at room temperature (25°C) using previously described methods (17, 49, 51). The recording pipettes contained (in mM) 115 KCl, 15 KOH, 10 EGTA, 10 HEPES, and 5 glucose (pH 7.2; 300–310 mosM). The bath solution contained (in mM) 140 NaCl, 4 KCl, 2 MgCl₂, 1 CaCl₂, 0.2 CdCl₂, 0.02 tetrodotoxin (TTX), 10 HEPES, and 5 glucose (pH 7.4; 300–310 mosM). Outward K⁺ currents were evoked during 500-ms or 4.5-s depolarizing voltage steps to test potentials between –40 and +60 mV from a HP of –70 mV after a 20-ms prepulse to –20 mV to eliminate the residual (20 μM TTX insensitive) component of the Na⁺ current. Inwardly rectifying K⁺ currents (I_K1) were recorded in response to hyperpolarizing voltage steps to test potentials between –90 and –120 mV from a HP of –70 mV. For action potential recordings, the TTX and the CdCl₂ were omitted from the bath solution, and action potentials were recorded at 25°C and 35°C in response to brief (1 ms) depolarizing current injections delivered at 1 Hz (25°C) or 10 Hz (35°C).

Cryostat sectioning and immunohistochemical analysis. For the isolation of hearts for the preparation of cryostat sections, animals were anesthetized with pentobarbital sodium, and the hearts were perfused with 4% paraformaldehyde in 0.2 M PB and subsequently immersed in 30% sucrose in PBS (pH 7.4) at 4°C overnight. The sucrose-equilibrated tissue was incubated in OCT tissue embedding media (Sakura Finetek) for ~6 h at 4°C and frozen in a dry ice-isopentane bath at –40°C. Whole frozen heart blocks were placed on a tissue holder using OCT, and cryostat sections were cut at 15 μm, collected and air-dried. Dried sections were mounted directly and propidium iodide (PI) (Vector Laboratories) was included in mounting media. For immunohistochemical analysis of isolated myocytes, cells were fixed with 2% paraformaldehyde in PB and then incubated in anti-GFP antibody solution (diluted 1:500 in PBS) overnight at 4°C. After washing was completed, cells were placed in Cy3-conjugated goat anti-rabbit (for Kv1.5 visualization) or a Cy3-conjugated goat anti-mouse (for GFP visualization) secondary antibody. For photography, sections/cells were placed on the stage of an Olympus Fluoview inverted confocal microscope and viewed through a ×40 objective.

**Echocardiograms and electrocardiograms.** Adult wild-type and Kv1.5-GFP-expressing animals were examined by non-invasive, transthoracic echocardiography using previously described methods (18, 38). For this procedure, the animals were anesthetized with Avertin (2.5%) (0.01 ml/g) and were examined using an Acuson Sequoia 256 Echocardiography system equipped with a 15-MHz transducer. Images obtained from each mouse were recorded on optical disks and subsequently stored on CD-ROM disks for off-line analysis; the means ± SE of the measurements made on transgenic (n = 6) and nontransgenic (n = 6) littermates are reported in Table 1.

Echocardiographic recordings were obtained from adult Kv1.5-GFP-expressing transgenic mice and from nontransgenic littermates using Data Sciences International Implantable Physioloty TA10ETA-F20 radio frequency transmitters and receivers (placed under cages) (17, 51). To implant the transmitters, animals were first anesthetized by intraperitoneal injection of ketamine (80 mg/kg) and xylazine (16 mg/kg). After the chest was opened, transmitters were placed in the abdominal cavity, and the echocardiographic leads were tunneled under the skin, sutured, and glued (veterinary tissue adhesive) to muscles in the thorax. Cathodal leads were routinely placed on the upper right portion of the thorax, and anodal leads were placed on the chest wall near the apex of the heart. Approximately 24 h later, analog electrocardiographic signals were collected, digitized at 1 kHz using a 16-bit Dataquest A.R.T. Gold acquisition analog-to-digital converter (Data sciences), and stored on CD-ROM disk. Three-minute recordings every hour for 48 consecutive hours were obtained from each animal. Unfiltered data were analyzed, and Q-T, QRS, R-P, and R-R intervals were measured; average values for individual animals were determined, and mean ± SE values for experiments completed on transgenic (n = 4) and nontransgenic (n = 5) littermates are reported.

**Data analysis.** Voltage-clamp and current-clamp data were compiled and analyzed using Clampfit (Axon Instruments) and Excel (Microsoft). Whole cell ventricular myocyte membrane capacitances were determined by integration of the capacitative transients evoked during brief (25 ms) subthreshold (± 10 mV) voltage steps from a HP of –70 mV. The

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All values are means ± SE; n, number of mice. GFP, green fluorescent protein; HR, heart rate; LVIDd and LVPd, end-diastolic and end-systolic left ventricular (LV) internal dimensions, respectively; IVSd and LVPd, end-diastolic intraventricular septal thickness and LV posterior wall thickness, respectively; LVM, LV mass: (LVPWd + IVSd + LVIDd)² – (LVIDd)² × 1.04; RWT, relative wall thickness: (LVPWd + IVSd)/LVIDd; FS, fractional shortening: (LVIDd – LVPd)/LVIDd; and LVMJ, which is derived from LVM divided by body weight.
means ± SE whole cell membrane capacitances of ventricular cells isolated from wild-type and Kv1.5-GFP-expressing transgenic animals were 140 ± 7 pF (n = 25) and 155 ± 10 pF (n = 15), respectively. The means ± SE input resistance of adult mouse wild-type (n = 25) and Kv1.5-GFP-expressing (n = 15) ventricular myocytes were 0.89 ± 0.24 and 0.74 ± 0.15 GΩ, respectively. The plateau outward K⁺ current in each cell was defined as the current remaining 3 s after the onset of the depolarizing voltage steps, and the peak outward current was defined as the maximum value of the outward K⁺ current during the 500-ms voltage steps. Current amplitudes, measured in individual cells, were normalized to cell size (whole cell membrane capacitance), and current densities (in pA/pF) are reported (52). Activation time constants were determined from single exponential fits to the rising phases (after the initial delay) of the outward K⁺ currents evoked during depolarizing voltage steps to test potentials between +10 and +60 mV from a HP of −70 mV (52). Inactivation time constants (τ) were determined from (single or double) exponential fits to the decay phases of the outward K⁺ currents recorded during 4.5-s depolarizing voltage steps (52).

RESULTS

Kv1.5-GFP expression in transiently transfected HEK-293 cells. Preliminary experiments were aimed at determining whether the properties of the voltage-gated K⁺ channels encoded by Kv1.5 are affected by the presence of GFP at the COOH terminus of the protein. For this purpose, HEK-293 cells were transiently transfected (51) with the Kv1.5-GFP construct or with separate plasmids encoding Kv1.5 and GFP. In both cases, GFP fluorescence was detected within 24–36 h, although the labeling appears quite different in cells transfected with Kv1.5-GFP compared with the cells transfected with the separate Kv1.5 and GFP cDNAs (Fig. 1A). In cells in which GFP was cotransfected with Kv1.5, the fluorescence was distributed throughout the cytoplasm of the transfected cells (Fig. 1A), whereas in the Kv1.5-GFP-expressing cells, the fluorescence appears to be localized primarily at the plasma membrane (Fig. 1B). These observations suggest that the presence of the COOH terminal 27-kDa protein does not affect the time or voltage-dependent properties of Kv1.5. A and B: GFP fluorescence is readily detected in human embryonic kidney (HEK)-293 cells cotransfected with wild-type Kv1.5 and GFP (A) or Kv1.5-GFP (B). In co-transfected cells, the fluorescence is cytosolic (A), whereas in cells transfected with Kv1.5-GFP (B), the fluorescence is associated with the membrane. The cell surface expression of the Kv1.5 α-subunit, therefore, is not affected by the presence of the COOH terminal GFP. C and D: whole cell outward K⁺ currents recorded from GFP-positive HEK-293 cells transiently transfected with wild-type Kv1.5 (C) or Kv1.5-GFP (D). Currents were evoked during 140-ms depolarizing voltage steps to potentials between −50 and +60 mV from a holding protein (HP) of −70 mV. Scale bars are 3 nA and 20 ms (E). Means ± SE peak outward current densities in HEK-293 cells transfected with wild-type Kv1.5 (○; n = 12) and Kv1.5-GFP (●; n = 12) are not significant. E: means ± SE time constants of activation, determined from single exponential fits to the rising phases of the outward K⁺ currents at +10 mV (in records such as those in C and D), are similar in cells expressing wild-type Kv1.5 (○; n = 12) and Kv1.5-GFP (●; n = 12).
GFP protein does not adversely affect the cell surface expression of Kv1.5 α-subunits.

The functional properties of Kv1.5-GFP were then assessed in electrophysiological experiments on transiently transfected, GFP-positive HEK-293 cells. As illustrated in Fig. 1, outward K⁺ currents recorded from Kv1.5-GFP-expressing HEK-293 cells (Fig. 1D) are similar to those recorded from cells expressing wild-type Kv1.5 (Fig. 1C). The means ± SE (n = 12) peak outward K⁺ current densities (Fig. 1E) and τ of outward current activation (Fig. 1F) in cells expressing wild-type Kv1.5 (Fig. 1C) and GFP-tagged Kv1.5 (Fig. 1D) are indistinguishable. In addition, the voltage dependences of activation of the Kv1.5-GFP (V_{1/2} = 35.2 mV; k = 10.4 mV) and Kv1.5 (V_{1/2} = 34.6 mV; k = 10.9 mV) are not significantly different where V_{1/2} is the half-inactivation voltage and K is the slope factor of the curve. Taken together, these results demonstrate that the GFP-tagged Kv1.5 α-subunits assemble properly and form functional voltage-gated K⁺ channels in HEK-293 cells, and that the time- and voltage-dependent properties of the Kv1.5-induced currents are not measurably affected by the presence of the COOH-terminal GFP tag (see DISCUSSION).

**Generation and characterization of transgenic mice expressing Kv1.5-GFP.** For the generation of transgenic mice, the GFP-tagged Kv1.5 α-subunit coding sequence was subcloned downstream of the cardiac-specific α-MHC promoter in the α-MHC expression vector. Previous work (35, 39) has shown that this promoter is cardiac specific and that constitutive expression of transgenes driven by this promoter is detectable in mouse atria and ventricles from the time of birth. A 9-kb fragment from this construct containing the α-MHC promoter, the Kv1.5-GFP coding sequence, and the hGH polyadenylation signal sequence was isolated and injected into C57BL6 mouse blastocytes. For screening, genomic tail DNA was prepared, and transgene incorporation was assessed by PCR using primers directed against the GFP coding sequence. A total of 45 offspring were obtained and screened, and three founders were identified, although transgene expression was successfully transmitted in only two of these founders. Two lines (lines 1 and 9) of Kv1.5-GFP-expressing transgenic mice were established and characterized.

On gross examination, there are no apparent differences between the Kv1.5-GFP-expressing transgenic animals and nontransgenic littermates (in both lines). Means ± SD body weights, for example, were 25.4 ± 3.2 g (n = 5) and 27.6 ± 3.6 g (n = 6) for 8-wk (adult) wild-type and Kv1.5-GFP-expressing animals, respectively. Heart weights were also not significantly different with means ± SD values of 95 ± 8 mg (n = 5) and 104 ± 13 mg (n = 6) for wild-type and Kv1.5-GFP-expressing animals, respectively. Heart-to-body weight ratios in nontransgenic and transgenic animals, therefore, were also indistinguishable. Consistent with these observations, echocardiographic (M mode) measurements in situ revealed no significant differences in cardiac structure or function in Kv1.5-GFP-expressing animals compared with wild-type control animals (Table 1). Means ± SE left ventricle mass index values in transgenic and nontransgenic littermates, for example, are indistinguishable (Table 1). In addition, histological examination of hearts from Kv1.5-GFP-expressing animals revealed no detectable morphological differences from controls (not shown). Thus, although there have been reports of cardiac hypertrophy and compromised cardiac output in transgenic mice expressing a truncated K⁺ (Kv4.2) channel α-subunit (47) or GFP alone (20), there are no obvious deleterious effects of Kv1.5-GFP expression in the transgenic lines established and characterized here (see DISCUSSION).

**Functional expression of Kv1.5-GFP in mouse ventricular myocytes.** RT-PCR analysis revealed that Kv1.5-GFP, as well as endogenous (wild-type) Kv1.5, expression is readily detected in the ventricles of transgenic animals, whereas only wild-type Kv1.5 is detected in the ventricles of nontransgenic littermates (Fig. 2A). In brain samples prepared from Kv1.5-GFP-expressing mice, only wild-type Kv1.5 is evident (Fig. 2A), consistent with the cardiac-specific expression of transgenes driven by the α-MHC promoter (35, 39). Expression of the Kv1.5-GFP fusion protein was also readily detected in Western blots of ventricular membrane proteins prepared from the hearts of Kv1.5-GFP-expressing animals using either an anti-Kv1.5 (Fig. 2B, left) or anti-GFP (Fig. 2B, center) antibody. Endogenous Kv1.5 (Fig. 2B, left) and other Kv α-subunits, such as Kv2.1 (Fig. 2B, right), which likely encodes the tetraethylammonium (TEA)-sensitive component of mouse ventricular I_{K_{slow}} (51) are readily detected in Western blots of fractionated wild-type and Kv1.5-GFP-expressing ventricular membrane proteins. Expression of Kv2.1 and wild-type Kv1.5 therefore appears to be unaffected by the presence of the Kv1.5-GFP transgene and/or by the functional expression of Kv1.5-GFP fusion protein at the cell surface.

Under epifluorescence illumination, GFP fluorescence is readily detected in randomly dispersed left ventricular (LV) myocytes isolated from the Kv1.5-GFP-expressing transgenic animals (Fig. 3F). Unexpectedly, however, GFP fluorescence was not detected in all LV myocytes isolated from the Kv1.5-GFP-expressing animals (Fig. 3F). Indeed, cell counts revealed that only ~50% of the cells were clearly GFP positive (Fig. 3F). In addition, outward K⁺ currents recorded from LV myocytes with no detectable GFP fluorescence (Fig. 3, A and B) appear to be indistinguishable from the currents recorded from LV myocytes isolated from wild-type animals (52). The heterogeneous expression of GFP in LV myocytes in vitro (Fig. 3) likely does not reflect regional differences in expression in situ as evidenced by the fact that GFP-positive and GFP-negative cells are evident in cells dispersed from the LV apex, base, and septum (see also below). Taken together, these observations suggest either that transgene expression is heterogeneous in vivo or, alternatively, that expression is downregulated following isolation and/or maintenance of the cells in vitro (see below).
Fig. 2. Expression of Kv1.5-GFP is readily detected in the hearts of transgenic mice. A: RT-PCR analysis (see METHODS) revealed that Kv1.5 is readily detected in the ventricles and (the brains) of Kv1.5-GFP-expressing and nontransgenic animals, whereas Kv1.5-GFP is only detected in the hearts of the transgenic (Kv1.5-GFP-expressing) animals. B: ventricular membrane proteins were fractionated by SDS-PAGE, transferred to polyvinylidene difluoride membranes, and probed with an anti-Kv1.5, anti-GFP, or anti-Kv2.1 antibody (see METHODS). Solid arrows, wild-type Kv1.5 and Kv2.1 open arrows, Kv1.5-GFP fusion protein. As expected, the Kv1.5-GFP fusion protein is only detected in ventricular membrane proteins from transgenic hearts. Wild-type Kv1.5 and Kv2.1 are detected in the blots of fractionated wild-type and transgenic ventricular membrane proteins (see text).

Fig. 3. Outward K⁺ current waveforms are altered in Kv1.5-GFP-expressing ventricular myocytes. Whole cell, outward K⁺ currents, recorded from GFP-negative (A and B) and GFP-positive (C and D) ventricular myocytes isolated from adult C57BL6 Kv1.5-GFP-expressing transgenic animals, were evoked during 450 ms (A and C) or 4.5 s (B and D) depolarizing voltage steps to potentials between −50 and +60 mV from a HP of −70 mV. Waveforms of the currents in GFP-negative (A and B) and GFP-positive (C and D) myocytes are distinct. Peak outward K⁺ current amplitudes at all test potentials are higher in the GFP-positive cells (C and D) when compared with the currents recorded in GFP-negative cells (A and B) or with the currents typically recorded in myocytes isolated from nontransgenic animals (17, 52). E: means ± SE peak outward K⁺ current densities in GFP-negative (○; n = 18) and GFP-positive (●; n = 18) ventricular cells are significantly (P < 0.01) different at all test potentials (see text). F: representative field of ventricular myocytes isolated from an adult Kv1.5-GFP-expressing transgenic animal demonstrating heterogeneity in GFP fluorescence.
Whole cell, voltage-clamp recordings from LV myocytes from Kv1.5-GFP-expressing transgenic animals revealed that the waveforms of the depolarization-activated outward K+ currents in GFP-positive LV cells (Fig. 3, C and D) are distinct from those recorded in GFP-negative (wild-type) LV cells (Fig. 3, A and B). Peak outward K+ current amplitudes at all test potentials, for example, are higher in Kv1.5-GFP-positive LV myocytes (Fig. 3, A and B). The means ± SE peak outward K+ densities at +40 mV were 104 ± 7.8 pA/pF (n = 18) and 45 ± 5.3 pA/pF (n = 18) in GFP-positive and GFP-negative cells, respectively (Fig. 3E). The densities of the currents remaining at the end of long depolarizing voltage steps are also significantly (P < 0.01) higher in the GFP-positive (Fig. 3D) than in the GFP-negative (Fig. 3B) LV cells. Previous studies (52) have shown that the outward K+ currents in (most) wild-type mouse LV cells reflect the presence of (at least) three distinct Ca2+-independent, voltage-gated K+ currents: 1) a rapidly activating and inactivating transient outward current (I_{to,f}; 2) a rapidly activating and slowly inactivating current (I_{Kslow}); and, 3) a sustained current (I_{ss}). In LV septum cells, a slow transient outward K+ current (I_{to,s}) is also expressed (17, 52; see also below). As noted above, the currents in GFP-negative cells from the Kv1.5-GFP-expressing transgenics are indistinguishable from the currents in wild-type LV cells, and I_{to,f}, I_{Kslow}, and I_{ss} are clearly evident in these cells (Fig. 3, A and B). The I_{to,f} however, is not readily apparent in the GFP-positive myocytes (Fig. 3D). In addition, the decay phases of the outward currents in GFP-positive cells (Fig. 3D) are much slower than in the GFP-negative cells (Fig. 3B).

Selective increase in 4-AP-sensitive K+ currents in Kv1.5-GFP-expressing cells. In wild-type mouse LV cells, the I_{Kslow} is blocked selectively by low concentrations (in the range of 10–100 μM) of 4-aminoypyridine (4-AP) (15, 25, 52) and, by analogy to human atrial IK,ultrarrapid (IKur) (44), this component of I_{Kslow} was hypothesized to be encoded by Kv1.5 (15, 25, 53). Recently, direct experimental evidence in support of this hypothesis was provided with the demonstration that the micromolar 4-AP-sensitive component of I_{Kslow} is eliminated in LV cells isolated from Kv1.5/Kv1.1 SWAP mice, in which the Kv1.5 coding sequence has been removed and replaced with Kv1.1 (27). Subsequent experiments, therefore, examined the effects of 30 μM 4-AP on the outward K+ currents in GFP-positive (and GFP-negative) LV myocytes. This concentration of 4-AP was used because previous studies have shown that mouse ventricular I_{Kslow} is selectively attenuated by 10–100 μM 4-AP (15, 25, 52), whereas I_{to,f} (and I_{ss}) is largely unaffected (52). In addition, this concentration (30 μM) is close to the EC50 (of 50 μM) for 4-AP block of human atrial IKur (44).

Outward currents were recorded in the absence (Fig. 4, A and B) and presence (Fig. 4, C and D) of 30 μM 4-AP, and the waveforms of the 30 μM 4-AP-sensitive currents (Fig. 4, E and F) were obtained by offline digital subtraction. As illustrated in Fig. 4, E and F, rapidly activating and slowly inactivating outward currents are blocked by 30 μM 4-AP in GFP-negative and in GFP-positive LV cells. The density of the 30 μM 4-AP-sensitive currents, however, is significantly (P < 0.005) higher in GFP-positive (Fig. 4F) than in GFP-negative (Fig. 4E) LV cells. The means ± SE (peak) densities of the 30 μM 4-AP-sensitive outward currents at +50 mV, for example, were 64 ± 6.5 pA/pF (n = 7) and 9 ± 1 pA/pF (n = 12) in GFP-positive and GFP-negative LV myocytes, respectively. The voltage dependencies of activation of the 30 μM 4-AP-sensitive outward K+ currents in wild-type and GFP-expressing LV cells, however, are indistinguishable (not illustrated).

Analysis of the 30 μM 4-AP-sensitive currents in GFP-positive cells (Fig. 4F) revealed that the decay phases of the currents (at test potentials positive to +10 mV) are well described by a single exponential with a mean ± SE (n = 7) inactivation time constant (τ_{decay}) of 1,600 ± 74 ms. This value is significantly (P < 0.001) larger than the mean ± SE (n = 12) τ_{decay} of 830 ± 107 ms for the (4-AP sensitive) component of I_{Kslow} remaining in Kv2.1N206F lag-expressing ventricular cells (51). As is also evident in Fig. 4D, the rapidly inactivating I_{to,f} is unmasked in the Kv1.5-GFP-expressing cells in the presence of 30 μM 4-AP. Importantly, the means ± SE densities of I_{to,f} in GFP-positive (26.4 ± 3.1 pA/pF at +40 mV; n = 18) and GFP-negative (means ± SE = 28.2 ± 3.7 pA/pF at +40 mV; n = 18) LV myocytes are not significantly different. The density of the current remaining at the end of long depolarizing voltage steps, I_{ss}, in GFP-positive (Fig. 4D) and GFP-negative (Fig. 4C) cells are also similar (see DISCUSSION).

Electrophysiological experiments similar to those illustrated in Figs. 3 and 4 were also completed on cells isolated from the LV septum of the Kv1.5-GFP-expressing transgenics. As in the randomly dispersed LV myocyte preparations (Fig. 3), there was considerable variability evident in GFP expression among LV septum cells. Similar results are seen in cells isolated from the LV apex and base, suggesting that the heterogeneity in expression seen in randomly dispersed LV cells does not reflect region-specific differences in Kv1.5-GFP expression (see below). Previous studies have shown that, in addition to I_{Kslow}, I_{ss}, and (sometimes) I_{to,f} LV septum cells also express I_{to,s} (52) recently shown to be encoded by Kv1.4 (17, 18). Analysis of the 30 μM 4-AP-insensitive K+ currents in GFP-positive LV septum cells revealed that I_{to,s} densities (means ± SE = 7.9 ± 0.6 pA/pF; n = 7) in these cells are not significantly different from I_{to,s} densities (means ± SE at +40 mV = 7.4 ± 0.7 pA/pF; n = 26) in wild-type LV septum cells (17, 52). Taken together, these results demonstrate that the expression of Kv1.5-GFP does not measurably affect the expression of mouse ventricular I_{to,f}, I_{to,s}, or I_{ss} (see DISCUSSION). In addition, the finding that Kv1.5-GFP expression has no measurable effect(s) on I_{to,s} is consistent with previous suggestions.
that Kv1.5 and Kv1.4 do not coassemble in mouse ventricles in vivo (17, 18, 27) (see DISCUSSION).

Effect of Kv1.5-GFP-expression on ventricular action potentials. Whole cell current-clamp experiments (6, 17, 51) conducted at room temperature (25°C) revealed that action potential durations in Kv1.5-GFP-expressing adult mouse ventricular myocytes are attenuated significantly ($P < 0.001$) compared with action potentials recorded from wild-type cells (Fig. 5A). Means ± SE action potential durations at 50% and 75% repolarization, for example, were 5.3 ± 0.4 and 10.1 ± 1.1 ms ($n = 16$) in wild-type cells and 3.3 ± 0.4 and 5.8 ± 1.1 ms ($n = 12$) in Kv1.5-GFP-expressing cells (Table 2). In contrast, action potential amplitudes and resting membrane potentials in GFP-positive and GFP-negative ventricular myocytes are not significantly different (Table 2). Importantly, recent studies have documented marked differences in action potential waveforms in mouse LV myocytes as a function of recording temperature and stimulation frequency (18). As illustrated in Fig. 5B, action potentials recorded in GFP-expressing LV myocytes at 35°C (10 Hz) are briefer than the action potentials recorded from GFP-negative (wild type) cells under the same experimental conditions. In contrast to the results obtained at room temperature, however, action potential durations at 50% and 75% repolarization are similar in GFP-positive and GFP-negative LV myocytes (Table 2). At 35°C (10 Hz), action potential durations are significantly ($P < 0.005$) shorter in the Kv1.5-GFP-expressing (than in wild type) cells only when measured at 90% repolarization (Fig. 5B, Table 2). Thus, despite the large differences in outward $K^+$ current waveforms in GFP-positive and GFP-negative LV myocytes (Fig. 3), at physiological temperature, the effect of “overexpression” of Kv1.5-GFP-encoded $K^+$ channels is quite small (see DISCUSSION).

To explore the functional consequences of Kv1.5-GFP expression, telemetric electrocardiogram recordings (17, 51) were obtained from Kv1.5-GFP-expressing transgenic and nontransgenic littermates; representative electrocardiographic recordings are presented in Fig. 5, C and D. No significant differences in heart rates or in the appearances and/or morphologies of the P waves, QRS complexes, or T waves were evident in the recordings obtained from the Kv1.5-GFP-expressing, compared with wild-type, mice. There were also no significant differences in PQ, QRS, or Q-T intervals seen in the Kv1.5-GFP-expressing animals compared with controls; Q-T intervals, for example, were 27 ± 3.5 (n = 7) ms and 26 ± 3 ms (n = 4) in wild-type and transgenic mice, respectively.

Heterogeneous expression of Kv1.5-GFP in mouse ventricle in situ. As noted above, the initial electrophysiological experiments revealed that only ~50% of the ventricular myocytes isolated from adult Kv1.5-
GFP-expressing transgenic animals were fluorescent, i.e., GFP positive (Fig. 3F). Similar results were obtained when myocytes were isolated from different regions of the LV, as well as in experiments completed on animals from line 1 \((n = 5)\) and line 9 \((n = 5)\), suggesting that expression of the Kv1.5-GFP transgene is heterogeneous. Recently, however, it was reported that Kv1.5 mRNA in rat ventricular cells is downregulated rapidly following cell isolation, an effect attributed to the loss of cell-cell contact upon dispersion (19). These observations suggested that the heterogeneity in the expression of Kv1.5-GFP observed in the electrophysiological experiments here (when the cells are visualized 12–24 h after dissociation) could be an artifact of cell isolation. To test this hypothesis directly, myocytes were fixed immediately (within minutes) after isolation and visualized. Similar to the observations made on cells in vitro for 12–24 h, these experiments revealed that GFP fluorescence was clearly evident in only \(\leq 50\%\) of the cells freshly isolated from the Kv1.5-GFP-expressing animals (Fig. 6, A and B). The absence of detectable GFP fluorescence

![Fig. 5. Functional consequence(s) of Kv1.5-GFP expression. A and B: whole cell current-clamp recordings reveal that action potentials in Kv1.5-GFP-expressing ventricular myocytes are shortened relative to those typically recorded in wild-type ventricular myocytes at 25°C (1 Hz) \((A)\) or at 35°C (10 Hz) \((B)\) (see also Table 2). C and D: representative electrocardiogram recordings from wild-type \((C)\) and Kv1.5-GFP-expressing \((D)\) animals (see METHODS). No significant differences in P or T waves, QRST durations, R-R or Q-T intervals were evident (see text); Q-T intervals are labeled.](http://ajpheart.physiology.org/)

**Table 2. Resting and active membrane properties of wild-type and Kv1.5-GFP-expressing adult mouse ventricular myocytes**

<table>
<thead>
<tr>
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<th>25°C (1 Hz)</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>RMP, mV</td>
<td>APA, mV</td>
<td>APD(_{50}), ms</td>
<td>APD(_{75}), ms</td>
</tr>
<tr>
<td>Wild-type</td>
<td>16</td>
<td>(-76.1 \pm 1.1)</td>
<td>(135 \pm 4.1)</td>
<td>(5.3 \pm 0.4)</td>
<td>(10.1 \pm 1.1)</td>
</tr>
<tr>
<td>Kv1.5-GFP</td>
<td>12</td>
<td>(-74.5 \pm 1.5)</td>
<td>(120 \pm 5.5)</td>
<td>(3.3 \pm 0.4^*)</td>
<td>(5.8 \pm 1.1^*)</td>
</tr>
<tr>
<td>35°C (10 Hz)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild-type</td>
<td>5</td>
<td>(-78.3 \pm 1.2)</td>
<td>(130 \pm 5.7)</td>
<td>(2.7 \pm 0.4)</td>
<td>(5.5 \pm 1.0)</td>
</tr>
<tr>
<td>Kv1.5-GFP</td>
<td>5</td>
<td>(-75.6 \pm 1.6)</td>
<td>(124 \pm 3.8)</td>
<td>(2.5 \pm 0.1)</td>
<td>(4.5 \pm 0.3)</td>
</tr>
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</table>

All values are means \(\pm SE\); \(n\), number of myocytes. RMP, resting membrane potential; APA, action potential amplitudes; APD\(_{50}\), APD\(_{75}\), APD\(_{90}\), action potential depolarization at 50, 75, and 90% repolarization, respectively. *Values are significantly different \((P < 0.005)\) from those for wild-type at 25°C (1 Hz); †value is significantly different \((P < 0.005)\) from that for wild-type at 35°C (10 Hz).
in some cells likely does not simply reflect a low level of (Kv1.5-GFP) protein expression, because similar observations were made when isolated cells were probed with an anti-GFP antibody (Fig. 6, C and D). In addition, in cryostat sections of Kv1.5-GFP-expressing ventricles (n = 5), the heterogeneity in transgene expression is clear (Fig. 6, E–G). There is also heterogeneity in the intensity of the GFP fluorescence, from very bright in some cells to quite dim in others, and virtually undetectable in the rest (Fig. 6, E–G). These observations suggest that transgene expression is indeed heterogeneous in situ.

Fig. 6. Heterogeneous expression of Kv1.5-GFP. A and B: confocal images of ventricular myocytes freshly isolated from Kv1.5-GFP-expressing mice following fixation and counterstaining with propidium iodide (PI) to allow nuclei to be visualized (see METHODS). Although there are clearly 3 cells in the field (B) only two are GFP-positive (A). When freshly isolated myocytes are fixed and probed with an anti-GFP antibody (C and D), similar results are obtained. In the example shown (C and D), PI staining revealed 4 cells in the field (D), 2 of which are GFP-positive, as revealed by the endogenous GFP fluorescence (C) or on probing with the anti-GFP antibody (D). GFP expression in situ is also heterogeneous, as illustrated in representative cryostat sections (15 μm) from the septum (E), right ventricle (F) and left ventricle (G) of a Kv1.5-GFP-expressing adult mouse; all sections were counterstained with PI. To illustrate the heterogeneity of transgene expression, confocal images were obtained from the same field using the 494-nm or the 535-nm laser line for detection of GFP or PI fluorescence, respectively, and are superimposed here. Arrowheads indicate nuclei of cells that are not GFP positive, asterisks indicate cells in which the intensities of the GFP fluorescence are markedly different.
DISCUSSION

Expression of Kv1.5-GFP in transgenic mice. The goal of the experiments here was to explore the functional effects of cardiac-specific expression of a GFP-tagged Kv1.5 α-subunit in the mouse heart. GFP was chosen as the epitope tag to allow visualization and monitoring of Kv1.5 expression in vivo and in vitro. Heterologous expression of Kv1.5-GFP in HEK-293 cells reveals voltage-gated K⁺ channel currents with time- and voltage-dependent properties indistinguishable from wild-type Kv1.5, demonstrating that the presence of GFP tag does not adversely affect the expression or the functioning of Kv1.5 channels. The Kv1.5-GFP coding sequence was then cloned downstream from the α-MHC promoter in the α-MHC expression vector (35, 39); DNA was prepared and injected into C57BL6 mouse blastocytes, and two lines (lines 1 and 9) of Kv1.5-GFP-expressing transgenic mice were established and characterized.

Importantly, on gross examination, there were no obvious differences noted when Kv1.5-GFP-expressing and wild-type mice or isolated hearts were compared. Histological and echocardiographic studies revealed no evidence of ventricular hypertrophy, chamber dilation, and/or contractile dysfunction. With respect to structure and function, therefore, the experiments completed here demonstrate that the Kv1.5-GFP-expressing animals are phenotypically “normal.” Only the electrical properties of individual Kv1.5-GFP-expressing LV myocytes are affected in these animals. These findings suggest that the myocardial hypertrophy described in transgenic animals expressing wild-type GFP (20) does not simply reflect or result from the presence of the GFP protein in the myocardium, as previously suggested. There are several other possible interpretations of the rather striking phenotype reported by Huang et al. (20), including effects due to insertion site, copy number, or, perhaps, a direct toxic effect of the GFP construct employed. Regardless of the correct explanation, the findings here that the hearts of animals expressing Kv1.5-GFP are indistinguishable from wild-type hearts clearly demonstrate that the expression of GFP per se does not cause and need not be associated with pathology, as was previously suggested (20).

Heterogeneous expression of Kv1.5-GFP. Unexpectedly, GFP was not detected in all ventricular cells isolated from Kv1.5-GFP-expressing animals. On the basis of intrinsic GFP fluorescence or staining with a specific monoclonal anti-GFP antibody, ~50% of the ventricular myocytes isolated from these animals express the transgene. In addition, the heterogeneity in GFP fluorescence was correlated directly with the electrophysiology in that outward currents recorded from GFP-negative ventricular myocytes (isolated from the Kv1.5-GFP-expressing transgenics) are indistinguishable from those recorded in wild-type myocytes, whereas outward K⁺ current densities and waveforms in GFP-positive cells are markedly different from the currents in wild-type cells. The heterogeneity is also not an artifact of cell isolation because GFP is also only evident in ~50% of the cells in the ventricles (Fig. 6) and in the atria (not illustrated) of Kv1.5-GFP-expressing animals in situ.

In previous studies using the α-MHC expression vector, we have not observed heterogeneity in transgene expression (6, 18, 50, 51). To our knowledge, there have been no other prior reports of heterogeneity in the expression in the myocardium of transgenes driven by the α-MHC promoter (35, 39). Although it might certainly be suggested that small differences in expression might go undetected, particularly in functional assays, it is clear that marked heterogeneity, such as observed in the present study, would have been readily detected in previous studies (6, 18, 50, 51). The simplest interpretation of these observations, therefore, is that the lack of GFP fluorescence in some cells reflects silencing of transgene expression owing to (specific sequences in) the GFP itself. If this hypothesis is correct, then one would expect to see marked heterogeneity in the expression of other GFP-tagged transgenes, an effect that could seriously compromise the usefulness of GFP as a viable marker of in vivo or in vitro gene expression.

Selective increase in a “novel” 4-AP-sensitive K⁺ current in Kv1.5-GFP-expressing cells. Whole cell, voltage-clamp recordings from Kv1.5-GFP-expressing ventricular cells revealed that peak outward K⁺ currents are significantly higher and that the waveforms of the outward K⁺ currents are distinct from those in wild-type cells. In current-clamp recordings, action potential depolarization at 50% and 75% values are decreased significantly in Kv1.5-GFP-expressing ventricular myocytes, consistent with the observed increases in peak outward K⁺ current densities. Pharmacological experiments revealed that expression of Kv1.5-GFP results in a selective increase in the density of a I_{K,slow} sensitive to low (30 μM) concentrations of 4-AP. In the presence of 30 μM 4-AP, outward K⁺ current waveforms in GFP-positive and GFP-negative cells are indistinguishable (Fig. 4). In addition, the results of the experiments completed here demonstrate that the expression of Kv1.5-GFP and the large increase in the density of the slowly inactivating current in mouse ventricular cells do not measurably alter the functional expression levels or the properties of I_{to,5}, I_{to,s}, or I_{SS}. Previous studies have shown that targeted deletion of Kv1.4 (26) eliminates I_{to,s}, but does not affect I_{K,slow} (17, 18), suggesting that the endogenous Kv1.4 and Kv1.5 α-subunit proteins do not coassemble in mouse ventricle in vivo. The lack of effect of Kv1.5-GFP expression on I_{to,s} in GFP-positive ventricular myocytes suggests that the heterologously expressed Kv1.5-GFP also does not coassemble with the endogenous Kv1.4 protein.

Relationship to previous studies. It has previously been reported that I_{K,slow} is attenuated or eliminated in ventricular myocytes isolated from transgenic mice expressing a truncated Kv1.1 α-subunit Ko1.1N206Tag that functions as a dominant negative (25). In addition, Kv1.5 protein expression is decreased in the hearts of...
Kv1.1N206Tag-expressing transgenic mice (25). These observations, together with the finding that mouse ventricular \( I_{K_{\text{slow}}} \) is sensitive to micromolar concentrations of 4-AP (15), led to the hypothesis that Kv1.5 underlies \( I_{K_{\text{slow}}} \) (15, 25, 53). Subsequently, it was shown that there are two components of mouse ventricular \( I_{K_{\text{slow}}} \) (52), and that the TEA-sensitive component of \( I_{K_{\text{slow}}} \) is eliminated in ventricle myocytes isolated from transgenic mice expressing a mutant Kv2.1 α-subunit that functions as a dominant negative (51). The micromolar 4-AP-sensitive component of \( I_{K_{\text{slow}}} \), however, remains in ventricular myocytes from these animals (51). More recently, it was shown that the \( I_{K_{\text{slow}}} \) component sensitive to micromolar 4-AP is eliminated in ventricular myocytes isolated from animals in which Kv1.5 has been deleted and replaced with the Kv1.1 coding sequence (27), thereby directly demonstrating that Kv1.5 encodes the micromolar 4-AP-sensitive \( I_{K_{\text{slow}}} \).

Although the 30 μM 4-AP-sensitive currents in GFP-positive ventricular myocytes (Fig. 4P) activate fast and are similar to \( I_{K_{\text{slow}}} \) in wild-type cells, the decay rates of the currents are significantly slower than that of \( I_{K_{\text{slow}}} \) in wild-type cells. Because it has, as noted above, been shown that targeted deletion of Kv1.5 eliminates the micromolar 4-AP-sensitive component of \( I_{K_{\text{slow}}} \), the slow kinetics was unexpected and suggests differences in the molecular compositions/structures of the endogenous and heterologously expressed Kv1.5-encoded K⁺ channels. There are splice variants of the Kv1.5 mRNA (4), and it certainly seems possible that these could play a role in the generation of endogenous \( I_{K_{\text{slow}}} \) channels, whereas the heterologously expressed Kv1.5-GFP protein will be full length. Alternatively, there could be differences in posttranslational modifications, such as protein phosphorylation (1, 7, 30), and/or in the association of Kv1.5 with auxiliary (β and minK) subunits (12, 42) of the endogenous and heterologously expressed Kv1.5 subunit, and these could contribute to the observed differences in current properties. Association of β-subunits with some members of the Kv1 family, for example, results in αβ-heteromultimers with inactivation kinetics more rapid than those of the corresponding α-homomultimers (42). It is certainly possible, therefore, that the “overexpressed” Kv1.5 α-subunits in the Kv1.5-GFP-expressing ventricular cells form channels that do not display the same inactivation kinetics as those of wild-type Kv1.5-encoded \( I_{K_{\text{slow}}} \) channels, because necessary accessory subunits are not properly assembled. Further experiments aimed at examining directly the roles of posttranslational modifications of Kv1.5 and other Kv α-subunits and associations with accessory subunits in the generation of functional voltage cardiac K⁺ channels are clearly warranted.

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EXPRESSION OF KV1.5-GFP IN MOUSE HEART

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