Molecular basis of transient outward K^+ current diversity in mouse ventricular myocytes

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1. Two kinetically and pharmacologically distinct transient outward K^+ currents, referred to as $I_{to,f}$ and $I_{to,s}$, have been distinguished in mouse left ventricular myocytes. $I_{to,f}$ is present in all left ventricular apex cells and in most left ventricular septum cells, whereas $I_{to,s}$ is identified exclusively in left ventricular septum cells.

2. Electrophysiological recordings from ventricular myocytes isolated from animals with a targeted deletion of the *Kv1.4* gene ($Kv1.4^{-/-}$ mice) reveal that $I_{to,s}$ is undetectable in cells isolated from the left ventricular septum ($n = 26$). $I_{to,f}$ density in both apex and septum cells, in contrast, is not affected by deletion of *Kv1.4*.

3. Neither the 4-AP-sensitive, slowly inactivating K^+ current, $I_{K,slow}$, nor the steady-state non-inactivating K^+ current, I_{SS}, is affected in $Kv1.4^{-/-}$ mouse left ventricular cells.

4. In myocytes isolated from transgenic mice expressing a dominant negative Kv4.2 α subunit, Kv4.2W362F, $I_{to,f}$ is eliminated in both left ventricular apex and septum cells. In addition, a slowly inactivating transient outward K^+ current similar to $I_{to,s}$ in wild-type septum cells is evident in myocytes isolated from left ventricular apex of Kv4.2W362F-expressing transgenics. The density of $I_{to,s}$ in septum cells, however, is unaffected by Kv4.2W362F expression.

5. Western blots of fractionated mouse ventricular membrane proteins reveal a significant increase in Kv1.4 protein level in Kv4.2W362F-expressing transgenic mice. The protein levels of other Kv α subunits, Kv1.2 and Kv2.1, in contrast, are not affected by the expression of the Kv4.2W362F transgene.

6. The results presented here demonstrate that the molecular correlates of $I_{to,f}$ and $I_{to,s}$ in adult mouse ventricle are distinct. Kv1.4 underlies mouse ventricular septum $I_{to,s}$, whereas Kv α subunits of the Kv4 subfamily underlie mouse ventricular apex and septum $I_{to,f}$. The appearance of the slow transient outward K^+ current in Kv4.2W362F-expressing left ventricular apex cells with properties indistinguishable from $I_{to,s}$ in wild-type cells is accompanied by an increase in Kv1.4 protein expression, suggesting that the upregulation of Kv1.4 underlies the observed electrical remodeling in Kv4.2W362F-expressing transgenics.

The amplitudes and the durations of action potentials in cardiac cells are largely determined by voltage-gated K^+ channels and, in most cells, two types of voltage-gated K^+ channel currents have been distinguished based on differences in time- and voltage-dependent properties and pharmacological sensitivities: (1) rapidly activating and inactivating, 4-aminopyridine-(4-AP)-sensitive K^+ currents, referred to as I_{to} (transient outward); and (2) delayed, slowly inactivating and typically 4-AP-insensitive, K^+ currents, referred to as I_K (delayed rectifiers). These are broad classifications, however, and it is now clear that there are multiple components of I_K, and that the densities, properties and functional roles of I_{to} and I_K vary in cardiac cells isolated from different species, as well as in cells from different regions of the heart in the same species (Anumonwo *et al.* 1991; Antzelevitch *et al.* 1995; Barry & Nerbonne, 1996). There are also marked changes in the densities and the properties of voltage-gated K^+ currents in the heart during normal development (Wetzel & Klitzner, 1996; Nerbonne, 1998) and in various myocardial disease states (Ten Eick *et al.* 1989; Boyden & Jeck, 1995; Näbauer & Kaab, 1998). For all of these reasons, there is considerable

interest in defining the properties and the molecular correlates of cardiac I_{to} and I_K channels, and in delineating the molecular mechanisms regulating the functional expression of these channels.

Rapidly activating and inactivating Ca^{2+}-independent, depolarization-activated transient outward, K^+-selective currents, referred to as I_{to1}, I_{to} or I_t, have been identified in a number of cardiac cell types (Campbell *et al.* 1995; Barry & Nerbonne, 1996; Giles *et al.* 1996; Nerbonne, 1998). The time- and voltage-dependent properties of I_{to} in most cells are similar in that activation, inactivation and recovery from steady-state inactivation are all rapid (Campbell *et al.* 1995; Barry & Nerbonne, 1996; Giles *et al.* 1996). In rabbit atrial and ventricular myocytes, however, inactivation of I_{to} (I_t) is slow and recovery from steady-state inactivation is very slow, with complete recovery requiring seconds (Giles & Imaizumi, 1988; Fedida & Giles, 1991; Wang *et al.* 1999). Recently, it was also reported that there are regional differences in the properties of I_{to} in human (Näbauer *et al.* 1996), ferret (Brahmajothi *et al.* 1999), rat (Wickenden *et al.* 1999) and mouse (Xu *et al.* 1999) ventricles. The rates of I_{to} inactivation and recovery (from steady-state inactivation), for example, are significantly slower in human and ferret left ventricular endocardial (than epicardial) cells (Näbauer *et al.* 1996; Brahmajothi *et al.* 1999). In adult mouse ventricular myocytes, two transient outward K^+ currents, similar to those characterized in the human and ferret, have also been distinguished; they have been referred to as $I_{to,fast}$ ($I_{to,f}$) and $I_{to,slow}$ ($I_{to,s}$) (Xu *et al.* 1999). In addition, $I_{to,f}$ and $I_{to,s}$ are differentially distributed in mouse ventricle: $I_{to,f}$ is present in both left ventricular apex and septum cells, whereas $I_{to,s}$ is only identified in left ventricular septum cells (Xu *et al.* 1999). Ferret epicardial I_{to} and mouse ventricular $I_{to,f}$ have similar kinetic properties and both are blocked by nanomolar concentrations of *Heteropoda* toxins (HpTx) (Sanguinetti *et al.* 1997), whereas ferret endocardial I_{to} and mouse ventricular $I_{to,s}$ are characterized by slow inactivation and recovery (from steady-state inactivation) kinetics and both currents are unaffected by HpTx (Brahmajothi *et al.* 1999; Xu *et al.* 1999).

In ferret, there are also regional differences in the expression patterns of the voltage-gated (Kv), pore-forming (α) subunits Kv1.4, Kv4.2 and Kv4.3 (Brahmajothi *et al.* 1999). These observations have been interpreted as suggesting that distinct Kv α subunits underlie I_{to} in ferret left ventricular endocardial (Kv1.4) and epicardial (Kv4.2/Kv4.3) cells (Brahmajothi *et al.* 1999). Several recent studies have provided direct evidence to support a role for Kv α subunits of the Kv4 subfamily in the generation of the rapidly activating and inactivating current $I_{to,f}$ in rat and mouse ventricular myocytes (Fiset *et al.* 1997*b*; Johns *et al.* 1997; Barry *et al.* 1998), although the molecular identity of mouse ventricular $I_{to,s}$ has not been determined. To test directly the hypothesis that Kv1.4 underlies $I_{to,s}$ in mouse left ventricular septum cells, electrophysiological experiments were completed on myocytes isolated from the left ventricles of mice with a targeted deletion of the *Kv1.4* gene ($Kv1.4^{-/-}$; London *et al.* 1998*b*). Analysis of whole-cell voltage-clamp recordings from $Kv1.4^{-/-}$ ventricular myocytes revealed that $I_{to,s}$ in septum cells is selectively eliminated, whereas the currents in apex cells are unaffected by elimination of *Kv1.4*. In addition, in experiments conducted on left ventricular myocytes isolated from transgenic mice expressing a mutant Kv4.2 α subunit, Kv4.2W362F (Barry *et al.* 1998), $I_{to,f}$ is eliminated in both septum and apex cells, whereas $I_{to,s}$ in septum cells is unaffected. Analysis of the outward K^+ currents in Kv4.2W362F-expressing left ventricular apex cells reveals the presence of a 'novel' current indistinguishable from $I_{to,s}$ in septum cells. Importantly, however, $I_{to,s}$ density in septum cells is unaffected by Kv4.2W362F expression. Western blot analysis also reveals that Kv1.4 protein expression is increased in the ventricles of Kv4.2W362F-expressing animals. Taken together, these results demonstrate that Kv1.4 underlies mouse ventricular $I_{to,s}$, and suggest that upregulation of Kv1.4 in apex cells underlies the electrophysiological remodelling observed in Kv4.2W362F-expressing transgenic mice.

METHODS

Isolation of mouse ventricular myocytes

Ventricular cells were isolated from adult (6–8 weeks of age) wild-type C57BL6 mice ($n = 5$), mice with a targeted deletion of the *Kv1.4* gene ($Kv1.4^{-/-}$, $n = 2$, London *et al.* 1998*b*) and transgenic mice expressing a dominant negative Kv4.2 α subunit, Kv4.2W362F ($n = 3$, Barry *et al.* 1998) using a procedure previously developed and utilized to isolate rat cardiomyocytes (Xu *et al.* 1996). Briefly, hearts were excised from anaesthetized (5% halothane–95% O_2) animals, mounted on a Langendorf apparatus, and perfused retrogradely through the aorta with 40 ml of a Ca^{2+}-free Hepes-buffered Eagle's balanced salt solution (Gibco) supplemented with 6 mM glucose, amino acids and vitamins (Buffer A). Hearts were then perfused with 50 ml of Buffer A containing 0·8 mg ml^{-1} collagenase (Type II, Worthington) and 10 μM $CaCl_2$ (Buffer B), with the temperature of the perfusate and the tissue maintained at 37 °C. The enzyme solution was filtered (at 5 μm) and recirculated through the heart for approximately 15–20 min.

The heart was cut open following perfusion, and the ventricular septum and the top approximately 0·3 mm of tissue at the apex of the left ventricle were removed. These tissue pieces were placed separately in Buffer A supplemented with 1.25 mg ml^{-1} taurine, 5 mg ml^{-1} bovine serum albumin (BSA, Sigma) and 150 μM $CaCl_2$ (Buffer C). After mechanical dispersion by gentle trituration, cell suspensions were filtered to remove large undissociated tissue fragments, and the cells were collected by sedimentation. Isolated myocytes were resuspended in Buffer C, plated on laminin-coated 35 mm culture dishes, and kept in a 5% CO_2–95% air incubator at 37 °C. Approximately 30 min after plating, serum-free medium-199 (M-199, Irvine Scientific) supplemented with antibiotics (penicillin–streptomycin) was added. Ca^{2+}-tolerant ventricular myocytes attached to the laminin-coated dishes; damaged cells were removed by replacing the medium with fresh M-199 at 1 h after plating.

Cells were examined electrophysiologically within 36 h of isolation. No differences in the properties or in the densities of the currents were evident in cells examined at times varying from 1 to 36 h after isolation.

Electrophysiological recordings

The conventional whole-cell recording technique was employed to record Ca^{2+}-independent, depolarization-activated K^+ currents and action potential waveforms in isolated adult mouse left ventricular myocytes. Voltage-clamp and current-clamp recordings were obtained only from Ca^{2+}-tolerant, rod-shaped cells, and all experiments were conducted at room temperature (22–24 °C). For voltage-clamp recordings, the bath solution contained (mM): 136 NaCl, 4 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 5 $CoCl_2$, 10 Hepes, 0·02 tetrodotoxin and 10 glucose; pH 7·4 and 295–305 mosmol l^{-1}. In current-clamp experiments, the $CoCl_2$ and tetrodotoxin were omitted from the bath solution. Recording pipettes contained (mM): 135 KCl, 1 $MgCl_2$, 10 EGTA, 10 Hepes, 5 glucose; pH 7·2 and 300–310 mosmol l^{-1}. 4-Aminopyridine (4-AP, Sigma) and *Heteropoda* toxin-3 (HpTx-3, NPS Pharmaceuticals) stock solutions were prepared in the bath solution and diluted to the appropriate concentrations immediately before use. 4-AP and HpTx-3 were applied to isolated myocytes during recordings through narrow-bore capillary tubes (inner diameter 300 μm) placed within 200 μm of the cells.

Experiments were conducted using a Dagan 3900A amplifier (headstage gain $(\beta) = 1$, Dagan Corporation). Pipettes had resistances of 2–3 MΩ when filled with the recording solution. Junctional potentials were zeroed before gigaohm seals were formed. After establishing the whole-cell configuration, ±10 mV voltage steps from a holding potential of −70 mV were applied to allow measurements of whole-cell membrane capacitances and input resistances. Series resistances were estimated by dividing the time constant of the decay of the capacitive transient by the membrane capacitance. Whole-cell membrane capacitances and series resistances were routinely compensated electronically (≥ 85 %); voltage errors resulting from the uncompensated series resistance were ≤ 6 mV and were not corrected. Only data obtained from the cells with input resistance ≥ 0·7 GΩ were analysed. From a holding potential of −70 mV, voltage-gated outward K^+ currents were evoked during 400 ms or 4 s depolarizing voltage steps to potentials between −40 and +60 mV in 10 mV increments. The voltage steps were applied at 15 s intervals. To quantify the rates of recovery from steady-state inactivation, cells were first depolarized to +50 mV for 10 s to inactivate the currents (conditioning pulses), subsequently hyperpolarized to −70 mV for varying times ranging from 10 ms to 8 s, and finally stepped to +50 mV to activate the currents and assess the extent of recovery. In current-clamp recordings, action potentials were evoked by 3 ms suprathreshold current pulses at 1·0 Hz and recorded after reaching a steady state. Experimental data were collected using a Gateway microcomputer equipped with a Digidata 1200 Series analog/digital interface (Axon) and pCLAMP 7 software (Axon). Data were acquired at variable sampling frequencies and the current signals were filtered on-line at 5 kHz before digitization and storage.

Data analysis

Analysis of digitized data was completed using Clampfit 6.0.5 (Axon). Whole-cell membrane capacitances were calculated by integrating the capacitive transients elicited during ±10 mV voltage steps from a holding potential of −70 mV. Peak outward K^+ currents at each test potential were measured as the difference between the maximal outward current amplitudes and the zero current level. The waveforms of the 4-AP- and HpTx-3-sensitive currents were obtained in the same cells by offline digital subtraction of the currents recorded in the presence of 4-AP or HpTx-3 from the control currents.

The decay phases of the outward K^+ currents evoked during 4 s depolarizing voltage steps to test potentials between +10 and +60 mV (from a holding potential of −70 mV) can be fitted by the sum of two or three exponential functions using one of the following equations:

$$y(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + B$$

or

$$y(t) = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3 \exp(-t/\tau_3) + B,$$

where t is time; τ_1, τ_2, and τ_3 are the time constants of decay of the inactivating K^+ currents; A_1, A_2 and A_3 are the amplitudes of the inactivating current components; and B is the amplitude of the steady-state, non-inactivating component of the total outward K^+ currents. For all analyses, correlation coefficients (r) were determined to assess the quality of fits; r values for the fits reported here were ≥ 0·98. Although the decay phases of the outward K^+ currents in all left ventricular apex cells were well described by the sum of two exponentials, this was not the case for septum cells. For the majority of septum cells, the decay phases of the outward K^+ currents were not well fitted by two exponentials as judged by eye and by the correlation coefficients of the fits. For these cells, the quality of the fits was markedly improved when three exponentials were included. In contrast, the quality of the fits to the decay phases of the outward K^+ currents in apex cells was not improved by the inclusion of a third exponential. As reported previously (Xu *et al.* 1999), the time constants of decay of $I_{to,f}$, $I_{to,s}$ and $I_{K,slow}$ do not display any appreciable voltage dependence; all mean ± S.E.M. decay time constants (τ_{decay}) reported here were calculated from the decays of the total outward K^+ currents evoked at +40 mV in each cell. To quantify the rates of recovery from steady-state inactivation of $I_{to,f}$ and the slowly inactivating transient outward K^+ current ($I_{to,s}$) in wild-type and Kv4.2W362F transgenic animals, the decay phases of the currents following each recovery time were fitted to the sum of two exponentials to provide the amplitudes of $I_{to,f}$, $I_{to,s}$ and $I_{K,slow}$; the amplitudes of $I_{to,f}$ or $I_{to,s}$ were normalized to the amplitudes of these components determined during the corresponding conditioning pulses; mean ± S.E.M. normalized recovery data are plotted and fitted with single exponential functions. All average and normalized data are presented as means ± S.E.M. The statistical significance of observed differences between groups of cells or between different parameters describing the properties of the currents were evaluated using a one-way analysis of variance (ANOVA) followed by an F test or a two-tailed Student's t test; P values are presented in the text, and statistical significance was set at the $P < 0{\cdot}05$ level.

Western blots

Ventricular membrane proteins were prepared from adult wild-type, Kv1.4$^{-/-}$ and Kv4.2W362F transgenic mice using methods for the preparation of rat ventricular membrane proteins (Barry *et al.* 1995; Xu *et al.* 1996). Tissue samples used in the membrane preparations included the lower half of the heart from the apex and included both the left and right ventricles. The protein content of the solubilized membrane preparations was determined by using a Bio-Rad protein assay kit with BSA as the standard. For Western blots, membrane proteins (50 or 180 μg) were fractionated on 10 % SDS-polyacrylamide gels and transferred to Polyscreen PVDF membrane (DuPont). PVDF membrane strips were first incubated

Table 1. Comparison of K^+ current densities in wild-type, Kv1.4 knockout and Kv4.2W362F-expressing mice

	n	I_{peak} (pA pF^{-1})	$I_{to,f}$ (pA pF^{-1})	$I_{to,s}$ (pA pF^{-1})	$I_{K,slow}$ (pA pF^{-1})	I_{SS} (pA pF^{-1})
Wild-type						
Apex	43	57·4 ± 2·9	34·5 ± 2·3	—	17·4 ± 0·9	5·5 ± 0·4
Septum with $I_{to,f}$	28	31·1 ± 1·3	6·8 ± 0·4	7·5 ± 0·6	12·2 ± 0·5	4·6 ± 0·4
Septum without $I_{to,f}$	8	20·5 ± 2·2	—	5·5 ± 0·7	10·4 ± 1·3	4·7 ± 0·4
Kv1.4$^{-/-}$						
Apex	8	56·9 ± 1·4	34·7 ± 2·5	—	17·0 ± 1·4	5·2 ± 0·4
Septum with $I_{to,f}$	20	25·0 ± 1·1	9·1 ± 0·6	—	11·0 ± 0·6	4·9 ± 0·2
Septum without $I_{to,f}$	6	15·5 ± 0·9	—	—	10·2 ± 0·8	5·2 ± 0·3
Kv4.2W362F						
Apex	11	33·4 ± 3·5	—	12·5 ± 1·7	15·9 ± 1·8	5·0 ± 0·6
Septum with $I_{to,f}$	—	—	—	—	—	—
Septum without $I_{to,f}$	13	21·8 ± 1·9	—	6·4 ± 0·6	11·4 ± 1·4	4·0 ± 0·5

Current densities were determined from analyses of records obtained on depolarization to +40 mV from a holding potential of −70 mV.

in 0·2% I-Block (Tropix) in PBS containing 0·1% Tween 20 (blocking buffer) for 1 h at room temperature, followed by overnight incubation at 4 °C with polyclonal antibodies directed against Kv1.4 (Alamone Labs), Kv1.2 (Alamone Labs) and Kv2.1 (Upstate Biotechnology). All antibodies were tested for specificity and cross reactivity, as described previously (Barry *et al.* 1995). The anti-Kv1.4, anti-Kv1.2 and anti-Kv2.1 antibodies were used at dilutions of 1:200, 1:100 and 1:100, respectively, in blocking buffer. After washing (twice), the strips were incubated for 2 h at room temprature in alkaline phosphatase-conjugated goat anti-rabbit IgG (Tropix) diluted in 1:5000 in blocking buffer. Strips were washed three times in blocking buffer and twice in assay buffer (0·1 M diethanolamine and 1 mM $MgCl_2$, Tropix). Bound antibodies were detected by using the CPSD (Tropix) chemiluminescent alkaline phosphate substrate.

RESULTS

Regional differences in action potential waveforms in adult mouse ventricle

Recently it was reported that the waveforms of the Ca^{2+}-independent, depolarization-activated K^+ currents in cells isolated from mouse left ventricular apex and septum are distinct (Xu *et al.* 1999). In addition, peak outward K^+ current densities in cells isolated from the apex are significantly ($P < 0·001$) higher than in cells isolated from the septum (Table 1). In cells isolated from the apex, the rapidly inactivating and the slowly inactivating K^+ currents, $I_{to,f}$ and $I_{K,slow}$, are evident (Table 1), and there is a steady-state, non-inactivating component of the outward K^+ currents, referred to as I_{SS} (Xu *et al.* 1999). On average,

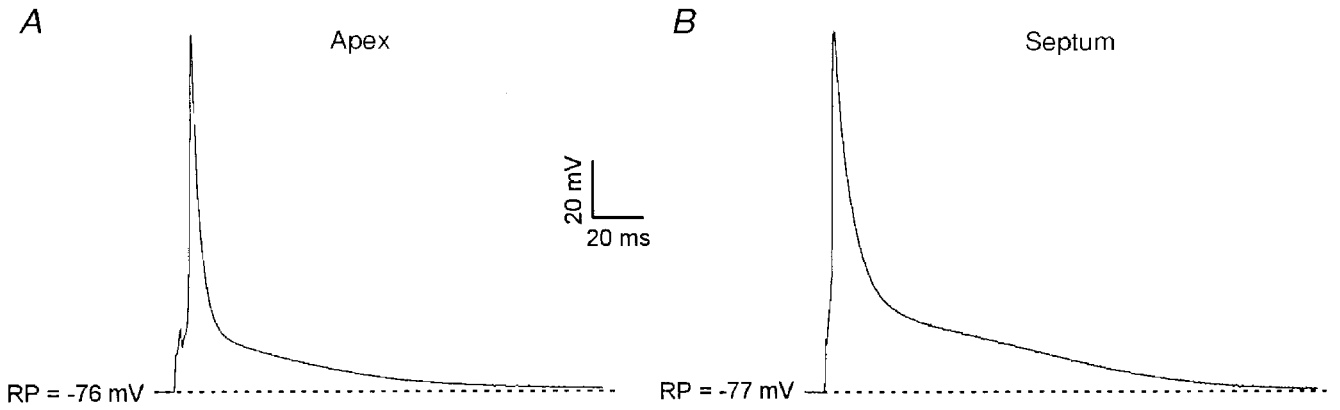

Figure 1. Action potential waveforms are distinct in apex and septum cells isolated from adult mouse left ventricle

Under current-clamp, action potentials evoked by 3 ms suprathreshold current pulses were recorded from adult mouse left ventricular apex (*A*) and septum (*B*) cells. Stimuli were applied at 1·0 Hz and action potentials were recorded after reaching a steady state. Action potentials are significantly broader in the septum cells; similar results were obtained in recordings from 7 apex and 8 septum cells.

Table 2. Comparison of action potential parameters in adult mouse left ventricular apex and septum cells

	APD_{25} (ms)	APD_{50} (ms)	APD_{75} (ms)	RP (mV)	APA (mV)
Apex ($n = 7$)	$2{\cdot}3 \pm 0{\cdot}1$	$4{\cdot}5 \pm 0{\cdot}3$	$10{\cdot}0 \pm 1{\cdot}2$	$-76{\cdot}2 \pm 0{\cdot}9$	$125{\cdot}6 \pm 2{\cdot}7$
Septum ($n = 8$)	$4{\cdot}0 \pm 0{\cdot}2$	$8{\cdot}5 \pm 0{\cdot}3$	$20{\cdot}9 \pm 1{\cdot}3$	$-77{\cdot}9 \pm 0{\cdot}7$	$128{\cdot}2 \pm 2{\cdot}6$

Mean ± S.E.M. action potential durations (APD) at 25% (APD_{25}), 50% (APD_{50}) and 75% (APD_{75}) of repolarization, resting membrane potentials (RP) and action potential amplitudes (APA) in apex and septum cells are summarized.

$I_{to,f}$, $I_{K,slow}$ and I_{SS} contribute ~60, 30 and 10%, respectively, to the peak outward K^+ currents in adult mouse left ventricular apex cells (Table 1). In approximately 80% of the septum cells studied, the decay phases of the outward K^+ currents were well described by the sum of three exponentials reflecting the presence of $I_{to,f}$, $I_{K,slow}$ and a distinct, slowly inactivating transient outward K^+ current, referred to as $I_{to,s}$ (Xu *et al.* 1999). Importantly, $I_{to,s}$ is present in septum cells but not in apex cells. In the remaining ~20% of septum cells, $I_{to,f}$ is absent and only $I_{to,s}$, $I_{K,slow}$ and I_{SS} are evident (Table 1). The densities of $I_{to,f}$ (when expressed) and $I_{K,slow}$ in septum cells are significantly ($P < 0{\cdot}01$) lower than in apex cells, whereas the densities of I_{SS} in apex and septum cells are similar (Table 1).

In mouse ventricular myocytes, $I_{to,f}$ plays a prominant role in action potential repolarization (Barry *et al.* 1998). The finding that the density of $I_{to,f}$ (and $I_{K,slow}$) is significantly lower in septum cells than in apex cells (Table 1), therefore, suggested that action potentials would probably be prolonged in septum cells, relative to action potentials in apex cells. Current-clamp recordings from isolated adult mouse left ventricular apex and septum cells confirmed this hypothesis (Fig. 1*A* and *B*), and quantitive analysis of action potential durations at 25% (APD_{25}), 50% (APD_{50})

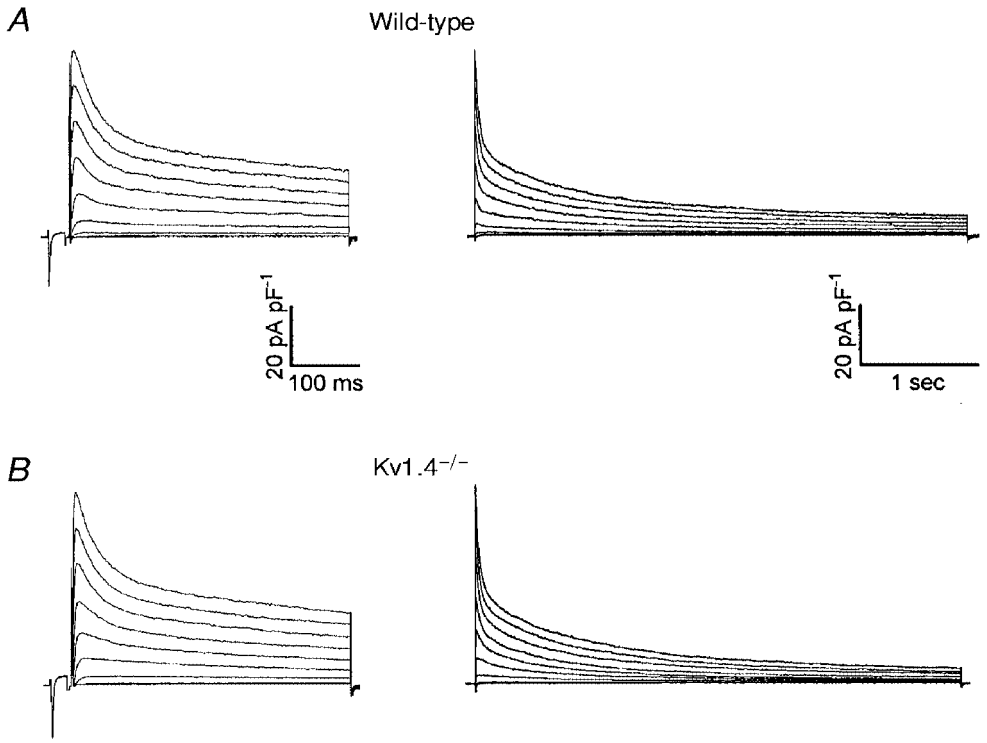

Figure 2. The waveforms of the Ca^{2+}-independent, depolarization-activated K^+ currents in myocytes isolated from the apex of wild-type (*A*) and Kv1.4$^{-/-}$ (*B*) adult mouse left ventricles are indistinguishable

Whole-cell outward K^+ currents were evoked during 400 ms (left) and 4 s (right) depolarizing voltage steps to potentials between −40 and +40 mV from a holding potential of −70 mV in 10 mV increments. Each trial was preceded by a short (25 ms) depolarization to −20 mV to eliminate contamination of inward Na^+ currents not completely blocked by TTX. The records shown in the left and right panels in *A* and *B* were obtained from the same cell.

and 75% (APD_{75}) repolarization revealed that action potentials are significantly ($P < 0{\cdot}01$) longer in septum than in apex cells (Table 2). In contrast, no differences in resting membrane potentials or in action potential amplitudes in apex and septum cells were observed (Table 2).

$I_{to,s}$ is eliminated in the ventricles of Kv1.4$^{-/-}$ mice

Although recently it was demonstrated that Kv4 α subunits underlie $I_{to,f}$ in mouse ventricular myocytes (Barry *et al.* 1998), the molecular identity of $I_{to,s}$ has not previously been defined. The slow rates of inactivation and recovery from steady-state inactivation of $I_{to,s}$ (Xu *et al.* 1999), however, are similar to the kinetic properties of one of the Kv α subunits, Kv1.4 (Rasmusson *et al.* 1995; Peterson & Nerbonne, 1999). In addition, Kv1.4 has recently been suggested to underlie the slow transient outward K^+ current in rabbit atrial myocytes (Wang *et al.* 1999), as well as in ferret (Brahmajothi *et al.* 1999) and human (Näbauer *et al.* 1998) left ventricular endocardial myocytes. To test directly the hypothesis that Kv1.4 also underlies $I_{to,s}$ in adult mouse left ventricular septum cells, electrophysiological experiments were completed on left ventricular myocytes isolated from mice with a targeted deletion of the *Kv1.4* gene (Kv1.4$^{-/-}$; London *et al.* 1998*b*). As illustrated in Fig. 2*B*, the waveforms of the outward voltage-gated K^+ currents in apex cells from Kv1.4$^{-/-}$ mice are similar to those recorded from wild-type animals (Fig. 2*A*). Similar results were obtained in eight cells and mean ± S.E.M. peak outward K^+ current densities in wild-type and Kv1.4$^{-/-}$ apex cells are indistinguishable (Table 1). There are also no measurable effects of the elimination of Kv1.4 on the densities (Table 1) or the kinetic properties of $I_{to,f}$, $I_{K,slow}$ and I_{SS} in apex cells.

In contrast to the findings in apex cells, peak outward K^+ current densities are significantly ($P < 0{\cdot}05$) lower in Kv1.4$^{-/-}$ septum cells than in wild-type septum cells (Table 1). As in wild-type septum cells (Fig. 3*A*), two populations of septum cells are isolated from Kv1.4$^{-/-}$ animals: cells with $I_{to,f}$ (Fig. 3*Ba*) and cells lacking $I_{to,f}$ (Fig. 3*Bb*). In approximately ~20% (6 of 26) of the Kv1.4$^{-/-}$ septum cells studied, outward current waveforms similar to those in Fig. 3*Bb* were recorded. This subset of cells lacks $I_{to,f}$ and there is a single component of outward current decay with a mean ± S.E.M. τ_{decay} of 1248 ± 73 ms (Fig. 3*Bb*). This slowly inactivating K^+ current is selectively blocked by micromolar concentrations of 4-aminopyridine (4-AP) similar to $I_{K,slow}$ in wild-type cells (Fiset *et al.* 1997*a*; London *et al.* 1998*a*; Zhou *et al.* 1998; Xu *et al.* 1999). Analysis of the outward K^+ currents in the Kv1.4$^{-/-}$ septum cells lacking $I_{to,f}$ under control conditions and following exposure to 50 μM 4-AP reveals that only $I_{K,slow}$ and I_{SS} are present; there is no evidence for $I_{to,s}$ in these cells (Fig. 4*A*).

In the majority (20 of 26) of the Kv1.4$^{-/-}$ septum cells studied, outward currents similar to those shown in Fig. 3*Ba* were recorded. In these $I_{to,f}$-expressing septum cells, the decay phases of the outward K^+ currents were well described by two exponentials with mean ± S.E.M. τ_{decay} values of

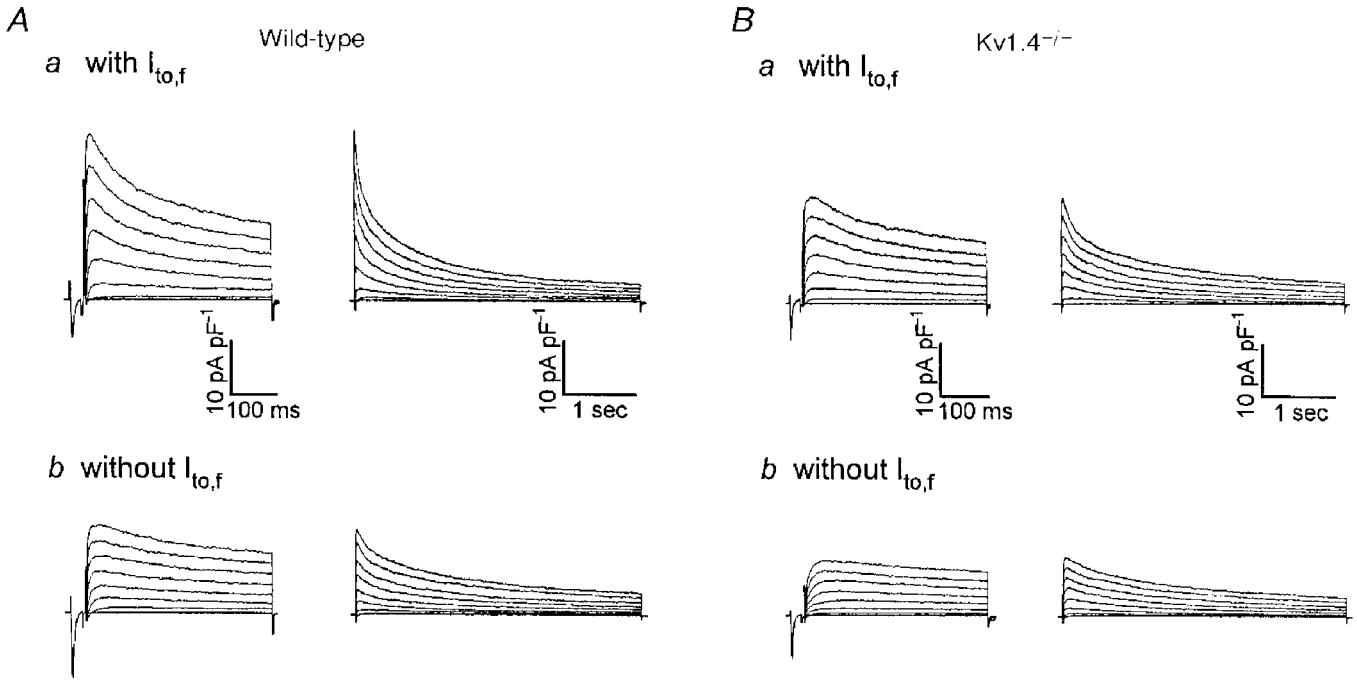

Figure 3. $I_{to,s}$ is eliminated in left ventricular septum cells isolated from Kv1.4$^{-/-}$ mice

Whole-cell outward K^+ currents were recorded as described in the legend to Fig. 2 from left ventricular septum cells isolated from wild-type (*A*) and Kv1.4$^{-/-}$ (*B*) mice. In cells isolated from the left ventricular septum of Kv1.4$^{-/-}$ mice, $I_{to,s}$ is undetectable (*B*); Kv1.4$^{-/-}$ septum cells express either only $I_{K,slow}$ and I_{SS} (*Bb*) or $I_{to,f}$, $I_{K,slow}$ and I_{SS} (*Ba*). In addition to the elimination of $I_{to,s}$, $I_{to,f}$ inactivation is slowed in septum cells isolated from Kv1.4$^{-/-}$ mice (see text).

122 ± 3 and 1336 ± 37 ms; these components contribute 36 and 44% to the peak outward K^+ currents, respectively. Although the slower time constant (1336 ± 37 ms) of current decay corresponds to $I_{K,slow}$, the faster time constant (τ_{decay} = 122 ± 3 ms) is significantly ($P < 0{\cdot}001$) slower than $I_{to,f}$ in wild-type septum cells (τ_{decay} = 53 ± 2 ms). This observation suggests either that a novel current is expressed in Kv1.4$^{-/-}$ septum cells or that the kinetics of $I_{to,f}$ inactivation is slowed in these animals. Experimental support for the latter hypothesis was provided in pharmacological experiments with *Heteropoda* toxin-3 (HpTx-3), which reportedly inhibits $I_{to,f}$ without measurably affecting $I_{to,s}$ (Xu *et al.* 1999). As illustrated in Fig. 4*B*, 300 nM HpTx-3 completely blocks the fast component of outward current decay in wild-type and in Kv1.4$^{-/-}$ septum cells. The mean ± S.E.M. τ_{decay} of the HpTx-3-sensitive currents in the Kv1.4$^{-/-}$ septum cells is 116 ± 17 ms (n = 3), a value that is significantly slower than the τ_{decay} values of the HpTx-3-sensitive current in wild-type cells. This τ_{decay} value (116 ± 17 ms) is, however, similar to the fast component of decay (122 ± 3 ms) derived from the double exponential fits to the decay phases of the outward currents in Kv1.4$^{-/-}$ septum cells. The simplest interpretation of these results is that 300 nM HpTx-3 also blocks $I_{to,f}$ in Kv1.4$^{-/-}$ septum cells but that the rate of $I_{to,f}$ decay is slowed in cells lacking Kv1.4 ($I_{to,s}$). Consistent with this hypothesis, analysis of the rates of recovery from steady-state inactivation of the outward K^+ currents in Kv1.4$^{-/-}$ septum cells revealed that recovery was characterized by the sum of two exponentials with recovery time constants of 42 ms and 1 s (n = 3, data

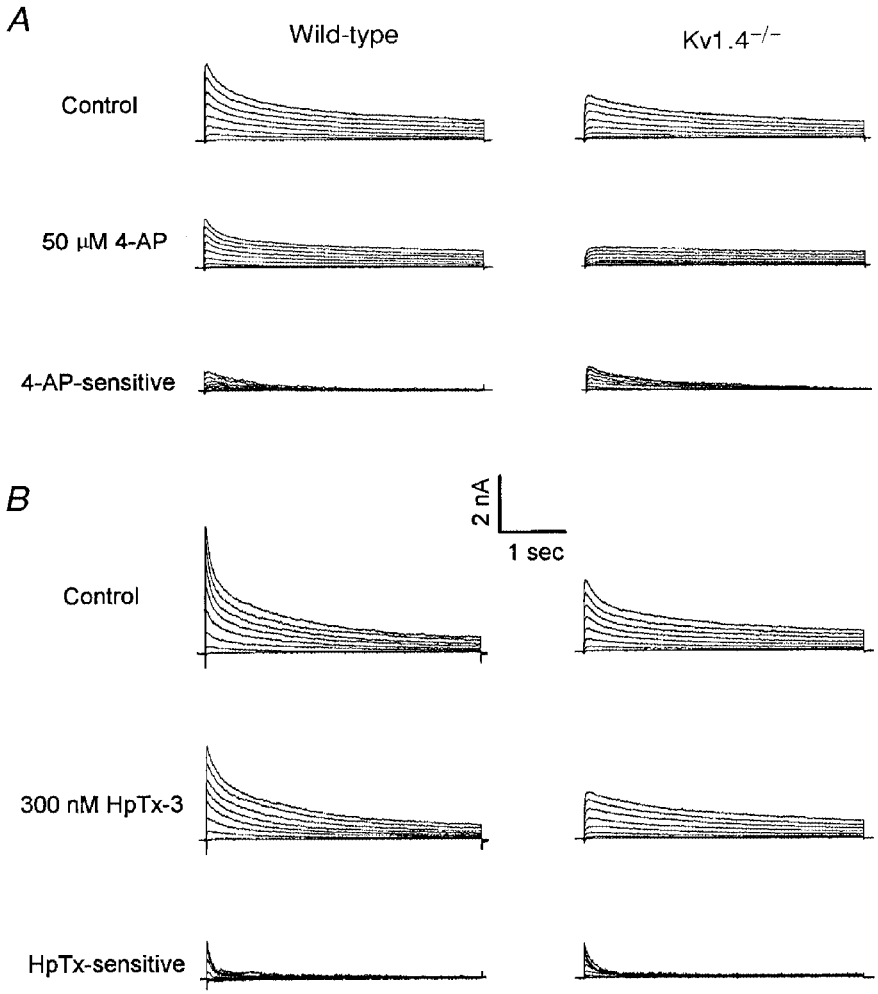

Figure 4. Effects of 50 μM 4-AP and 300 nM HpTx-3 on the outward K^+ currents in wild-type and Kv1.4$^{-/-}$ mouse left ventricular septum cells

Representative whole-cell outward K^+ currents recorded from adult mouse left ventricular septum cells lacking and expressing $I_{to,f}$ are displayed in *A* and *B*, respectively. To determine the effects of 50 μM 4-AP (*A*) and 300 nM HpTx-3 (*B*), control currents were recorded before exposure to the drug, and when the effect reached a steady state, the currents were recorded again. The waveforms of the 50 μM 4-AP- and 300 nM HpTx-3-sensitive currents were obtained by offline digital subtraction of the currents in the presence of 50 μM 4-AP or 300 nM HpTx-3 from the controls. Note that $I_{to,s}$ is clearly evident in wild-type septum cells, but undetectable in Kv1.4$^{-/-}$ septum cells, when $I_{K,slow}$ or $I_{to,f}$ is selectively blocked by micromolar concentrations of 4-AP (*A*) or nanomolar concentrations of HpTx-3 (*B*). Similar results were obtained in experiments on 3 cells of each group.

not shown); these values are indistinguishable from those of $I_{to,f}$ and $I_{K,slow}$ recovery in wild-type septum cells (Xu *et al.* 1999). Taken together, these results indicate that $I_{to,s}$ is absent and the kinetics of $I_{to,f}$ inactivation is altered (slowed) in Kv1.4$^{-/-}$ septum cells, whereas the kinetics of $I_{to,f}$ recovery are not affected. Further experiments will be necessary to explore the underlying mechanisms involved in mediating the selective modification of $I_{to,f}$ inactivation kinetics. It is of interest to note that, in spite of the loss of $I_{to,s}$ in Kv1.4$^{-/-}$ septum cells, the densities of $I_{to,f}$ (when evident), $I_{K,slow}$ and I_{SS} are not significantly different from those in wild-type septum cells (Table 1).

Elimination of $I_{to,f}$ and upregulation of $I_{to,s}$ in Kv4.2W362F-expressing mice

It has been demonstrated that the kinetic and pharmacological properties of $I_{to,f}$ in myocytes isolated from adult mouse left ventricular apex and septum are indistinguishable, suggesting that the molecular basis of $I_{to,f}$ in apex and septum cells is the same (Xu *et al.* 1999). This hypothesis was tested directly in experiments on myocytes isolated from the left ventricular apex and septum of transgenic mice expressing a mutant Kv4.2 α subunit, Kv4.2W362F, that functions as a dominant negative (Barry *et al.* 1998). In a previous study on myocytes randomly dispersed from the ventricles of Kv4.2W362F-expressing animals, it was reported that I_{to} ($I_{to,f}$) is eliminated and that a 'novel' slowly inactivating transient outward K^+ current (not detectable in wild-type cells) was evident (Barry *et al.* 1998). Representative outward K^+ current waveforms recorded from left ventricular apex (Fig. 5*A*) and septum (Fig. 5*B*) cells isolated from adult Kv4.2W362F transgenic mice are shown. As reported previously (Barry *et al.* 1998), peak outward K^+ current densities in these cells are substantially lower than in wild-type myocytes (Table 1). In all left ventricular septum cells examined ($n = 13$), the decay phases of the outward K^+ currents were well described by the sum of two exponentials with mean ± S.E.M. τ_{decay} values of 230 ± 21 and 1055 ± 67 ms, corresponding to $I_{to,s}$ and $I_{K,slow}$, respectively; $I_{to,f}$ was not detected in any of these cells. In addition, the densities of $I_{to,s}$, $I_{K,slow}$ and I_{SS} in Kv4.2W362F-expressing septum cells are not significantly different from the densities of these currents determined in wild-type septum cells (Table 1).

Analysis of the decay phases of the outward K^+ currents in left ventricular apex cells isolated from the Kv4.2W362F transgenics also revealed that $I_{to,f}$ is undetectable ($n = 11$, Table 1). In addition, 300 nM HpTx-3 had no measurable effect on the outward K^+ currents in these apex cells (Fig. 6*A*), consistent with the absence of $I_{to,f}$. Similar to the findings in randomly dispersed cells (Barry *et al.* 1998), a slowly inactivating transient outward K^+ current with a mean ± S.E.M. τ_{decay} of 209 ± 20 ms was detected in all Kv4.2W362F-expressing apex cells ($n = 11$). The pharmacological properties of this current are distinct from both $I_{to,f}$ and $I_{K,slow}$ in wild-type apex cells: the currents are not blocked by nanomolar concentrations of HpTx-3 which block $I_{to,f}$ (Fig. 6*A*) or by micromolar concentrations of 4-AP which block $I_{K,slow}$ (Fig. 6*B*). The τ_{decay} (209 ± 20 ms) for

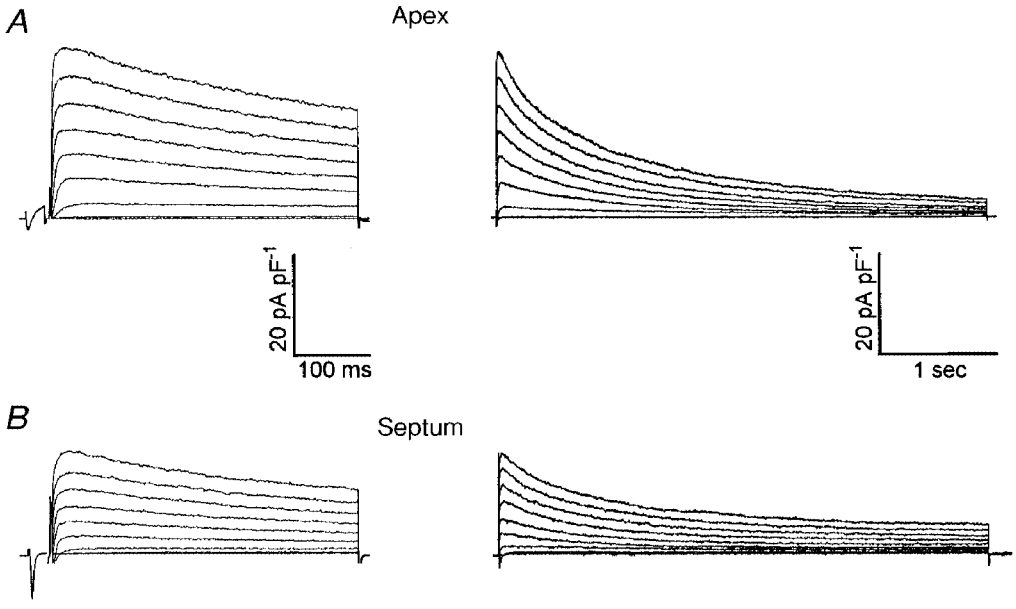

Figure 5. $I_{to,f}$ is eliminated in ventricular myocytes isolated from both the apex and the septum of adult Kv4.2W362F transgenic mice

Whole-cell outward K^+ currents were recorded from adult mouse left ventricular apex (*A*) and septum (*B*) cells as described in the legend to Fig. 2. The records shown in the left and right panels in *A* and *B* were obtained from the same cell, and only the durations of the voltage steps are different. As is evident, $I_{to,f}$ is undetectable in both apex and septum cells. Note that a slowly inactivating transient outward K^+ current ($I_{to,s}$), which was not detected in wild-type apex cells (see Fig. 2*A*), is evident in Kv4.2W362F-expressing apex cells.

this current, however, is similar to that of $I_{to,s}$ in wild-type septum cells (231 ± 11 ms, $n = 36$) and the currents are blocked by higher concentrations (0·5 mM) of 4-AP (data not shown). In addition, the slowly inactivating transient outward K^+ current evident in Kv4.2W362F-expressing apex cells recovers slowly from steady-state inactivation with a time constant of 1·0 s (Fig. 7*B* and *C*). This recovery rate is significantly slower than the rate of $I_{to,f}$ recovery ($\tau_{rec} = 28$ ms) (Fig. 7*A* and *C*), but is similar to the rate of $I_{to,s}$ recovery ($\tau_{rec} \approx 1{\cdot}3$ s, Xu *et al.* 1999). Taken together, these observations suggest that the slow transient outward K^+ current in Kv4.2W362F apex cells corresponds to $I_{to,s}$. If this interpretation is correct, the results further suggest that $I_{to,s}$ is selectively upregulated in the left ventricular apex, i.e. not in the left ventricular septum in the Kv4.2W362F-expressing transgenics (see Discussion).

Upregulation of Kv1.4 protein in Kv4.2W362F transgenic mice

The electrophysiological experiments described above revealed the presence of a slowly inactivating transient outward K^+ current in Kv4.2W362F-expressing left ventricular apex cells with kinetic and pharmacological

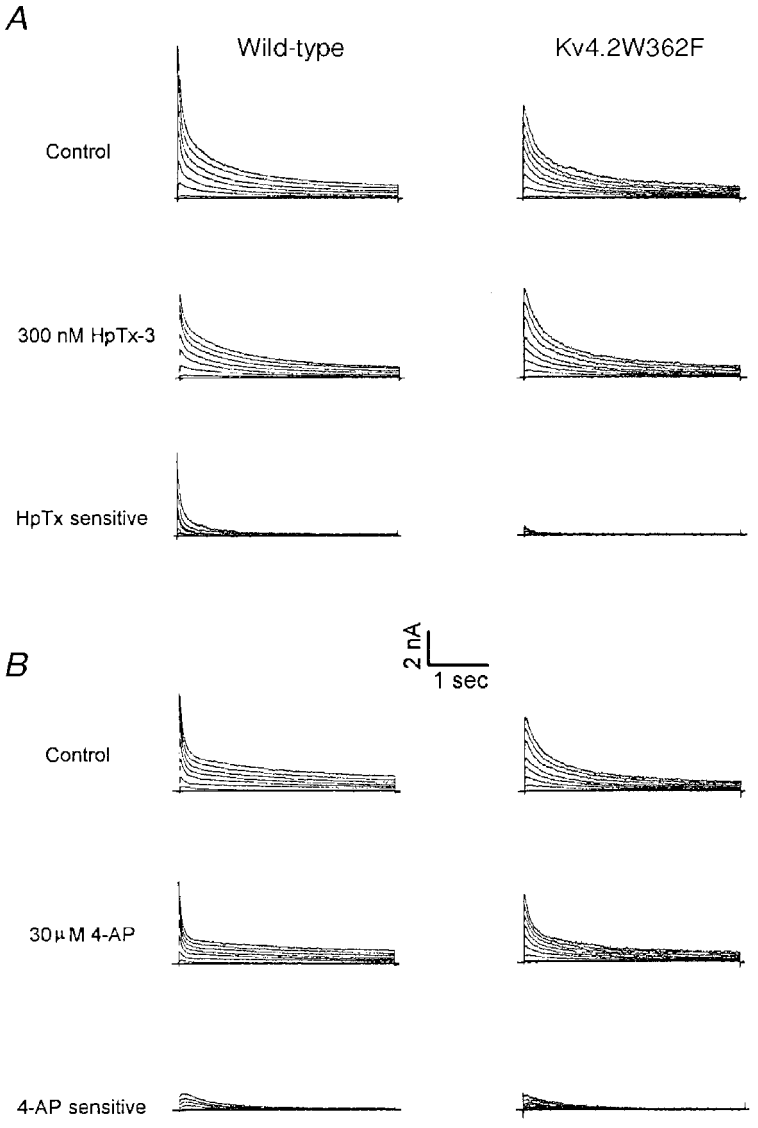

Figure 6. Effects of 300 nM HpTx-3 and 30 μM 4-AP on the outward K^+ currents in wild-type and Kv4.2W362F-expressing mouse left ventricular apex cells

Whole-cell outward K^+ currents were recorded as described in the legend to Fig. 2 from left ventricular apex cells isolated from wild-type and Kv4.2W362F transgenic mice. After recording control currents, cells were exposed to either 300 nM HpTx-3 (*A*) or to 30 μM 4-AP (*B*), and when the effects reached a steady state, the currents were recorded again. The waveforms of the 300 nM HpTx-3- and 30 μM 4-AP-sensitive currents were obtained by offline digital subtraction of the currents in the presence of HpTx-3 or 4-AP from the controls. Note that the slowly inactivating transient outward K^+ current in Kv4.2W362F-expressing apex cells is insensitive to nanomolar concentrations of HpTx-3 and micromolar concentrations of 4-AP. Similar results were obtained in experiments on 4 cells of each group.

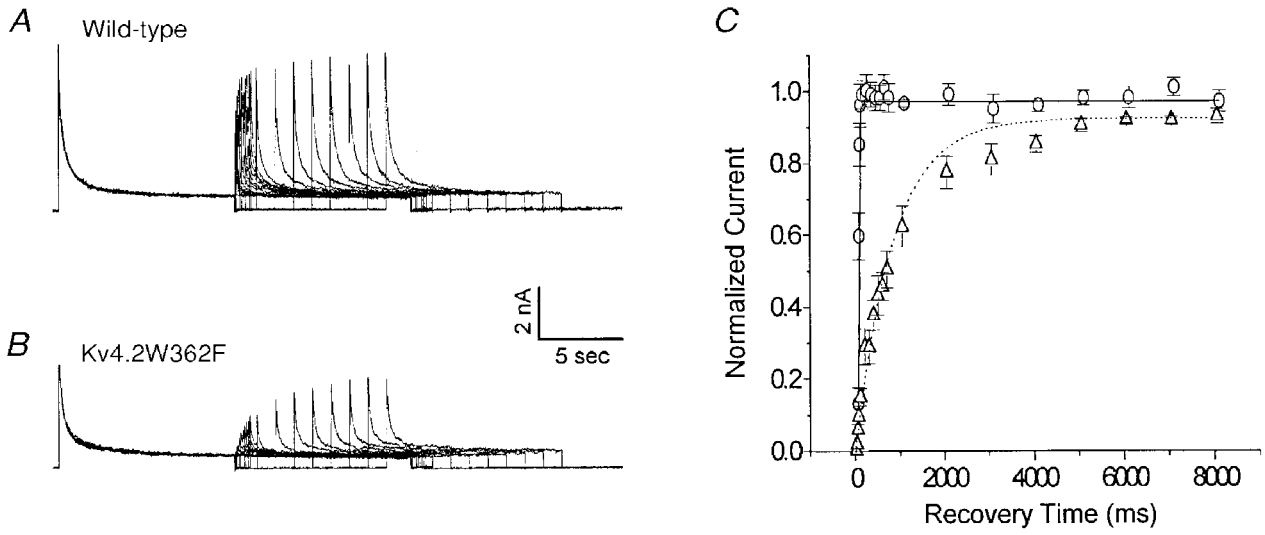

Figure 7. The slowly inactivating transient outward K^+ current in Kv4.2W362F-expressing mouse left ventricular apex cells recovers slowly from steady-state inactivation

After inactivating the currents by 10 s prepulses to +50 mV, adult mouse left ventricular apex cells were hyperpolarized to −70 mV for varying times (ranging from 10 ms to 8 s) before 10 s test depolarizations to +50 mV to assess the extent of recovery. Typical current waveforms recorded from wild-type and Kv4.2W362F transgenic animals are illustrated in *A* and *B*, respectively. The amplitudes of $I_{to,f}$ in wild-type apex cells and the slow transient outward K^+ current ($I_{to,s}$) in transgenic apex cells evoked at +50 mV following each recovery period were determined from double exponential fits to the decay phases of the outward currents as described in Methods, and then normalized to the current amplitudes recorded during the 10 s prepulses. Mean ± S.E.M. normalized currents ($n = 5$) for $I_{to,f}$ (○) and $I_{to,s}$ (△) are plotted as a function of recovery time in *C*. All recovery data are well described by single exponentials (continuous and dotted lines) although the recovery rates for $I_{to,f}$ and $I_{to,s}$ are distinct (see text).

properties similar to $I_{to,s}$ in wild-type left ventricular septum cells. In addition, the experiments on cells isolated from the Kv1.4$^{-/-}$ mice revealed that $I_{to,s}$ is eliminated thereby demonstrating that $I_{to,s}$ is encoded by Kv1.4. Based on these experimental observations, it seemed reasonable to speculate that upregulation of Kv1.4 (in apex cells) occurs in Kv4.2W362F animals in which $I_{to,f}$ channels are reduced/eliminated. Consistent with this hypothesis, Western blot analysis of fractionated mouse ventricular membrane proteins revealed that Kv1.4 protein is increased in Kv4.2W362F-expressing animals relative to wild-type non-transgenic littermates (Fig. 8). Using a polyclonal antibody

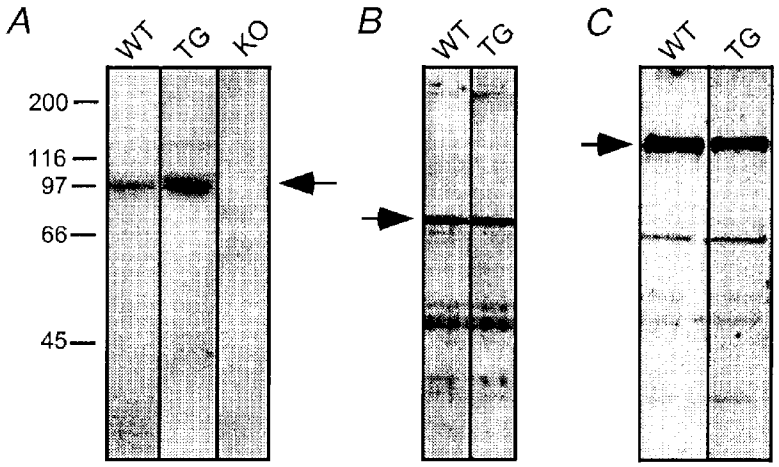

Figure 8. Western blots reveal that Kv1.4 protein expression is increased in Kv4.2W362F-expressing transgenic mice

Ventricular membrane proteins (*A*: 180 μg loaded in each lane; *B* and *C*: 50 μg loaded in each lane) were fractionated on SDS-PAGE gels, transferred to PVDF membranes and immunoblotted with the polyclonal anti-Kv1.4 (*A*), anti-Kv1.2 (*B*) or anti-Kv2.1 (*C*) antibodies (see Methods). WT, TG and KO indicate ventricular membrane proteins prepared from adult wild-type, Kv4.2W362F-expressing and Kv1.4$^{-/-}$ mice, respectively. The arrows show the specific bands recognized by the antibodies.

directed against the N terminal sequence of Kv1.4, a single protein band at 97 kDa was evident in blots of fractionated wild-type mouse ventriclar membrane proteins. This band is undetectable in membrane proteins prepared from the Kv1.4$^{-/-}$ ventricles, consistent with the deletion of the *Kv1.4* gene in these animals. As is evident in Fig. 8*A*, the amount of the Kv1.4 protein is greater in the Kv4.2W362F-expressing mouse ventricles than in wild-type ventricles; similar results were obtained in three separate experiments. In contrast to the findings with Kv1.4, however, the expression levels of two other Kv α subunits, Kv1.2 (Fig. 8*B*) and Kv2.1 (Fig. 8*C*), are not measurably different in wild-type and Kv4.2W362F-expressing animals.

DISCUSSION

Molecular correlates of mouse ventricular $I_{to,f}$ and $I_{to,s}$ are distinct

The marked differences in the kinetic and pharmacological properties of mouse ventricular $I_{to,f}$ and $I_{to,s}$ described previously (Xu *et al.* 1999) suggested that the molecular correlates of these two conductance pathways are distinct. Consistent with this hypothesis, the experiments here revealed that $I_{to,s}$ is absent in cells isolated from the left ventricular septum of Kv1.4$^{-/-}$ mice, thereby documenting a functional role of Kv1.4 in the generation of mouse ventricular septum $I_{to,s}$ channels. In contrast, no effects on $I_{to,f}$, $I_{K,slow}$ or I_{SS} densities were seen in apex or septum cells from Kv1.4$^{-/-}$ animals (see Table 1). In approximately 20% of the Kv1.4$^{-/-}$ septum cells, only $I_{K,slow}$ and I_{SS} were detected. In the other ~80% of the Kv1.4$^{-/-}$ septum cells studied, two exponentials with mean ± S.E.M. τ_{decay} values of 122 ± 3 and 1336 ± 37 ms were required to fit the decay phases of the outward K^+ currents. Although the slower time constant corresponds to $I_{K,slow}$, the inactivation time constant of the faster component is distinct from that of both $I_{to,f}$ (τ_{decay} = 53 ± 2 ms) and $I_{to,s}$ (τ_{decay} = 231 ± 11 ms) in wild-type septum cells. This current is selectively blocked by 300 nM HpTx-3 and recovers rapidly from steady-state inactivation, consistent with $I_{to,f}$. Importantly, the properties of $I_{to,f}$ in wild-type and Kv1.4$^{-/-}$ apex cells are indistinguishable, suggesting that $I_{to,f}$ modulation is selective for septum cells. Further experiments will be necessary to explore the underlying mechanisms involved in mediating the slowing of $I_{to,f}$ inactivation kinetics in septum cells.

The observation that the properties of $I_{to,f}$ in mouse left ventricular apex and septum are indistinguishable suggested that the molecular correlates of $I_{to,f}$ in these two regions would also be the same (Xu *et al.* 1999). The findings here that $I_{to,f}$ is eliminated in both apex and septum cells isolated from Kv4.2W362F-expressing transgenic mice confirm this hypothesis. Since Kv4.2W362F functions as a dominant negative when coexpressed with Kv4.2 or Kv4.3 (Barry *et al.* 1998), however, further experiments will be necessary to determine if both Kv4.2 and Kv4.3 contribute to mouse ventricular $I_{to,f}$ in both the apex and the septum and, in addition, to determine if differences in Kv α subunit composition contribute to regional heterogeneity in $I_{to,f}$ density in mouse left ventricular apex and septum.

In left ventricular apex cells isolated from Kv4.2W362F-expressing mice, however, a slowly inactivating transient outward K^+ current undetectable in wild-type apex cells with properties similar to $I_{to,s}$ in wild-type septum cells is evident. Interestingly, $I_{to,s}$ in left ventricular septum cells is unaffected by Kv4.2W362F expression. These findings are interpreted as suggesting that $I_{to,s}$ is upregulated in apex cells in Kv4.2W362F transgenic mice, perhaps as a result of the absence of $I_{to,f}$. Consistent with the upregulation of $I_{to,s}$, Kv1.4 protein levels are increased in Kv4.2W362F-expressing mouse ventricles. Importantly, the electrophysiological data indicate that this electrical remodelling is only evident in apex cells, which normally lack $I_{to,s}$, and is not seen in septum cells, which normally express $I_{to,s}$. Further experiments will be necessary to test directly the hypothesis that the molecular identity of the novel current in Kv4.2W362F-expressing apex cells and $I_{to,s}$ in wild-type septum cells is indeed the same.

Comparison with previous studies

In a previous study of ventricular myocytes isolated from Kv1.4$^{-/-}$ animals, it was reported that the transient outward K^+ current, which we now refer to as $I_{to,f}$ (Xu *et al.* 1999) was unaffected by the targeted deletion of Kv1.4 (London *et al.* 1998*b*). Interestly, however, London and colleagues (1998*b*) did report a statistically significant decrease in the density of the current remaining 800 ms after the onset of the depolarizing voltage steps in cells isolated from Kv1.4$^{-/-}$ animals relative to the currents in wild-type (control) cells. Based on the present results, it might be suggested that this observation probably reflected the loss of $I_{to,s}$ in a subset of the cells studied although no attempts were made in the previous study to examine regional differences in K^+ channel expression or properties.

Topographic heterogeneity of I_{to} expression, similar to that described here in adult mouse left ventricle, has been previously reported in other species. In rat ventricle, for example, I_{to} densities in left ventricular apex and right ventricular free wall are significantly higher than in septum (Bénitah *et al.* 1993; Gómez *et al.* 1997; Wickenden *et al.* 1999). In addition, inactivation of I_{to} in rat ventricular septum cells is markedly slower than in apex cells (Bénitah *et al.* 1993). In both human and ferret left ventricles, the density of I_{to} in endocardial myocytes is significantly lower than in epicardial myocytes. In addition, endocardial I_{to} inactivates at more negative potentials and recovers more slowly from steady-state inactivation than epicardial I_{to} (Näbauer *et al.* 1996, 1998; Brahmajothi *et al.* 1999). Endocardial I_{to} in ferret heart is also not blocked by HpTx (Brahmajothi *et al.* 1999). The properties of human and ferret endocardial I_{to}, therefore, are similar to $I_{to,s}$ in mouse left ventricular septum cells. These observations, together with the finding that Kv1.4 is expressed in the endocardium

but not in the epicardium of ferret left ventricle (Brahmajothi *et al.* 1999), suggest that ferret endocardial I_{to} and mouse $I_{to,s}$ are similar and that Kv1.4 also probably underlies endocardial I_{to} in ferret. Further experiments will be necessary to test this hypothesis directly. As noted previously, the properties of the transient outward K^+ currents in rabbit atrium and ventricle are distinct from those in other species in that inactivation and recovery from steady-state inactivation are slow (Fedida & Giles, 1991; Wang *et al.* 1999). The properties of I_{to} in rabbit heart, therefore, are also similar to $I_{to,s}$ in mouse left ventricular septum. These observations make it tempting to speculate that Kv1.4 also underlies rabbit I_{to} and experimental evidence supporting this hypothesis in rabbit atria has been recently provided (Wang *et al.* 1999).

Physiological and pathological implications

Studies focussed on defining regional differences in the expression and/or the properties of ionic currents in the myocardium are important for a detailed understanding of normal heart functioning, as well as changes in cardiac function that occur in myocardial disease states. Differential expression of $I_{to,f}$ and $I_{to,s}$, for example, is expected to contribute to regional heterogeneity in action potential repolarization in normal ventricle (Antzelevitch *et al.* 1995; Rosen *et al.* 1998). In addition, the slow recovery kinetics of $I_{to,s}$ from steady-state inactivation suggests that, at normal heart rates, particularly in the mouse, $I_{to,s}$ in the septum (or endocardium) could be largely inactivated. At reduced heart rates, however, $I_{to,s}$ is expected to have a more pronounced (and frequency-dependent) regulatory effect on action potential durations than the much more rapidly recovering $I_{to,f}$ in apex (or epicardium).

Defining the molecular basis of I_{to} ($I_{to,f}$ and $I_{to,s}$) heterogeneity could also have important implications for understanding various myocardial diseases. Reductions in transient outward K^+ currents have been found in a variety of cardiac pathologies, including pressure overload-induced myocardial hypertrophy (Tomita *et al.* 1994), post-myocardial infarction remodelling (Qin *et al.* 1996), short-term diabetes (Shimoni *et al.* 1994) and chronic atrial fibrillation (Van Wagoner *et al.* 1997). In addition, Gómez *et al.* (1997) recently reported that in pressure overload-induced myocardial hypertrophy in the rat, there is a marked decrease in I_{to} density in myocytes from the left ventricular apex, whereas there is no significant effect on I_{to} density in septum cells. It has been reported that Kv4 α subunit mRNA and protein expression are downregulated by a number of myocardial hypertrophic factors, whereas Kv1.4 expression is not affected (Takimoto *et al.* 1997; Guo *et al.* 1998). Clearly, further studies aimed at delineating the molecular mechanisms regulating the functional expression of cardiac voltage-gated K^+ channels and K^+ channel-forming subunits in the normal and diseased myocardium are warranted.

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