Heterogeneity of Kv2.1 mRNA Expression and Delayed Rectifier Current in Single Isolated Myocytes From Rat Left Ventricle

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Abstract—Expression of the voltage-gated K⁺ channel Kv2.1, a possible molecular correlate for the cardiac delayed rectifier current (I\(_K\)), has recently been shown to vary between individual ventricular myocytes. The functional consequences of this cell-to-cell heterogeneity in Kv2.1 expression are not known. Using multiplex single-cell reverse transcriptase–polymerase chain reaction (RT-PCR), we detected Kv2.1 mRNA in 47% of isolated midmyocardial myocytes from the rat left ventricular free wall that were positive for α-myosin heavy chain mRNA (n=74). Whole-cell patch-clamp recordings demonstrated marked differences in the magnitude of I\(_K\) (200 to 1450 pA at V\(_{\text{pset}}\)=40 mV) between individual myocytes of the same origin. Furthermore, the tetraethylammonium (TEA)–sensitive outward current (I\(_{\text{TEA}}\)), known to be partly encoded by Kv2.1 in mice, revealed a wide range of current magnitudes between single cells (150 to 1130 pA at V\(_{\text{pset}}\)=40 mV). Combined patch-clamp recordings and multiplex single-cell RT-PCR analysis of the same myocytes, however, showed no differences in I\(_K\) or I\(_{\text{TEA}}\) magnitude or inactivation kinetics between myocytes expressing Kv2.1 mRNA and those that did not express Kv2.1 mRNA. In contrast, in all midmyocardial myocytes expressing the transient outward potassium current (I\(_{\text{to1}}\)), Kv4 mRNA, which has been shown to underlie I\(_{\text{to1}}\), was detected (n=10). These results indicate that I\(_K\) heterogeneity among individual left ventricular myocytes cannot be explained by the distribution pattern of Kv2.1 mRNA. Other mechanisms besides Kv2.1 mRNA expression appear to determine magnitude and kinetics of I\(_K\) in rat ventricular myocytes. (Circ Res. 2001;88:483-490.)

Key Words: voltage-gated K⁺ channels ■ single-cell reverse transcriptase–polymerase chain reaction ■ Kv2.1 mRNA expression ■ K⁺ currents ■ delayed rectifier

Depolarization-activated Ca\(^{2+}\)-independent outward K⁺ currents recorded from rat ventricular myocytes have been separated into 2 components, as follows: first, a transient outward current (I\(_{\text{to1}}\)) that activates and inactivates rapidly and is inhibited by millimolar concentrations of 4-aminopyridine (4-AP), and second, a delayed rectifier current (I\(_K\)) that also activates rapidly but inactivates slowly and is blocked by millimolar concentrations of tetraethylammonium (TEA). Recent work has suggested that I\(_K\) does not represent a uniform entity but is composed of at least 3 pharmacologically and kinetically different components. Similarly, the molecular correlate of I\(_K\) has not been clearly determined. Possible candidates for I\(_K\) are members of the Kv1, Kv2, and Kv3 gene families (Kv1.2, Kv1.5, Kv2.1, Kv2.2, Kv3.1, and Kv3.2). These α subunits have all been detected at significant levels in rat left ventricle and share functional characteristics with the native I\(_K\) when expressed in heterologous systems. Recent investigations of transgenic mice expressing a dominant negative Kv2.1 subunit showed a reduction of the inactivating component (I\(_{\text{K,slow}}\)) of I\(_K\) in left ventricular myocytes. These observations strongly suggest that Kv2.1 contributes to I\(_K\).

K⁺ channels are not uniformly distributed in the heart (for review, see Nerbonne). Extensive diversity in K⁺ channel gene expression as well as in K⁺ currents has been identified between atria and ventricles, but also in circumscribed regions such as the left ventricular free wall, where I\(_{\text{to1}}\) current and its underlying genes are differentially expressed between endocardial and epicardial regions. Furthermore, K⁺ channel gene expression may even vary from cell to cell. Using in situ hybridization on single isolated myocytes of the ferret left ventricle, Brahmajothi et al detected Kv2.1 mRNA in only 65% and Kv2.2 mRNA in only 15% of individual myocytes. The present study was undertaken to further characterize possible cell-to-cell differences in Kv2 channel gene expression and its functional consequences by combining patch-clamp experiments with single-cell reverse transcriptase–polymerase chain reaction (RT-PCR) analysis in isolated rat left ventricular myocytes.

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Materials and Methods

Isolation of Myocytes

Female Wistar rats weighing 180 to 240 g were anesthetized by IP injection of Trapanal (thiopental-sodium, Byk Gulden), 100 mg/kg body mass. The heart was quickly excised and midmyocardial myocytes were isolated as described previously.14 Cells were stored at 22°C to 26°C in modified Tyrode’s solution containing 100 μmol/L CaCl2. Only single rod-shaped cells with clear cross striations and no spontaneous contractions were used for experiments.

Patch-Clamp Technique

The ruptured-patch whole-cell configuration was used as described previously.2,14 Myocytes were transferred to an elongated chamber (2.5×20 mm) mounted on the stage of an inverted microscope (Axiovert 25, Zeiss). Patch pipettes were pulled from borosilicate glass (GC150-15, Clark Electromedical Instruments) using a P-87 Puller (Sutter Instruments). Currents were recorded using an EPC-9 amplifier (HEKA Elektronik) controlled by a Power-Macintosh computer (Apple Computers) and the Pulse Software (HEKA Elektronik). Membrane capacitance (Cmem) and series resistance (Rs) were calculated using the automated capacitance compensation procedure of the EPC-9 amplifier. When filled with potassium glutamate, pipette resistance averaged 3.9±0.1 MΩ (n=129). Rf averaged 6.2±0.2 MΩ (n=130) and was compensated by 85% leading to an average effective Rf of 0.9 MΩ. Cm averaged 140±3 pF (n=129). Pipette potential (Vpip) and membrane potential (Vm) were corrected for a liquid junction potential (13.3 mV for pipette solution versus control solution). Whole-cell data were analyzed using the PulseFit software (HEKA Elektronik) and IGOR (WaveMetrics). Data are given as mean±SEM unless stated otherwise. Statistical significance was calculated using the appropriate version of the Student t test using the software PRISM (GraphPad Inc). Differences with P<0.05 were considered statistically significant. All experiments were performed at room temperature (22°C to 26°C).

Solutions and Chemicals

Seals (Ω) were obtained in modified Tyrode’s solution (control solution), containing (in mmol/L) NaCl 138, KCl 4, MgCl2, 1, Na2HPO4 0.33, CaCl2 2, glucose 10, and HEPES 10, titrated to pH 7.30 with NaOH. To inhibit Ca2+ currents, CaCl2 0.3 mmol/L was added. Cd2+ slightly increased (~10%), n=8) the slowly inactivating component of IKs but had no effect on its TEA-sensitive component (n=23, data not shown). TEA (10 mmol/L) was added to control solution to estimate the TEA-sensitive current. The pipette solution contained (in mmol/L) glutamic acid 120, KCl 10, MgCl2 2, EGTA 10, Na2-ATP 2, and HEPES 10, titrated to pH 7.20 with KOH. For cDNA synthesis the following reagents and solutions were used: 200 U/μL reverse transcriptase Moloney murine leukemia virus (GIBCO), 40 U/μL ribonuclease inhibitor (Promega), 100 mmol/L dTTP, and 5× buffer-primer-dNTP mix (25 μmol/L hexamer random primers [Boehringer] and 2.5 mmol/L of each deoxyribonucleotide [Pharmacia] in 10 mmol/L Tris-HCl [pH 8.3], 1.5 mmol/L MgCl2, and 0.1 mmol/L diethylpyrocarbonate [DEPC]). For PCR amplification the following solutions were used (in mmol/L): 10× buffer (Tris-HCl 200 [pH 8.3] and KCl 500; GIBCO), MgCl2, 50, and dNTP mix 10, and 5 U/μL Taq DNA polymerase (GIBCO).

Single-Cell RT-PCR

Under visual control (using 100× magnification), a single myocyte was sucked into a micropipette (tip opening ~30 μm) filled with 6 μL of 0.1 mol/L DEPC. The pipette content was ejected into a reaction cup (200-μL cups) and after short centrifugation the following (in μL) were added, up to a total volume of 10 μL: RNase inhibitor 0.5, buffer/primer-dNTP mix 2, DTT 1, and reverse transcriptase 0.5 (for stock solution concentrations see Solutions and Chemicals, above). After reverse transcription at 37°C for 60 minutes in a water bath, the cup was stored at −20°C until PCR amplification.

Single-cell RT-PCR (PE-2400, Applied Biosystems) was performed as described previously.16,17 The cDNA reaction (10 μL) was used directly for PCR amplification. For each PCR reaction, the following (in μL) were mixed and filled up to a reaction volume of 100 μL: 10× buffer 10, MgCl2 solution 3, dNTP mix 3, each amplification primer (10 μmol/L solution) 6, and Taq DNA polymerase 0.5 (see Solutions and Chemicals). The cycle conditions were as follows: 94°C for 5 minutes, hot start, 35 step cycles (94°C for 30 seconds, 57°C for 30 seconds, and 68°C for 30 seconds), and 68°C for 7 minutes. Subsequently, 2 consecutive rounds of PCR with heminested primer pairs were formed. Degenerate primer pairs were used to amplify both members of the Kv2 family in the same reaction. The template for the second PCR was 1 μL derived from the first PCR. For each round of PCR, amplification controls for possible contamination were performed. If controls were positive (<10% of all amplifications), the complete RT-PCR analysis was discarded. To verify specificity of amplification, PCR products were probed by sequencing after subcloning or Southern blotting with radiolabeled oligonucleotides. Primer pairs were intron-overspanning to prevent amplification of genomic DNA. Positive controls for primer efficiency were run using plasmids at several dilutions (down to 0.1 fg plasmid DNA). After 2 rounds of PCR (35 cycles each), Kv2.1 and Kv2.2 mRNAs were amplified at similar efficiency. Sequences and locations of the following primers for Kv2.1 (X16476) and Kv2.2 (M77482) refer to sequences and locations published in GenBank (National Center for Biotechnology Information, available at http://www.ncbi.nlm.nih.gov): upper primer, 5′-TCTGGGACATTCCGAAGA-3′ (797–816 and 1342–1361); lower primer, 5′-AGGATGCGTGGAGCAGATG-3′ (104–1126 and 1443–1465); and lower nested primer, 5′-CGAGAATCTGCAGACCCGGA-3′ (889–908 and 1218–1237). Oligonucleotides for Southern blotting were the following: Kv2.1, 5′-CTCTGGGCAACTTGTTAGGC-3′ (654–677); and Kv2.2, 5′-GTTGGCACACTCATGTCCCT-3′ (983–1006). The washing conditions were 0.5× SSC at 55°C. To determine whether the fraction of Kv2 mRNA-positive cells declines as a function of time after cell isolation, single-cell RT-PCR was performed immediately (<1 hour) and 8 to 10 hours after cell isolation. Kv2 mRNA expression rates were similar in both groups (0 to 1 hour, 40% [n=30]; 8 to 10 hours, 40% [n=40]).

Multiplex Single-Cell RT-PCR

For multiplex single-cell RT-PCR, an intron-overspanning primer pair specific for α-myosin heavy chain (α-MHC) was added to the reaction cup in addition to the primers for the Kv2 subfamily (1 μL of each primer out of 10 μmol/L solution). Conditions for the first round of PCR were identical to those described above for single-cell RT-PCR. The second round of PCR amplification was performed separately for Kv2 and α-MHC with cycling conditions as described above. Primers for α-MHC (KO1464) were the following: upper primer, 5′-AGCCCCATACTCCCGCAAG-3′ (1624–1644); lower primer, 5′-TTGACGGTGACACAGAAGAGGC-3′ (3269–3277); and upper nested primer, 5′-CTCTGGCAGAGACACAGAGT-3′ (1678–1698).

To estimate the detection threshold of the multiplex single-cell RT-PCR, we performed control reactions using cloned Kv2.1, Kv2.2, and α-MHC plasmids. After mixing in one reaction cup, all genes were consistently amplified from an amount of 0.1 fg, which corresponds to <10 copies of each transcript. A similar sensitivity of single-cell RT-PCR has been reported previously.16,17 To exclude a possible amplification of mRNA fragments from the surrounding bath solution, tissue components of myocytes were analyzed by single-cell RT-PCR under identical conditions. All of these controls gave negative results (n=8, data not shown).

Multiplex single-cell RT-PCR for Kv4 and α-MHC was performed under the same cycle conditions as described above. Degenerate primer pairs were used to amplify all members of the Kv2 family (Kv4.1, Kv4.2, and Kv4.3) in the same reaction as described previously.17 Primers for Kv4.1 (M64226), Kv4.2 (M64320), and Kv4.3 (U75448) were the following: upper primer, 5′-TCTATCGACTCGTG- TGGCCCATCT-3′ (797–816, 1342–1361, and 802–821); upper nested
primer, 5'-TACAC(AC)CT(CG)AAGAGCTGTGC-3' (943–962, 1513–1532, and 954–973); and lower primer, 5'-TGGTAGAT(CG)-(GT)(AG)CT(AG)AAGTT-3' (1228–1247, 1773–1792, and 1223–1242).

Combined Patch-Clamp Recordings and Single-Cell RT-PCR
Using the whole-cell patch-clamp configuration, depolarization-activated outward currents were recorded in the absence and in the presence of TEA, which required ≈5 minutes. Subsequently, a second pipette filled with DEPC-water (0.1 mol/L) was forwarded to the myocyte. After flushing the cell and its surroundings with DEPC, the myocyte was lifted from the bottom of the chamber by elevating the patch pipette and then sucked into the second pipette. The amount of the remaining DEPC was ≈6 µL. The content of the second pipette was expelled into a reaction cup and transferred to multiplex single-cell RT-PCR analysis.

Results
Detection of Kv2 mRNA in Individual Myocytes
Figure 1A shows the results of a single-cell RT-PCR analysis performed on 6 individual myocytes. In 2 myocytes (2 and 3), a PCR product with the expected length of ≈540 bp was amplified. In the remaining myocytes as well as in the 2 controls (C1 and C2), amplification yielded no detectable products. Overall, a PCR product was amplified in 6 out of a total of 18 myocytes (33%) investigated. Figures 1B and 1C display Southern blots of the single-cell RT-PCR products shown in Figure 1A using specific probes for Kv2.1 (Figure 1B) and Kv2.2 (Figure 1C). In both myocytes, only Kv2.1 mRNA was identified. The same result was obtained in all other myocytes with a positive PCR amplification for Kv2 mRNA.

Multiplex Single-Cell RT-PCR
In 12 of 18 myocytes (67%), no Kv2-related mRNA could be detected. A myocyte that yielded no amplification product by single-cell RT-PCR could actually not express Kv2 channel genes; alternatively, the amplification process might not have been successful. We therefore decided to coamplify α-MHC mRNA, which is constitutively expressed in cardiac myocytes, as an internal control for the process of cell transfer and amplification. Figure 2 shows the results of a multiplex single-cell RT-PCR analysis performed on 7 myocytes (lanes 1 through 7). Six of these myocytes (lanes 1 through 4, 6, and 7) were positive for α-MHC (≈300 bp, Figure 2A), indicating that the process of cell transfer and amplification did work. In 3 of the myocytes positive for α-MHC (lanes 2, 3, and 6), a PCR product of the expected size for Kv2 cDNA was detected and later identified as Kv2.1 by sequencing. In total, 74 of the 77 myocytes (96%) investigated were positive for α-MHC. Kv2.1 mRNA was identified in 35 of the 74.
most myocytes by fitting the current decay with the sum of 3 exponential functions: a short time constant ($\tau_1$) in the range of 40 ms, an intermediate time constant ($\tau_2$) in the range of 300 ms, and a long time constant ($\tau_3$) in the range of 2500 ms. $\tau_1$ describes the decay of $I_{\text{fast}}$, $\tau_2$ may represent the rapidly inactivating $I_K$ component described by Himmel et al., whereas $\tau_3$ describes the more slowly inactivating component of $I_K$. In 28% of the myocytes, only the current components inactivating with $\tau_1$ and $\tau_3$ could be detected. Similar results have been obtained in ventricular myocytes of mice, in which the depolarization-activated outward current was divided into $I_{\text{fast}}$ ($\tau_1=70$ ms), $I_{\text{slow}}$ ($\tau_2=200$ ms, not present in all myocytes), and $I_{\text{slow,s}}$ ($\tau_3=1200$ ms). It is therefore likely that the current component inactivating with $\tau_1$ corresponds to $I_{\text{slow,slow}}$ observed in mice. This current has been shown to be partially encoded by Kv2.1.8 We therefore further analyzed this component. Figure 3B shows an average current-voltage relation of recordings similar to those shown in Figure 3A. Activation of the current started at $-20$ mV, and the current magnitude increased linearly with depolarization. Similar properties have been described for recombinant Kv2.1 heterologously expressed in Xenopus oocytes19 or in mammalian cells.4

If Kv2.1 contributed to $I_K$ and was present in only about half of the myocytes, as suggested by the results above, one should expect a broad distribution of $I_K$ magnitudes. The average current-voltage relation shown in Figure 3B indeed reveals a large variation of $I_K$ magnitude among individual myocytes. Figure 3C depicts a histogram that plots the number of occurrences versus the magnitude of $I_K$ at $V_{\text{pp}}=40$ mV. The histogram shows a broad distribution of $I_K$ magnitudes with no clear indication for separate populations of myocytes. It is likely, however, that >1 current component is coding for $I_{\text{slow,s}}$, and it might well be that, in myocytes in which Kv2.1 mRNA is absent, an increase of the remaining component(s) may compensate for at least part of the current component normally encoded by Kv2.1. We therefore determined the TEA-sensitive current component ($I_{\text{TEA}}$) to further isolate Kv2 currents.

Figure 4A shows current traces recorded using the same voltage-pulse protocol as applied in Figure 3A. The recordings shown in Figure 4B were obtained in the presence of 10 mmol/L TEA in the bath solution. $I_K$ was markedly reduced, whereas $I_{\text{fast}}$ remained unaffected by TEA. Figure 4C depicts $I_{\text{TEA}}$, calculated by subtracting the current traces shown in Figure 4B from those shown in Figure 4A. $I_{\text{TEA}}$ is eliminated completely as a result of its insensitivity to TEA. $I_{\text{TEA}}$ activates rapidly and inactivates slowly within seconds, until a steady state is reached. Inactivation of $I_{\text{TEA}}$ followed a monoexponential time course with an average inactivation time constant of $\tau_{\text{TEA}}=2157\pm86$ ms ($V_{\text{pp}}=40$ mV; n=63). In some recordings, in which a part of $I_{\text{in}}$ had escaped subtraction (probably because of an incompletely compensated $R_{\text{L}}$), the current decay was fitted with a double-exponential function to exclude a contribution of $I_{\text{in}}$. The magnitude of $I_{\text{TEA}}$ was estimated as the current component ascribed to $\tau_{\text{TEA}}$ and averaged $540\pm27$ pA ($V_{\text{pp}}=40$ mV; n=63). Because $\tau_{\text{TEA}}$ was similar to $\tau_1$ and $I_{\text{TEA}}$ reached $\approx80\%$ of the current that inactivated with $\tau_1$, it is likely that $I_{\text{TEA}}$ largely underlies the slowly inactivating component of $I_K$. Currents were intention-

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**Pattern of Delayed Rectifier Current**

To investigate whether the difference in Kv2.1 mRNA expression between individual myocytes is associated with differences in functional properties, we analyzed depolarization-activated outward currents recorded from myocytes isolated from the same region as above. Figure 3A shows current traces recorded by depolarizing voltage steps from a holding potential of $V_{\text{pp}}=90$ mV to potentials ranging from $V_{\text{pp}}=60$ to $-40$ mV in steps of $-20$ mV. The initial phase of the current is dominated by $I_{\text{fast}}$. After the inactivation of $I_{\text{fast}}$, ie, after $\approx200$ ms, $I_K$, which inactivates much more slowly, becomes visible. The rat $I_K$ has recently been further divided into 3 components, as follows: a rapidly inactivating component ($\tau=400$ ms), a more slowly inactivating component ($\tau$ in the range of seconds), and a steady-state component.2 In agreement with that study, 3 inactivating components of the outward current could be identified in myocytes positive for $\alpha$-MHC (47%). Kv2.1 mRNA was not detected in myocytes negative for $\alpha$-MHC. These data indicate that Kv2.1 mRNA is expressed in only 40% to 50% of midmyocardial myocytes of the rat left ventricular free wall.

**Figure 3.** Depolarization-activated outward currents recorded from a left ventricular myocyte of midmyocardial origin. A, Currents activated by 5000-ms voltage pulses from a holding potential of $V_{\text{pp}}=90$ mV to values ranging from $+60$ mV to $-40$ mV in steps of $-20$ mV. Voltage pulses were delivered at 0.1 Hz. B, Average current-voltage relation (n=88), obtained by plotting the current magnitude fitted by $\tau_3$ and recorded at $V_{\text{pp}}=40$ mV (n=89).
Figure 4. Depolarization-activated outward currents recorded from a left ventricular myocyte of midmyocardial origin. A. Currents activated by $I_{\text{TEA}}$ voltage pulses from a holding potential of $V_{\text{hp}}=-90$ mV to values ranging from $+60$ mV to $-40$ mV in steps of $-20$ mV. Voltage pulses were delivered at 0.1 Hz. B. Similar recording as in panel A, in the presence of 10 mmol/L TEA in the bath solution; C, $I_{\text{TEA}}$ obtained by subtracting the current traces recorded in the absence of TEA (panel A) from those recorded in its presence (panel B). D, Average current-voltage relation of $I_{\text{TEA}}$ ($n=57$), obtained by plotting current magnitude fitted by $\tau_{\text{TEA}}$, the inactivation time constant of $I_{\text{TEA}}$. Error bars indicate SD. E, Histogram plotting number of occurrences vs magnitude of $I_{\text{TEA}}$ fitted by $\tau_{\text{TEA}}$. Data were fitted with linear regression and revealed a significant correlation of $I_{\text{TEA}}$ with its occurrence ($P=0.045$, $r^2=0.064$). Dashed lines represent the 95% confidence interval of the linear regression (solid line; $n=63$).

Figure 5. $I_{\text{TEA}}$ and expression of $\alpha$-MHC mRNA and Kv2 mRNA in 2 representative midmyocardial myocytes (left and right columns). A, $I_{\text{TEA}}$ recorded under conditions as described in Figure 4. B and C, Ethidium bromide–stained gels with single-cell RT-PCR products of $\alpha$-MHC mRNA (B) and Kv2 mRNA (C). C1 and C2 indicate negative controls for each round of PCR amplification; M, molecular weight marker.

36 myocytes. Linear regression analysis revealed a weak but significant positive correlation ($P=0.045$, $r^2=0.064$) between $I_{\text{TEA}}$ and $\tau_{\text{TEA}}$. Thus, in some myocytes a faster inactivation of $I_{\text{TEA}}$ may be explained by a lack of Kv2.1 expression.

Collectively, these data are consistent with the concept that expression of Kv2.1 mRNA in myocytes may cause a larger magnitude and a slower inactivation of $I_k$.

Combined Patch-Clamp Recordings and Multiplex Single-Cell RT-PCR

To test this hypothesis directly, we combined analysis of current properties and Kv2 mRNA expression in the same myocytes. $I_{\text{TEA}}$ was estimated in the whole-cell configuration; thereafter, the myocyte was sucked into a second pipette and transferred to multiplex single-cell RT-PCR analysis for Kv2 mRNA (see Materials and Methods). Figure 5 shows current recordings and gene expression in 2 representative ventricular myocytes. $I_{\text{TEA}}$ was similar and $\alpha$-MHC mRNA expression was detected in both myocytes, but only the myocyte shown on the left expressed Kv2.1 mRNA. This combined analysis was repeated in a total of 27 myocytes. $\alpha$-MHC mRNA expression was detected in all myocytes investigated, whereas Kv2 mRNA was found in 9 cells (33%). Figure 6 compares the distribution of the magnitude and inactivation of $I_{\text{TEA}}$ in both groups. Both variables were equally distributed in Kv2.1 mRNA–positive and Kv2.1 mRNA–negative myocytes ($I_{\text{TEA}}$, $576\pm62$ versus $570\pm52$ pA; $\tau_{\text{TEA}}$, $2494\pm203$ versus $2439\pm171$ ms). Note that $C_m$ (Figure 6C) and the current ascribed to $\tau_0$ of the total outward current (data not shown) were also equally distributed in both groups. These data indicate that the expression pattern of Kv2.1 mRNA

ally not corrected for cell capacitance to allow for a direct correlation with the presence of Kv2.1 mRNA expression in individual myocytes, which cannot be corrected for cell size (see next section). Figure 4D shows the average current-voltage relation of $I_{\text{TEA}}$ calculated from recordings similar to those shown in Figure 4C. Similarly to Figure 3B, the current activated at $V_{\text{hp}}=-20$ mV and increased rather linearly with depolarization. Figure 4E depicts a histogram of the magnitudes of $I_{\text{TEA}}$. $I_{\text{TEA}}$ was distributed over a wide range of magnitudes from 150 to 1130 pA ($V_{\text{hp}}=40$ mV). Xu et al. reported that the inactivating component of $I_{\text{TEA}}$ was largely reduced in left ventricular myocytes isolated from mice expressing a dominant negative mutant of Kv2.1. Accordingly, an explanation for the wide range of $I_{\text{TEA}}$ may be that myocytes that express Kv2.1 mRNA display a large $I_{\text{TEA}}$, whereas myocytes that do not express Kv2.1 mRNA have a small $I_{\text{TEA}}$.

Mice expressing a dominant negative Kv2.1 subunit displayed an accelerated inactivation of $I_{\text{slow}}$. We therefore correlated the inactivation time constant of $I_{\text{TEA}}$ with its magnitude. This relation is depicted in Figure 4F for a total of 63 myocytes. Linear regression analysis revealed a weak but significant positive correlation ($P=0.045$, $r^2=0.064$) between $I_{\text{TEA}}$ and $\tau_{\text{TEA}}$. Thus, in some myocytes a faster inactivation of $I_{\text{TEA}}$ may be explained by a lack of Kv2.1 expression.

Collectively, these data are consistent with the concept that expression of Kv2.1 mRNA in myocytes may cause a larger magnitude and a slower inactivation of $I_k$. 
among individual myocytes does not account for the differences in $I_{\text{TEA}}$ or its inactivation time constant.

To confirm that a direct correlation of ionic currents with the expression of specific mRNAs in individual cardiac myocytes is possible, we analyzed $I_{\text{to1}}$ and Kv4 mRNA expression, which has been shown to encode for $I_{\text{to1}}$ (for review, see Reference 9). Multiplex single-cell RT-PCR amplifying both Kv4 mRNA and $\alpha$-MHC mRNA revealed that 87% of myocytes positive for $\alpha$-MHC also expressed Kv4. mRNA Figure 7 displays the results of combined patch-clamp recordings and multiplex single-cell RT-PCR analysis.

$I_{\text{to1}}$ was present in all myocytes with a magnitude of 1140 to 5880 pA (estimated as peak current after 600 ms at $V_{\text{Pip}} = 40$ mV), which is in good agreement with results we have previously obtained in midmyocardial myocytes.\(^{14}\) In all of the myocytes investigated, PCR fragments of the expected size for Kv4 and $\alpha$-MHC were amplified.

**Discussion**

**Methodological Limitations**

In a substantial fraction of myocytes (\(~59\%)\), single-cell RT-PCR failed to detect Kv2 mRNA transcripts. The successful amplification of $\alpha$-MHC in 96% of all myocytes revealed that the process of cell transfer, reverse transcription, and PCR amplification was successful. A difficulty of single-cell RT-PCR is mRNA degradation by RNases.\(^{20}\) Because the total amount of $\alpha$-MHC mRNA is probably larger than that of Kv2 mRNA, the latter may become degraded to an extent that does not allow its detection by single-cell RT-PCR, whereas $\alpha$-MHC mRNA is still detectable. This might lead to an underestimation of Kv2 mRNA–positive myocytes. If this were the case, however, the number of Kv2 mRNA–positive myocytes should be reduced considerably further in experiments in which single-cell RT-PCR was preceded by electrophysiological measurements, because in these experiments the procedure of cell harvesting was substantially prolonged. The finding of similar fractions of Kv2 mRNA–positive myocytes in all experimental series suggests that mRNA degradation does not account for the heterogeneity of Kv2 mRNA expression among left ventricular myocytes. Furthermore, our data are in agreement with previous results obtained from ferret left ventricular myocytes using in situ hybridization.\(^{13}\) Thus, Kv2.1 mRNA appears to be present in only \(~40\%\) to 50% of midmyocardial myocytes from the rat left ventricular free wall.

**Identification of Kv2.1 Currents**

When expressed in heterologous systems, Kv2.1 currents are activated by depolarizing voltage steps exceeding $V_m = -20$ mV; display a linear current-voltage relationship\(^{4,19}\); and are sensitive to several substances such as TEA,\(^{21}\) 4-AP,\(^{4}\) hantatoxins,\(^{22}\) and propafenone.\(^{23}\) Unfortunately, Kv2.1 shares these properties with several other Kv channels expressed in the mammalian heart. Further candidates possibly underlying $I_K$, such as Kv1.5, Kv3.1, and Kv3.2 are also inhibited by TEA.\(^{24,25}\) Members of the Kv4 family, which probably underlie $I_{\text{to1}}$, are also inhibited by 4-AP and propafenone.
similar concentrations as Kv2.1,²³ and hanatoxins are known to block Kv4.2.²²

In rat left ventricular myocytes, we have found \( I_{\text{TEA}} \) to share many properties with heterologously expressed Kv2.1 currents; the current-voltage relation and inactivation time constants were similar, and \( \approx 75\% \) of the current component inactivating with the slow time constant was inhibited by 10 mmol/L TEA, which would also be expected for Kv2.1 currents (\( K_{\text{p}} = 4 \text{ mmol/L} \)).²¹²⁶ In an independent series of experiments, we have found steady-state inactivation and recovery from inactivation of \( I_{\text{TEA}} \) to be in the range observed for Kv2 currents (data not shown). Most importantly, the inactivating component of \( I_{\text{TEA}} \) has been reported to be absent in mice expressing a dominant negative Kv2.1 subunit.⁸ However, because TEA also inhibits other ionic currents present in cardiac myocytes, which share kinetic properties with Kv2, we cannot exclude that \( I_{\text{TEA}} \) may also contain current components other than Kv2.

**Discordance Between Kv2.1 mRNA Expression Pattern and \( I_{\text{TEA}} \) Magnitude**

Although it has been shown in mice that \( I_{\text{TEA}} \) is significantly altered in ventricular myocytes expressing a dominant negative Kv2.1 subunit,⁸ we could not detect a correlation between magnitude or kinetics of \( I_{\text{TEA}} \) and the expression of Kv2.1 mRNA in the present study. Several factors might account for this discrepancy. The presence of Kv2.1 mRNA might not reflect the amount of the corresponding protein present in the cell membrane. In neurons, however, the method of combined electrophysiological characterization and single-cell RT-PCR has repeatedly and successfully been used to ascribe specific electrical behavior to the presence or absence of ion channels.¹⁶¹⁷ Furthermore, we could demonstrate the presence of both \( I_{\text{mol}} \) and Kv4 mRNA in all 10 myocytes in which a combined analysis of \( I_{\text{mol}} \) and Kv4 mRNA was achieved. This suggests that also in cardiac myocytes a relation of channel mRNA and corresponding ionic current is likely. We cannot exclude, however, that protein turnover of Kv2.1 in cardiac myocytes is very slow and thus might lead to an mRNA level too low to be detected by single-cell RT-PCR even in myocytes expressing a large \( I_K \). Another possibility is that in rat left ventricular myocytes Kv2.1 mRNA is expressed at levels too low to significantly contribute to \( I_K \). In view of the demonstration of significant Kv2 expression levels in the rat left ventricle,¹ however, this seems to be unlikely. Thus, the large difference in \( I_{\text{TEA}} \) between individual rat midmyocardial myocytes may rather be ascribed to factors other than Kv2.1 mRNA expression. Another delayed rectifier component encoded by Kv1.5, Kv3.1, or Kv3.2, or an as-yet-unidentified \( \alpha \) subunit, could vary in magnitude or could account for a larger fraction of total \( I_K \), thus making it impossible to identify the contribution of Kv2.1 mRNA to \( I_K \) magnitude. Alternatively, intracellular signaling mechanisms as well as expression of additional subunits may primarily determine magnitude and kinetics of Kv2.1-generated currents. Kv2.1 polypeptides are extensively serine phosphorylated at their C-terminal domain,²⁷ and it has been demonstrated that Kv2.1 channels can coassemble with other Kv channel \( \alpha \) subunits such as Kv5.1,²⁸ Kv6.1,²⁶ Kv6.2,²⁹ or Kv9.1 to Kv9.3.²⁸⁻³⁰ Heteromultimerization with these subunits could significantly modulate the electrical and pharmacological properties of Kv2.1 currents.

**Conclusions**

By using single-cell RT-PCR, which is the current most sensitive technique to identify mRNA in single cells, the present study demonstrates that Kv2.1 mRNA can be detected in 41% (78/189) of individual midmyocardial myocytes isolated from the rat left ventricular free wall. Although the TEA-sensitive component of \( I_K \), which is known to be at least partly encoded for by Kv2.1 in mice, showed considerable differences among individual myocytes, a combined molecular and electrophysiological approach failed to detect a correlation of \( I_{\text{TEA}} \) magnitude or inactivation kinetics with the expression of Kv2.1 mRNA. In contrast, Kv4 mRNA was detected in all myocytes in which \( I_{\text{mol}} \) was identified. These results indicate that additional mechanisms besides expression of Kv2.1 mRNA shape \( I_K \) in the rat left ventricle.

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**References**


Heterogeneity of Kv2.1 mRNA Expression and Delayed Rectifier Current in Single Isolated Myocytes From Rat Left Ventricle

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