Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle

PETER MELNYK,1,3 LIMING ZHANG,1 ALVIN SHRIER,5 AND STANLEY NATTEL1,2,4
1Department of Medicine and Research Center, Montreal Heart Institute, Montreal, Quebec H1T 1C8; 2Department of Medicine, University of Montreal, Montreal, Quebec H3C 3J7; and Departments of 3Pathology, 4Pharmacology, and 5Physiology, McGill University, Montreal, Quebec H3G 1Y6, Canada

Received 30 October 2001; accepted in final form 8 May 2002

Melnyk, Peter, Liming Zhang, Alvin Shrier, and Stanley Nattel. Differential distribution of Kir2.1 and Kir2.3 subunits in canine atrium and ventricle. Am J Physiol Heart Circ Physiol 283:H1123–H1133, 2002. First published May 16, 2002; 10.1152/ajpheart.00934.2001.—Ventricular inward rectifier K⁺ current (Iₖ₁) is substantially larger than atrial, producing functionally important action potential differences. To evaluate possible molecular mechanisms, we recorded Iₖ₁ with patch-clamp techniques and studied Kir2.1 and Kir2.3 subunit expression. Iₖ₁ density was >10-fold larger in the canine ventricle than atrium. Kir2.1 protein expression (Western blot) was 78% greater (P < 0.01) in the ventricle, but Kir2.3 band density was 228% greater (P < 0.01) in the atrium. Immunocytochemistry showed transverse tubular localization of Kir2.1 in 89% (17 of 19) of atrial and 22% (4 of 18, P < 0.001) in the ventricle. Kir2.1 protein expression (Western blot) was 78% greater (P < 0.01) in the ventricle, but Kir2.3 band density was 228% greater (P < 0.01) in the atrium. Immunocytochemistry showed transverse tubular localization of Kir2.1 in 89% (17 of 19) of ventricular and 26% (5 of 19, P < 0.0001) of atrial cells. Both exhibited a weakly positive Kir2.1 signal at intercalated disks. Kir2.3 was strongly expressed at the intercalated disks in all cells and in the transverse tubular regions in 78% (14 of 18) of atrial and 22% (4 of 18, P < 0.001) of ventricular cells. Tissue immunohistochemical results qualitatively resembled isolated cell data. We conclude that the expression density and subcellular localization of Kir2.1 and Kir2.3 subunits differ in the canine atrium versus ventricle. Overall protein density differences are insufficient to explain Iₖ₁ discrepancies, which may be related to differences in subcellular distribution.

inward rectifier potassium current; ion channels; cell biology; electrophysiology

WEIDMANN FIRST OBSERVED inward rectification in Purkinje fibers in 1955 (27). Inward rectification, due to internal voltage-dependent block by Mg²⁺ (23) and cytoplasmic polyamines (11), is important in the action potential (AP) profile of cardiomyocytes. During diastole, the large K⁺ conductance provided by open inward rectifier K⁺ channels [inward rectifier K⁺ current (Iₖ₁)] is responsible for maintaining the resting potential. After depolarization, inward rectification allows the AP plateau to be maintained without an excessive repolarizing influence and prevents excessive K⁺ loss. Electrophysiological studies of Iₖ₁ in atrial and ventricular cells reveal important differences (24, 26). Iₖ₁ density is smaller in the atrium than ventricle, and atrial cells exhibit very limited outward current at voltages positive to the reversal potential.

The molecular identity of Iₖ₁ is incompletely understood. It is thought that more than one inwardly rectifying K⁺ channel contributes to macroscopic Iₖ₁ (28) and that members of the Kir2 subfamily play an important role (12). The mRNA expression of various members of the Kir2 family has been studied in the human atrium and ventricle to evaluate possible molecular mechanisms of the differences between ventricular and atrial Iₖ₁ (26). Kir2.3 message was found to be nearly 10-fold more concentrated in the atria. Kir2.1 mRNA was much more abundant than other Kir species in both atria and ventricles, but no significant atrial-ventricular differences in Kir2.1 expression were noted. Antisense oligonucleotides directed against Kir2.1 significantly reduce whole cell Iₖ₁ in rat ventricular myocytes (14). A greater abundance of Kir2.1 subunits in ventricular myocytes would provide a possible explanation for the observed current differences, but Kir2.1 mRNA concentrations are comparable in the two regions in humans (26).

It is known that protein expression may not parallel that of corresponding mRNA (1). In addition, subcellular compartmentalization of proteins may have important consequences for their physiological function. We were unable to identify studies of Kir2 subunit protein expression in the heart. We therefore performed the present study to evaluate the expression levels and cellular distribution of Kir2.1 and Kir2.3 proteins in the canine atrium and ventricle.

METHODS

Canine atrial and ventricular tissue samples were obtained from excised canine hearts after euthanasia with overdoses of pentobarbital. All animal handling procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the institutional animal research ethics committee.
Western Blotting

Canine atrial and ventricular tissue were dissected from eight explanted hearts and frozen in liquid nitrogen. Sarcolemmal preparations were partially purified by a high salt (0.6 M KI) wash followed by differential high-speed centrifugation (Optima Ultracentrifuge, Beckman; Palo Alto, CA). To limit the effects of proteases, the lysis buffer contained the following protease inhibitors (Sigma; Oakville, Ontario, Canada): 10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride. A phosphatase inhibitor, sodium orthovanadate (1 mM, Sigma), was also added to the solution. Total protein concentration for each sample was determined by Bradford assay to ensure equal protein loading. The samples were loaded on 8% polyacrylamide gels in an alternating atrial-ventricular manner to minimize systematic artifacts due to differences in electrophoretic conditions. After the proteins were electrophoretically separated, they were transferred to polyvinylidene difluoride membranes (Millipore; Bedford, MA). After transfer, the membranes were blocked in 5% nonfat dry milk (NFDM) in Tris-buffered saline (TBS) with 0.1% Tween 20 (TTBS). The primary antibodies [anti-Kir2.1 (1:1,000) or anti-Kir2.3 (1:1,000), Alomone Labs; Jerusalem, Israel] were diluted with 1% NFDM in TTBS and incubated with the membrane for 60 min at room temperature. After three washes in TTBS and a further blocking of the membranes with 1% NFDM in TTBS, the secondary antibody [anti-rabbit IgG-horse radish peroxidase (1:7,500), Santa Cruz; Santa Cruz, CA] was diluted in 5% NFDM and applied to the membranes for 45 min at room temperature. After three further washes in TTBS, the membranes were incubated with Western Blot Chemiluminescence Reagent Plus (New England Nuclear Life Science Products; Boston, MA), and the results were recorded on Biomax ML film (Eastman Kodak; Rochester, NY). After digital scanning of the Western blot films, band density was quantified using Quantity One software (PDI; Huntington Station, NY). The software contains background subtraction algorithms.

Immunocytochemistry

Ventricular and atrial myocytes were isolated from seven hearts as described previously (29). Briefly, after excision, the hearts were placed in room temperature Tyrode solution. Solutions were equilibrated with 100% O₂. The right coronary artery was cannulated, and the right atrium was dissected free and perfused with Tyrode solution (37°C, 5 min). The tissue was perfused at 12 ml/min with nominally Ca²⁺-free Tyrode solution for 20 min. Any leaking arterial branches were ligated. The tissue was then perfused for 40 min with Tyrode solution containing collagenase (100 U/ml, CLSII, Worthington Biochemical; Freehold, NJ), and the results were recorded on Biomax ML film (Eastman Kodak; Rochester, NY). After digital scanning of the Western blot films, band density was quantified using Quantity One software (PDI; Huntington Station, NY). The software contains background subtraction algorithms.

Immunohistochemistry

Atrial and ventricular tissues were dissected from five explanted canine hearts and placed in isobutanol precooled to −80°C, which was then cooled further in liquid nitrogen to ensure rapid and even freezing. Frozen tissue was subsequently stored at −80°C until cryosectioning, which took place at −20°C. Serial 14-μm sections were cut with a Leica CM1900 cryostat and left to dry before storage at −80°C. Before the application of antibodies to the sections, the tissue was fixed for 20 min with 3%-paraformaldehyde solution. The primary antibodies [anti-Kir2.1, anti-Kir2.3, or anti-N-cadherin] were in contact with the sections for 90 min at room temperature. This was followed by three washes with PBS and the application of the secondary antibodies [anti-rabbit IgG-Cy3 or anti-mouse IgM-Cy5] for 45 min at room temperature. After being washed, the sections were mounted using Immuno Floures (ICN; Aurora, OH).

Confocal Microscopy

Images of tissue sections and isolated cells were obtained with a Zeiss LSM 510 inverted confocal microscope in conjunction with LSM 510 software. To study labeling with Cy3 (excitation: 553 nm, emission: 575 nm), a 543-nm HeNe laser was applied; to visualize Cy5 (excitation: 651 nm, emission: 674 nm), a 633-nm HeNe laser was used.

Electrophysiology

After cell isolation from 10 hearts, rod-shaped quiescent cells were used for voltage-clamp recording. An aliquot of the solution containing the isolated cells was placed in a 1-ml chamber mounted on the stage of an inverted microscope. After 5 min (for cell adhesion), the cells were superfused at 3 ml/min with Tyrode solution for recording currents (see Solutions and Reagents). Experiments were carried out at 35°C. IK1 was defined as 1 mM Ba²⁺-sensitive current upon depolarization and hyperpolarization from a holding potential of −40 mV. Voltage clamp was performed as previously described (29, 31). IK1 was measured from the holding current to the peak value during a test pulse (peak IK1). Junction potentials averaged 10 ± 2 mV and were not corrected.

Solutions and Reagents

Tyrode solution contained (in mM) 126 NaCl, 2 CaCl₂, 5.4 KCl, 0.8 MgCl₂, 0.33 NaH₂PO₄, 10 dextrose, and 10 HEPES; pH 7.4 (adjusted with NaOH). The high-K⁺ storage solution contained 1% albumin and (in mM) 20 KCl, 10 KH₂PO₄, 10 dextrose, 70 glutamic acid, 10 β-hydroxybutyric acid, 10 taurine, and 10 EGTA; pH 7.4 (adjusted with KOH). The pipette solution used to study IK1 contained (in mM) 0.1 GTP, 110 potassium aspartate, 20 KCl, 1 MgCl₂, 5 Mg-ATP, 10 HEPES, 5 Na₂-phosphocreatine, and 10 EGTA; pH 7.4 (adjusted with KOH). For current recording, the Tyrode solution was modified by adding Cd²⁺ (200 μM) to block Ca²⁺ current and Ca²⁺-dependent Cl⁻ current and atropine (200 nM) to
block any basal activity of acetylcholine-dependent $K^+$ current. TBS contained (in mM) 20 Tris-HCl and 500 NaCl; pH 7.4 (NaOH). The primary antibodies included a polyclonal antibody raised in the rabbit against an epitope corresponding to amino acid residues 392–410 of human Kir2.1 (Alomone Labs), a polyclonal antibody raised in rabbit against an epitope corresponding to amino acid residues 418–437 of rat Kir2.3 (Alomone Labs), and a mouse monoclonal IgG1 antibody generated against a peptide corresponding to the COOH-terminus of chicken N-cadherin (Sigma).

Data Analysis

Results are presented as means ± SE. Statistical comparisons between group means were by t-test, and contingency comparisons were by $\chi^2$-test. Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Electrophysiology

Figure 1, A and C, shows examples of Ba$^{2+}$-sensitive $I_{K1}$ recorded from a canine atrial and ventricular myocyte, respectively. Atrial currents were clearly much smaller than ventricular. Figure 1B shows means ± SE $I_{K1}$ density in nine atrial cells. The outward component was small as previously reported (26) but was clearly present, as can be appreciated from the expanded outward current section in the inset. The uncorrected reversal potential was in the range of −70 mV; with correction for junction potential, the true reversal potential was negative to −80 mV. Mean (±SE) $I_{K1}$ density in 16 ventricular myocytes is shown in Fig. 1D (filled symbols). For purposes of comparison, corresponding data from atrial myocytes are reproduced on the same graph as open symbols. $I_{K1}$ was substantially and significantly larger in ventricular cells. To quantify the relative size of $I_{K1}$ in the atrium versus ventricle over a broad voltage range, we determined the slope conductance by linear regression of current against voltage at test potentials negative to the reversal potential. The mean slope conductance of inward current in ventricular myocytes (1.23 ± 0.05 nS/pF) was almost 14-fold larger than that in atrial cells (0.09 ± 0.01 nS/pF, $P < 0.001$).

Western Blotting

Figure 2 illustrates the results of Western blot studies of Kir2.1 and Kir2.3 protein expression. Gels from one heart showing typical signals detected with Kir2.1 antibodies are shown in Fig. 2A. Bands are seen at the expected molecular mass of ~55 kDa. In the individual example shown, the signal in ventricular tissue was 81% stronger than that in atrium tissue. Figure 2B shows a representative gel for Kir2.3, with bands detected at the expected molecular mass of just over 55 kDa. In contrast to the results for Kir2.1, the atrial signal was stronger than the ventricular one, by 244% in the example shown. Mean data for Kir2.1 in eight hearts are shown in Fig. 2C and indicate that the Kir2.1 signal intensity in ventricular myocardium was on average 78% stronger ($P < 0.01$) than in atrial myocardium. The normalized band density for atrial Kir2.1 averaged 1.00 ± 0.04 optical density (OD)·mm$^2$, whereas ventricular Kir2.1 band density was 1.78 ± 0.04 OD·mm$^2$. Mean data for Kir2.3 in eight hearts are shown in Fig. 2D and indicate Kir2.3 to be 228% more...
abundant ($P < 0.01$) in the atrial homogenates ($3.28 \pm 0.22$ OD·mm$^2$) compared with the ventricular ones ($1.00 \pm 0.03$ OD·mm$^2$).

**Immunocytochemistry**

**Kir2.1.** Figure 3A is a fluorescent image of two ventricular cardiomyocytes. The fluorescent image is typical of the images we obtained in ventricular cells and shows the subcellular distribution of Kir2.1. The Kir2.1 staining appeared predominantly in a transversely striated pattern, sometimes with an increased signal at the longitudinal ends of cells. More intense signal densities were also found randomly distributed within cells. In a minority of ventricular cells, no clear transverse striations were seen, as illustrated in Fig. 3B. As can be seen from the negative control experiment (antibody preincubated with antigenic peptide) illustrated in Fig. 3C, the random densities represent nonspecific signals. Of 19 ventricular myocytes studied, 17 (89%) exhibited a transversely striated Kir2.1 localization. In contrast to ventricular myocytes, the majority of atrial cells had a diffuse Kir2.1 signal, as shown in Fig. 3D. Transversely striated Kir2.1 localization was occasionally seen in atrial myocytes, as illustrated in Fig. 3E. However, transverse localization was seen in only 26% of the atrial cells (5 of 19, $P < 0.0001$ vs. ventricular cells). Nonspecific signal densities were also observed in atrial cells (Fig. 3F).

The distances between the Kir2.1 transverse signal striations ranged from 1.4 to 1.9 μm. These values are consistent with a transverse tubular distribution (21). To obtain more information about the subcellular distribution of Kir2.1 and Kir2.3, we used the LSM 510 software to obtain optical sections of labeled myocytes. A series of 25–30 images was taken at equal intervals through the horizontal plane. The fluorescent signals at transverse striations were observed throughout the depth of the cells, not only at the cell surface. This observation supports a transverse tubular localization pattern. To obtain additional confirmation of transverse tubular signal localization, we performed double labeling of myocytes with anti-Kir2.1 (Fig. 4A) and WGA (Fig. 4B). WGA labels membrane surface structures, including transverse tubules (21). The orange-colored striations in Fig. 4A represent Kir2.1 expression, and the blue-colored striations in Fig. 4B indicate WGA localization. In the double-labeled image (Fig. 4C), colocalization is seen as a superposition of the colors, resulting in a red-violet coloration. The high degree of colocalization evident in Fig. 4C indicates that ventricular Kir2.1 staining is concentrated in transverse tubules. Colabeling studies in seven other cells provided similar results.

**Kir2.3.** In all ventricular myocytes studied, a Kir2.3 signal was apparent at the longitudinal ends of cells, as illustrated by the example in Fig. 5A. In the majority of cells, there was little additional specific cytoplasmic staining. In 6 of 18 ventricular cells (33%), striated signal patterns were observed in addition to the strong cell-end fluorescence, as shown by the example in Fig. 5B. Negative controls (antibody preincubated with peptide) showed only rare, scattered speckled staining in ventricular myocytes (Fig. 5C). When probed with anti-Kir2.3, all atrial myocytes had labeling at cell ends. In addition, 14 of 18 (78%, $P < 0.001$ vs. ventricular) atrial myocytes exhibited regularly spaced transverse striations (Fig. 5D), whereas a much smaller proportion (4 of 18, 22%) showed only end labeling (Fig. 5E). Some limited nonspecific signal was also observed in atrial myocytes, as illustrated by control samples exposed to antibody preincubated with antigen (Fig. 5F). To determine whether the striated atrial Kir2.3 pattern is due to transverse tubular localization of Kir2.3, double-labeling experiments were performed with anti-Kir2.3 and WGA (Fig. 6). Kir2.3 localization is indicated by green coloration (Fig. 6A), and WGA is indicated by blue coloration (Fig. 6B). Figure 6C shows simultaneous imaging for both, with colocalization in...
dicated by a white signal. As can be seen in Fig. 6C, the atrial Kir2.3 transverse striations colocalize with WGA. Similar results were obtained in four other cells.

To determine whether the predominant end-label Kir2.3 signal was localized to the intercalated disk region, we probed myocytes with a monoclonal antibody directed against N-cadherin. With this approach, N-cadherin was detected specifically at cell ends (Fig. 7A). Kir2.3 also localized to cell ends (Fig. 7B), colocalizing with N-cadherin in double-labeling experiments, as illustrated by Fig. 7C. Similar results were obtained in studies with 12 other cells.

**Immunohistochemistry**

Cell isolation requires the use of cell-dissociating enzymes that strip cell-connecting proteins. Cell isolation could conceivably alter the distribution of ion channel subunits. To determine whether our results are relevant to Kir2.1 and Kir2.3 subunit distribution in intact tissue, we performed immunohistochemistry on fast-frozen cardiac tissue samples. As in immunocytochemical studies, cross-striated Kir2.1 distribution was commonly seen in ventricular sections (Fig. 8A), with a minority of ventricular sections lacking the evident transverse striation pattern of Kir2.1 (Fig. 8B). Most atrial sections lacked transverse staining (Fig. 8D), but some transverse Kir2.1 signal was seen in a minority of atrial sections (Fig. 8E). Preincubation of the Kir2.1 antibody with the antigen removed most of the signal (Fig. 8, C and F). Similar results were obtained in five hearts. Immunohistochemical results for Kir2.1 distribution in the atrium differed from isolated cell data primarily by virtue of the presence of stronger signals at the intercalated disk regions of tissue sections.

As was observed with isolated ventricular myocytes, most of the Kir2.3 signal was localized to the intercalated disk region of ventricular tissue sections, with
some diffuse labeling of the rest of the cell (Fig. 9A). Less commonly, ventricular sections exhibited a signal at both the intercalated disks and organized striations (Fig. 9B). The majority of the atrial tissue sections probed with anti-Kir2.3 had transversely oriented striations of signal density (Fig. 9D) as well as more dense expression at the intercalated disks. Some atrial tissue sections did not exhibit transversely localized striations (Fig. 9E). Similar results were obtained in all three hearts examined.

DISCUSSION

In the present study, we compared the protein expression levels and subcellular distributions of two members of the Kir2 family of strongly inwardly rectifying $K^+$ channel subunits. We found that Kir2.1 and Kir2.3 are differentially distributed in the canine atrium versus ventricle. These differences in the distribution of Kir2 subunits, which likely play a crucial role in forming $I_{K1}$ channels, likely contribute to atrial-ventricular $I_{K1}$ differences.

Differences in Expression Level and Subcellular Distribution

Our Western blot results show a significantly greater degree of Kir2.1 expression in the canine ventricle compared with the atrium. The greater concentration of Kir2.1 subunits in the ventricle likely contributes to the greater ventricular $I_{K1}$ density. However, the quantity of Kir2.1 in the ventricle exceeds the quantity in the atrium by only 78%, whereas ventricular $I_{K1}$ density is ~14 times greater than that in atrial $I_{K1}$. Another factor to be considered is the subcellular localization of channel subunits. The transverse tubular membranes depolarize with the AP to excite the cell interior (25). Consequently, transverse tubules are crucial to excitation-contraction coupling. It is quite likely that current across transverse tubule membranes contributes to transmembrane potential changes. Indeed, various ion channels have been localized to this region (4, 20, 21). In a study of rabbit ventricular myocytes, Christie (4) found that the loss of $I_{K1}$ in cultured cells paralleled the loss of transverse...
Kir2.3

Fig. 5. Immunocytochemical detection of Kir2.3 in isolated ventricular (A and B) and atrial (D and E) myocytes. The majority (14 of 18 cells) of ventricular myocytes showed signals only at the cell ends, as in A, whereas most (14 of 18) atrial myocytes showed both a transverse striated and cell-end localization, as in D. Negative (antigen-preincubated) controls are shown in C (ventricular) and F (atrial).

Fig. 6. A and B: immunolocalization of Kir2.3 (A) and WGA (B) in an atrial myocyte. C: composite image, consisting of A and B superimposed. Similar results were obtained in a total of 5 cells.
tubules, possibly indicating a transverse tubular localization of one or more $I_{K1}$ subunits (4). It has been suggested that a significant portion of conductances that are active at hyperpolarized potentials are localized at the transverse tubules (12).

Our results show that the Kir2.1 subunit localizes predominantly to the transverse tubules and intercalated disks of canine ventricular myocytes. That most atrial myocytes have a diffuse distribution of Kir2.1 not associated with transverse tubules is an interesting observation, given the much smaller current density in these cells. Although less extensive than in ventricular myocytes, a transverse tubular system is clearly present in atrial myocytes, as shown by the atrial WGA staining pattern (Fig. 6) and the prominent transverse tubular localization of Kir2.3 in the atrium (Figs. 5 and 6). Thus the lack of atrial transverse tubular localization of Kir2.1 is not due to a lack of transverse tubules per se but presumably to differential targeting of subcellular Kir2.1 expression in the ventricle versus atrium. Furthermore, the colocalization of Kir2.3 and WGA in isolated atrial myocytes

### Kir2.1 in Tissue

![Images of Kir2.1 in Tissue](http://ajpheart.physiology.org/)

Fig. 8. Immunohistochemical detection of Kir2.1 in ventricular (A and B) and atrial (D and E) tissue. Negative controls (treated with antibody preincubated with the antigenic peptide) are shown in C (ventricle) and F (atrium). Similar results were obtained in 5 hearts.
Kir2.3 in Tissue

(Fig. 6) demonstrates that atrial myocytes do have the ability to preferentially cluster Kir2 family channels at the transverse tubules. It is possible that the transverse tubular localization of Kir2.1 in ventricular myocytes concentrates these subunits in a membrane compartment in which they can more readily form functional channels. The lack of such localization may therefore contribute significantly to the much smaller $I_{K1}$ in the atrium.

Biophysical Properties

When Kir2.3 subunits are expressed in Xenopus oocytes, the resulting channels have a slope conductance of 13 pS (16), a value that is much lower than for the other members of the Kir2 subfamily. Under similar conditions, Kir2.1 was found to have a single channel conductance of 36 pS (28). The greater expression of Kir2.3 protein in atrial tissue, as well as the smaller single channel slope conductance of this subunit, raises the possibility that Kir2.3 contributes to atrial $I_{K1}$ and to the smaller $I_{K1}$ density in canine atrial myocytes. To evaluate this possibility further, it would be necessary to know more about the single channel conductances and open probabilities in atrial versus ventricular myocytes. Evidence for a role of Kir2.3 subunits in $I_{K1}$ comes from experiments with polyunsaturated fatty acids. Kir2.3 is the only member of the Kir2.0 family to be modulated by arachidonic acid (10). The potentiation of Kir2.3 current by arachidonic acid may underlie some of the effects of polyunsaturated fatty acids on rat myocytes, such as effects typically modulated by $I_{K1}$, like resting membrane potential hyperpolarization (7).

Functional evidence for the involvement of more than one subunit in the genesis of $I_{K1}$ comes from studies using antisense oligonucleotides targeted against Kir2.1 to knock down Kir2.1 expression (14). In ventricular myocytes treated with anti-Kir2.1 antisense oligonucleotides, a significant reduction in the expression of a 21-pS (believed to be Kir2.1) channel was observed, whereas the opening frequency of a 13.5-pS channel was not significantly affected (14). $I_{K1}$ density was substantially reduced, consistent with mRNA data suggesting that Kir2.1 is the most strongly expressed Kir2 subunit in this species.

Sato and Koumi (18) described only a single $I_{K1}$ unitary channel type in both the human atrium and ventricle, with a single channel conductance of 27–28 pS. Wible et al. (28) recorded $I_{K1}$ channels with conductances of 41, 35, 21, and 9 pS from the human atrium. The most commonly observed channel type,
seen in 77% of patches, was the 21-pS conductance channel believed to correspond to Kir2.1. In guinea pig ventricular myocytes, however, only three groups of \( I_K \) conductances were reported: 34.0, 23.8, and 10.7 pS (9). In comparing native \( I_K \) with the results of Kir2 subunit expression in a heterologous expression system, it is difficult to identify native channels with those formed by homomeric subunits because of the large variability in the reported properties of the expressed channels. Single channel conductances for gpKir2.1, gpKir2.2, and gpKir2.3 expressed in HEK cells were reported to be 40.0, 30.6, and 14.2 pS, respectively, different from those of native \( I_K \) channels in the same species (9). In addition, inward rectifier channels formed by a single subunit may be able to show more than one conductance. For example, Picones et al. (17) found that homomeric expression of mouse Kir2.1 in HEK cells or oocytes leads to the formation of channels with a wide range of unitary conductances varying from 2 to 33 pS.

**mRNA Levels**

Transcripts encoding Kir2.1, Kir2.2, and Kir2.3 subunits have been identified in the heart (9, 26, 30). Kir2.4 mRNA is not expressed in guinea pig ventricular myocytes (9). The strongest mRNA expression is observed for Kir2.1 (26, 30), with transcript concentrations an order of magnitude greater than that for other Kir2 subfamily members. Our results demonstrate a significantly greater degree of Kir2.1 subunit protein expression in the canine ventricle compared with the atrium, a difference not seen at the mRNA level in human myocardium (26). Kir2.3 mRNA has been reported to be ~12-fold more abundant in the human atrium than ventricle (26). We found Kir2.3 protein expression to be 228% greater in canine atrial homogenates compared with ventricular ones, qualitatively consistent with human mRNA data.

**Possible Role for Kir2 Heterotetramers**

In myocytes from mice that have undergone a targeted homozygous deletion of Kir2.1 (Kir2.1
\(^{-/-}\)), \( I_K \) was not detectable in 4 mM extracellular \([K^+]_o\)-containing solutions, although a small residual \( I_K \) could be detected in the presence of 60 mM extracellular \([K^+]_o\) (30). Experiments by the same group with Kir2.2
\(^{-/-}\) mice found a 50% reduction in \( I_K \) (30). These results suggest that the Kir2.1 subunit is essential for the formation of virtually all \( I_K \) channels in the mouse but that other subunits (specifically Kir2.2) may also play an important role. The fact that Kir2.1 subunits seem to be an integral component of all \( I_K \) channels, whereas Kir2.2 may be involved in approximately half of murine ventricular \( I_K \) could be explained by the formation of heteromeric channels involving both subunits. The coexpression of a dominant-negative Kir2.1 subunit with Kir2.2 had no effect on resulting \( I_K \) recorded in *Xenopus* oocytes (19, 22), suggesting that at least in this heterologous expression system Kir2.1 and Kir2.2 subunits do not coassemble. Thus the association of Kir2.1 with Kir2.2 subunits may require the presence of an as-yet-unidentified protein.

Some evidence suggests that coassociation between different members of the Kir2 family can occur. Coexpression of G\( \beta \gamma \) subunits with Kir2.1 has no effect on the resulting current, whereas G\( \beta \gamma \) expression abolishes Kir2.3 currents (5). When Kir2.1 and Kir2.3 are coexpressed with G\( \beta \gamma \), almost no current can be recorded, suggesting coassembly of Kir2.1 and Kir2.3, producing channels with a dominant G\( \beta \gamma \) inhibitory phenotype (5). Heteromeric assembly of Kir2.1 and Kir2.3 appears to be dependent on the NH2-terminal ends of the channels (6). The different subcellular localization of Kir2.1 and Kir2.3 subunits that we saw in the canine atrium versus ventricle argues against preferential Kir2.1/2.3 heteromultimer formation, but of course this is only circumstantial evidence.

Members of the membrane-associated guanylate kinase (MAGUK) family can modulate the localization of ion channels. Kir2.1, Kir2.2, and Kir2.3 have a PDZ-binding motif in their COOH termini important for protein-protein interactions with MAGUK proteins. Although Kir2.1 and Kir2.3 have been shown to bind PSD-95 (15), this MAGUK protein has been reported to be present in the brain but not the heart. Kir2.1, Kir2.2, and Kir2.3 have also been shown to interact with the MAGUK protein synapse-associated protein 97 (SAP97) (8). The colocalization of SAP97 and Kir2.2 has been demonstrated in the transverse tubules of rat ventricular myocytes (8). Although SAP97 does not appear to control the distribution of Kir2.2 directly, it may, in its capacity as an intracellular scaffolding molecule, help to create favorable conditions for the formation of heteromultimers. It would be very interesting to understand the potential role of scaffolding proteins like the MAGUK family in targeting Kir2 proteins to cardiac transverse tubules in atrial and ventricular tissues.

**Potential Significance of Our Findings**

The present study is the first of which we are aware to assess the differential atrial-ventricular distribution of Kir2.1 and Kir2.3 proteins. Our results suggest that both the density and the subcellular distribution of Kir2.1 and Kir2.3 differ between the canine atrium and ventricle. This differential distribution may contribute to atrioventricular \( I_K \) density differences. The larger ventricular \( I_K \) likely explains the more negative resting potential and more rapid phase 3 repolarization in the ventricle compared with the atrium. Thus the atrial-ventricular differences in Kir2 subunit protein expression that we noted may be of functional importance.

**Potential Limitations**

Kir2.1 encodes the inward rectifier potassium channel in arterial smooth muscle cells in rats (2). To isolate sufficient protein for Western blotting quickly enough to minimize protein degradation, we dissected myocardial regions of interest and froze them immediately with liquid nitrogen. The frozen tissue was pulverized and sus-
pended in buffer. We cannot exclude a contribution from smooth muscle cells to the total Kir2.1 expression that we measured by Western blot. This would not, of course, be expected to have affected the immunocytochemical or immunohistochemical findings.

Immunocytochemical studies provide qualitative information about the presence of antigenic proteins in subcellular compartments but not precise information on their quantity. We cannot exclude the possibility that our inability to detect Kir2.1 or Kir2.3 in transverse tubules in certain cells and tissues was due to expression at levels below the detection threshold rather than their absence. Neither Western blots nor immunocytochemical studies provide functional information. Further biophysical work would be very interesting to elaborate the potential functional bases of atrial-ventricular $I_{K1}$ differences. In addition to the quantity, subcellular localization and subunit composition of $I_{K1}$, differences in regulation of channel function could play a significant role.

The authors thank the Canadian Institutes of Health Research and the Quebec Heart Foundation for Research Funding, Louis Villeneuve for technical assistance with confocal microscopy, and France Thériault for secretarial help with the manuscript.

P. Melnyk was supported by a Heart and Stroke Foundation of Canada research studentship.

REFERENCES


