Endosomal K$_{\text{ATP}}$ channels as a reservoir after myocardial ischemia: a role for SUR2 subunits

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Bao L, Hadjiolova K, Coetzee WA, Rindler MJ. Endosomal K$_{\text{ATP}}$ channels as a reservoir after myocardial ischemia: a role for SUR2 subunits. Am J Physiol Heart Circ Physiol 300: H262–H270, 2011. First published October 22, 2010; doi:10.1152/ajpheart.00857.2010.—ATP-sensitive K$^+$ (K$_{\text{ATP}}$) channels, composed of inward rectifier K$^+$ (Kir)6.x and sulfonylurea receptor (SUR)x subunits, are expressed on cellular plasma membranes. We demonstrate an essential role for SUR2 subunits in trafficking K$_{\text{ATP}}$ channels to an intracellular vesicular compartment. Transfection of Kir6.x/SUR2 subunits into a variety of cell lines (including h9c2 cardiac cells and human coronary artery smooth muscle cells) resulted in trafficking to endosomal/lysosomal compartments, as assessed by immunofluorescence microscopy. By contrast, SUR1/Kir6.x channels efficiently localized to the plasma membrane. The channel turnover rate was similar with SUR1 or SUR2, suggesting that the expression of Kir6/SUR2 proteins in lysosomes is not associated with increased degradation. Surface labeling of hemagglutinin-tagged channels demonstrated that SUR2-containing channels dynamically cycle between endosomal and plasmalemmal compartments. In addition, Kir6.2 and SUR2 subunits were found in both endosomal and sarcolemmal membrane fractions isolated from rat hearts. The balance of these K$_{\text{ATP}}$ channel subunits shifted to the sarcolemmal membrane fraction after the induction of ischemia. The K$_{\text{ATP}}$ channel current density was also increased in rat ventricular myocytes isolated from hearts rendered ischemic before cell isolation without corresponding changes in subunit mRNA expression. We conclude that an intracellular pool of SUR2-containing K$_{\text{ATP}}$ channels exists that is derived by endocytosis from the plasma membrane. In cardiac myocytes, this pool can potentially play a cardioprotective role by serving as a reservoir for modulating surface K$_{\text{ATP}}$ channel density under stress conditions, such as myocardial ischemia.

cardiomyocytes; potassium channels; endocytosis; ischemia; electrophysiology; adenosine 5’-triphosphate-sensitive potassium channel; sulfonylurea receptor

ATP-sensitive K$^+$ (K$_{\text{ATP}}$) channels affect diverse physiological processes, ranging from the regulation of insulin secretion in pancreatic β-cells to the control of vascular tone. They act as metabolic sensors by controlling cellular excitability when opening in response to an impaired intracellular energetic status. The channel is composed of four inward rectifier K$^+$ (Kir6) subunits and four sulfonylurea receptor (SUR) subunits (34). There are two Kir6 subfamily members, Kir6.1 and Kir6.2. There are also two types of sulfonylurea (SUR) subunits, SUR1 and SUR2, that belong to the ATP-binding cassette superfamily (32). SUR2 has two major functionally relevant splice variants, SUR2A and SUR2B (33). Various native K$_{\text{ATP}}$ channels have different subunit compositions. Kir6.2 together with SUR1, for example, constitute the β-cell K$_{\text{ATP}}$ channel (1, 31), whereas the cardiac and smooth muscle K$_{\text{ATP}}$ channels are generally considered to consist of Kir6.2/SUR2A and Kir6.1/SUR2B subunit combinations, respectively (2, 26).

K$_{\text{ATP}}$ channels protect against ischemia-reperfusion injury (12). Pharmacological approaches imply a role for mitochondrial K$_{\text{ATP}}$ channels in protection and ischemic preconditioning (7). However, a definitive role for sarcolemmal K$_{\text{ATP}}$ channels has also been demonstrated (29, 35, 36). The cellular mechanisms by which sarcolemmal K$_{\text{ATP}}$ channels are protective may involve action potential shortening and prevention of intracellular Ca$^{2+}$ overload. However, the molecular signals responsible for the protective effect are less well established. One possibility is the increased activity of available K$_{\text{ATP}}$ channels mediated by intracellular protein kinase C (PKC) signaling pathways (15, 17). Another possibility is that the K$_{\text{ATP}}$ channel density may be altered through transcriptional or posttranscriptional mechanisms (3). Posttranscriptional K$_{\text{ATP}}$ channel regulatory mechanisms are not well characterized.

In addition to the role of SUR subunits in conferring distinct biophysical and pharmacological properties to the various types of K$_{\text{ATP}}$ channels, they also affect channel subcellular trafficking. Both SURx and Kir6.x subunits contain specific amino acid sequences that regulate the trafficking of newly synthesized K$_{\text{ATP}}$ channels to the cell surface (38). To date, however, there has been no evidence to indicate that SUR1 and SUR2 subunits behave differently in this regard. Our data demonstrate that SUR1 subunits direct surface expression, whereas the SUR2 subunit, mostly found in muscle (5), directs K$_{\text{ATP}}$ channel trafficking both to the surface and to intracellular compartments. Moreover, we demonstrate that the intracellular pool of K$_{\text{ATP}}$ channels is mobile and has the ability to translocate to the sarcolemma (SL) after ischemic episodes to increase the K$_{\text{ATP}}$ channel surface density. This ischemia-induced surface trafficking of K$_{\text{ATP}}$ channels should be considered as a possible molecular mechanism for their protective role in postischemic damage.

MATERIALS AND METHODS

Plasmids. pCS-MT-Kir6.1myc, pcDNA3-Kir6.2-hemagglutinin (HA), and a nonconductive mutant of mouse Kir6.2HA were constructed as previously described (28). pCI-Kir6.2-green fluorescent protein (GFP) (S65A) was from Dr. M. Takano (Jichi Medical School, Minamikawachi, Japan). Kir6.2-GFP(S65A)/SUR2A transfected cells exhibited K$_{\text{ATP}}$ channel activity with biophysical properties and ATP-sensitivity indistinguishable from wild-type (not shown). Kir6.2HA+11 was a gift of Dr. L. Jan (University of California, San Francisco, CA), and hamster SUR1 cDNA was a gift of Dr. J. Bryan (Baylor College of Medicine). Both were subcloned into pcDNA3.1. Rat SUR2A in pcMV6 was provided by Dr. S. Seino (Kobe Univer-
Cell culture, transfection, and immunofluorescence microscopy. Cells were transfected with the \( K_{\text{ATP}} \) channel subunit cDNAs using lipofectamine plus (HEK-293T), lipofectamine 2000 (COS-1L and h9c2), or nucleofection (smooth muscle cells) (see supplemental methods; note: all supplemental material may be found posted with the online version of this article). For immunofluorescence microscopy, cells were pretreated with cycloheximide (100 \( \mu \)M), a protein synthesis inhibitor, for 4 to 5 h before fixation with paraformaldehyde and staining. Images were acquired using confocal microscopy.

Pulse-chase labeling and immunoprecipitation and antibody uptake experiments. Cells were preincubated in methionine- and cysteine-free DMEM containing 15 mM HEPES and 5% dialyzed FBS and labeled with 250 \( \mu \)Ci/ml \([35S]\)Translabel (MP Biomedical) for 1 h. To chase, the cells were returned in growth medium supplemented with 5 mM methionine and cysteine. Immunoprecipitation was conducted with anti-myc antibodies, and the amount of Kir6.1myc in each sample was quantified by phosphorimaging after SDS-PAGE. Antibody uptake was conducted using the procedure described by Hu et al. (14) with modifications. The protocols used are described in detail in the supplemental methods.

Fractionation of cardiac tissue. All animal procedures were in accordance with National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of New York University School of Medicine. For experiments measuring \( K_{\text{ATP}} \) channel translocation in ischemic versus nonischemic rats, male Sprague-Dawley rats were overdosed with pentobarbital sodium and either euthanized immediately or 17–18 min after respiratory arrest. Membrane fractionation was performed using Optiprep gradients as described (21) with modifications (see supplemental methods). The plasma membrane and endosome-enriched fractions were subjected to Western blot analysis. The \( K_{\text{ATP}} \) subunit expression was normalized to that of the Na,K-ATPase.

Cardiac myocyte isolation and whole cell recordings. Ventricular myocytes were isolated after enzymatic digestion of adult rat hearts. Some hearts were made globally ischemic before cell isolation by stopping flow for 20 min. Whole cell patch-clamp recording, mRNA isolation, and quantitative RT-PCR were performed as described.

**Figure 1.** Sulfonylurea receptor (SUR) 2 targets inwardly rectifying K\(^+\) (Kir) 6.1 and Kir6.2 to intracellular vesicles. COS-1L cells were transfected with plasmids encoding Kir6.1myc (top) or Kir6.2-hemagglutinin (HA) (middle) together with SUR1 or SUR2A or in the absence of SURx (with pcDNA3). Cells were pretreated with cycloheximide for 4 h before staining for immunofluorescence microscopy (IFM). When expressed alone, Kir6.1 or Kir6.2 staining was in a reticular pattern consistent with retention in the endoplasmic reticulum. When expressed together with SUR1, both Kir6.1 and Kir6.2 were predominantly localized on plasma membranes (arrowheads). When expressed with SUR2, both Kir6.1 and Kir6.2 were detected in intracellular vesicles (arrows) and on the plasma membrane. **Bottom:** COS-1L cells were transfected with a 1:9 (left) or a 1:1 (right) ratio of Kir6.1:SUR2B plasmid DNA and stained for IFM using goat anti-Kir6.1 or SUR2B antibodies. The untagged Kir6.1 subunit was localized to intracellular vesicles (arrows). SUR2B was also localized to intracellular vesicles. Figure panels have been adjusted for brightness and contrast. Bars = 10 \( \mu \)m.
previously (13, 37). The detailed procedures are given in the supplemental methods.

**Data analysis.** Data are represented as means ± SE (n denoting the number of cells). Comparisons between groups were made using unpaired or paired Student’s t-tests (SigmaStat, Systat Software) with a P value of <0.05 considered as statistically significant.

**RESULTS**

**SUR2 subunits direct K\(_{\text{ATP}}\) channel trafficking to endosomal compartments.** We investigated the subcellular trafficking patterns of K\(_{\text{ATP}}\) channels that contain either SUR1 or SUR2\(_x\) subunits, initially using transfected cells. When COS-1L cells were transfected with epitope-tagged Kir6.1 or Kir6.2 cDNAs in the absence of an SUR subunit, the channel subunits were confined to the endoplasmic reticulum (ER) (Fig. 1) as confirmed by colocalization with the ER-specific marker ribophorin-2 (not shown). Consistent with the findings of others (38), we found predominant surface membrane labeling when coexpressing Kir6.1 or Kir6.2 together with SUR1 subunits (Fig. 1). However, when Kir6.x subunits were coexpressed with SUR2A subunits, a punctate intracellular staining pattern was observed in addition to the expression on the cell surface (Fig. 1). This subcellular localization pattern was also observed in other cell types (e.g., HEK-293T cells; supplemental Fig. S1) or when using untagged or GFP-tagged K\(_{\text{ATP}}\) channel constructs (e.g., Kir6.2-GFP; data not shown). Thus the vesicular staining pattern on SUR2A transfected cells did not depend on the nature of the Kir6.x subunit present, the cell type, or the method of detection.

We next investigated whether differences exist between SUR2A and SUR2B in terms of their subcellular trafficking; they differ from each other in the COOH-terminal 42 amino acids as a result of alternative splicing (33). In cells coexpressing Kir6.1/SUR2B subunits (the smooth muscle K\(_{\text{ATP}}\) channel subtype), both Kir6.1 and SUR2B subunits localized to intracellular vesicles (Fig. 1, bottom), suggesting trafficking to this subcellular compartment by the fully assembled heteromeric K\(_{\text{ATP}}\) channel. This finding demonstrates that SUR2B has the same trafficking properties as SUR2A.

To rule out the possibility that the experimental outcome was influenced by the ratio of subunits expressed, we investigated the effect of transfecting cells with different plasmid ratios. As the ratios of SUR\(_x\) to Kir6.1-encoding plasmids were increased over a 1,000-fold range, the localization changed from being exclusively in the ER (as expected from the limited
SUR levels where few channels can be assembled) to a largely plasmalemmal pattern for SUR1-containing channels or to a predominant vesicular staining pattern for SUR2-containing channels (supplemental Fig. S2). Thus the localization was not dependent on the subunit ratio. We also investigated whether functional channels are needed for these unique localization patterns by using a nonconducting Kir6.2-AAA pore mutant subunit (37). However, the trafficking patterns were unaltered (supplemental Fig. S3).

To determine the nature of the cellular organelles corresponding to the SUR2-containing vesicles, HEK-293T cells were transfected with Kir6.2-GFP and SUR2A cDNAs and stained with antibodies to human lysosomal-associated membrane protein-2 (LAMP2), a marker of endosomes and lysosomes (4). We found overlapping localization of LAMP2 and GFP (Fig. 2A), indicating that the SUR2-containing channels were targeted to the endosomal/lysosomal pathway. The vesicles generally did not correspond to those labeled with fluorescently tagged transferrin after a 15-min uptake period, indicating that KATP channels did not accumulate in early endosomes (supplemental Fig. S3). In addition, we did not observe any colocalization with mitotracker, a marker of mitochondria (supplemental Fig. S4).

**Turnover rate of KATP channel subunits.** The SUR2-dependent targeting to endosomes and lysosomes might be expected to accelerate channel turnover. However, the turnover rate of Kir6.1-myc subunits was comparable when expressed with SUR1 or SUR2A subunits as determined using a pulse-chase assay (the $t_{1/2}$ was ~20 and ~15 h for SUR2 and SUR1-containing channels, respectively; Fig. 2B). We also examined whether the differences in channel trafficking could be explained by an accumulation of SUR2-containing channels in lysosomes as a consequence of slower degradation. The cells were incubated with chloroquine, which neutralizes lysosomal pH, thereby blocking degradation and causing an accumulation of proteins in endosomes and lysosomes (11). Because chloroquine treatment was not well tolerated in HEK-293T cells, this experiment was performed in COS-1L cells. As shown in Fig. 2C, chloroquine did not greatly affect the appearance of SUR1-containing KATP channels, which did not accumulate in vesicles. Kir6.1/SUR2A channels were still localized to vesicles, which were enlarged in size. The data are consistent with the notion that the subcellular localization of SUR2 channels is due to a unique mode of trafficking to endosomes and lysosomes.

**Rapid internalization of Kir6.2/SUR2A KATP channels.** Previous reports have documented that KATP channels undergo endocytosis from the cell surface and that this process is greatly enhanced by the treatment of cells with phorbol 12-myristate 13-acetate (PMA), an activator of PKC (14, 22). To investigate whether there was an SUR-dependent difference in the rate of endocytosis, we used a Kir6.2 subunit containing an extracellular HA-epitope tag (Kir6.2HA+11). Live COS-1L cells were incubated with anti-HA antibodies, and the cells were either fixed immediately or after warming the cells to 37°C for 15 min. Internalization and vesicle trafficking were

![Fig. 3. Rapid internalization of SUR2-containing ATP-sensitive K⁺ (KATP) channels. COS1 cells transfected with plasmids encoding Kir6.2HA+11 and SUR1 (top) or SUR2A (bottom) were incubated for 2 h with anti-HA antibodies. Cells were warmed to 37°C for 15 min before fixation and staining with rhodamine-conjugated anti-rat antibodies. The anti-HA staining decorated the cell surface in SUR1-transfected cells, even after a 15-min incubation. By contrast, cell surface staining was initially observed in SUR2A-transfected cells, but vesicle staining was extensive after 15 min. Incubation of the cells with 100 nM PMA caused significant vesicle accumulation of antibody in SUR1-expressing cells but had little effect in SUR2-expressing cells. Figure panels have been adjusted for brightness and contrast. Bar = 10 μm.](http://ajpheart.physiology.org/)

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monitored by immunofluorescence microscopy. The SUR1-containing channels still predominantly localized at the cell surface, suggesting that the net uptake of the extracellular anti-HA antibody was slow (Fig. 3, top). By contrast, the extracellularly labeled SUR2-containing channels were initially observed at the surface, but by 15 min they were also observed in intracellular vesicles (Fig. 3, bottom), indicating that there was rapid and spontaneous antibody uptake. We also investigated the role of PKC activation. As previously reported (14), PMA dramatically accelerated the appearance of large labeled intracellular vesicles in SUR1-expressing cells (Fig. 3). However, this treatment had little effect in SUR2-expressing cells, most likely because of the already extensive internalization in the absence of PKC activation. The data demonstrate that SUR2-containing channels are present both on the cell surface and endosomal compartments and suggest that they dynamically cycle between these compartments.

SUR2-containing KATP channels localize to intracellular vesicles in smooth muscle and cardiac myocytes. SUR2 subunits are natively found in cardiac and smooth muscle cells, which respectively express SUR2A and SUR2B subunits (26). We therefore investigated whether this unique trafficking mode is recapitulated when SUR2-containing KATP channels are exogenously expressed in primary human coronary artery smooth muscle cells or in h9c2 cells, a cardiac cell line derived from embryonic rat heart (18). As shown in Fig. 4, exogenously expressed SUR2-containing KATP channels are exogenously expressed in primary human coronary artery smooth muscle cells or in h9c2 cells, a cardiac cell line derived from embryonic rat heart (18). As shown in Fig. 4, exogenously expressed SUR2-containing KATP channels targeted to intracellular vesicles in both cell types, whereas SUR1-containing channels were found primarily on the cell surface.

Rat heart KATP channels subunits translocate from endosomal membrane fractions to the SL following ischemia. To further investigate whether native KATP channel subunits are present in endosomal compartments, we evaluated their distribution in membrane preparations obtained from rat hearts using density gradient centrifugation. The Kir6.2 subunit was detectable in both SL and endosome-enriched fractions (Fig. 5A), which were identified using antibodies to the Na,K-ATPase and GLUT4, respectively. The ER marker ribophorin was widely distributed but best represented in the highest density fraction (data not shown), in contrast to Kir6.2. To examine the pathophysiological relevance of the endosomal localization of KATP channels in ventricular tissue, we subjected hearts to global ischemia before cell fractionation. The amount of Kir6.2 present in the SL fraction increased markedly after ischemia relative to the endosome-enriched fractions (Fig. 5B). Comparable results were obtained for SUR2A recovered in the SL fraction (23 vs. 58% after ischemia; data not shown). Similar results have been reported in isolated myocytes (3) and in Langendorff-perfused rat hearts (9).

**Increased sarcolemmal KATP channel density following ischemia.** A translocation of KATP channels to the SL after ischemia would be expected to lead to an increased KATP channel density. To measure this directly, we next performed patch-clamp recordings using ventricular myocytes isolated from rat hearts that were rendered globally ischemic before the cell isolation procedure. They were compared with myocytes isolated from nons ischemic hearts. In each group, myocytes were isolated from both the left and right ventricular free walls. There were no differences between the groups in cell capacitance (Table 1). Current-voltage relationships were recorded using slow voltage ramps. The current-voltage relationships were similar between groups as demonstrated by the absence of differences in the zero-current potential and currents recorded at $-5 \text{ mV}$. Dinitrophenol (DNP) was used to maximally activate KATP channel current. The application of DNP (100 $\mu\text{M}$) led to a large outward shift of membrane current at
voltages positive to the $K^+$ equilibrium potential ($E_k$), which reached to a plateau within 1 to 2 min (Fig. 6A). The reversal potential of the DNP-activated current was close to the $E_k$ (not shown) and was partially blocked by glibenclamide (2 $\mu$M), consistent with prior observations of an incomplete inhibition of K$_{ATP}$ channels by glibenclamide in metabolically impaired cells (10). Whereas the currents before the application of DNP were not significantly different between the ischemic and the nonischemic groups, DNP induced a larger current density in ischemic groups, DNP induced a larger current density in nonischemic control and ischemic rat hearts. The results from 3 experiments were quantified and plotted below as means ± SE. *$P < 0.05$. Figure panels have been adjusted for brightness and contrast.

### DISCUSSION

**Subcellular localization of K$_{ATP}$ channels.** K$_{ATP}$ channels were first discovered as ATP-gated $K^+$-selective channels present in isolated cardiac myocytes (27), and the sarcolemmal presence of K$_{ATP}$ channels has since then been confirmed by many others. The surface topology of K$_{ATP}$ channels has been mapped with high-resolution scanning ion conductance microscopy, and they were found to be organized in clusters and anchored in the $Z$-grooves of the SL (20). This is in keeping with the staining pattern observed using antibodies directed against the Kir6.2 subunit, which has been found to localize along $Z$-lines of isolated rat cardiac myocytes (25), suggesting an enrichment of K$_{ATP}$ channels in t-tubular structures. Collectively, these data provide clear evidence for the presence of sarcolemmal K$_{ATP}$ channels. Cell fractionation approaches have provided evidence that cardiac K$_{ATP}$ channel subunits are present in endosomal fractions (9) where they may function as a reservoir. The specific signaling mechanisms for the localization of K$_{ATP}$ channels to these subcellular fractions have not been identified. Our data provide novel mechanistic insight by demonstrating that the subtype of SUR subunit present in the K$_{ATP}$ Channel complex is responsible for its specific subcellular localization. Specifically, we show that, independent of the cell type, SUR2-containing K$_{ATP}$ channels localize to endosomal and lysosomal vesicles in transfected cells, whereas SUR1 consistently directed expression predominantly to the plasma membrane. The presence of SUR2-containing channels in endosomal/lysosomal compartments is not indicative of a degradation pathway since the protein turnover rate is similar when comparing SUR1 and SUR2-containing channels. Moreover, Western blot analysis of rat cardiac membrane

### Table 1. Electrical properties of ventricular cardiomyocytes isolated from ischemic rat hearts

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Values are means ± SE and are shown for nonischemic (control) and postischemic (ischemia) myocytes from the left (LV) or right (RV) ventricular free wall. Measurements include membrane capacitance ($C_m$), the zero-current voltage of the current-voltage relationship ($E_\text{m}$), and peak steady-state current density before (preDNP) and after (DNP) application of 100 $\mu$M dinitrophenol or glibenclamide (2 $\mu$M). *$P < 0.05$ compared with DNP group (paired t-test); †$P < 0.05$ comparing control and ischemic groups (t-test). There were no significant differences between LV and RV myocytes in either group.

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**Fig. 5.** Increase in cardiac myocyte sarcolemmal K$_{ATP}$ channels following global ischemia. A: cell fractionation was performed on rat ventricular tissue from nonischemic and ischemic rats using Optiprep gradient centrifugation. The sarcolemmal fraction (SL = 0%/5% interface), endosomal fraction (E = 5%/15% interface), and a loose pellet representing the highest density material (Hi) were subjected to Western blot analysis. B: representative Western blot of the SL and E fractions of membranes prepared from nonischemic control and ischemic rat hearts. The results from 3 experiments were quantified and plotted below as means ± SE. *$P < 0.05$. Figure panels have been adjusted for brightness and contrast.
fractions indicated that Kir6.2 and SUR2x subunits are both present in sarcolemmal as well as endosomal compartments. Collectively, these data indicate that KATP channels are not only expressed at the SL but are also present in endosomal compartments. The presence of a dynamic endosomal pool of SUR2-containing KATP channels suggests that SUR1 and SUR2 have evolved specialized trafficking and membrane regulatory pathways.

**The dynamic nature of sarcolemmal K$_{ATP}$ channel expression.** K$_{ATP}$ channel subunit expression is regulated at the transcriptional and/or translational level, for example as evidenced by alterations in their expression levels after hypoxia or ischemia (16, 24). Our data, along with previously published findings (3, 9), suggest an additional mechanism by which the functional sarcolemmal pool of K$_{ATP}$ channels is regulated. The picture that emerges is one of continuous and rapid cycling of K$_{ATP}$ channels from endosomal to membrane locations, with the balance between these compartments being shifted by events such as ischemia. Evidence favoring this mechanism of regulating the K$_{ATP}$ channel surface density includes our data demonstrating that extracellularly labeled SUR2-containing channels are rapidly internalized. A rapid internalization of K$_{ATP}$ channels, and their appearance in rab7-positive endosomes, has also been previously observed in COS-7 cells treated with PMA. This effect was blocked by the PKC inhibitor chelerythrine (14), which suggested an obligatory role of PKC activation in channel cycling. However, there is some evidence that PKC may actually promote cardiac myocyte K$_{ATP}$ channel translocation from endosomal to sarcolemmal compartments instead of causing internalization (9). Since our data were obtained under basal conditions and in the absence of exogenous stimuli, the role or PKC in K$_{ATP}$ channel internalization and cycling is not apparent in our studies. Although internalization and recycling of Kir6.2/SUR1 channels can occur (23), our results are consistent with a model for a trafficking mechanism, whereby both types of assembled channels are transported via the secretory pathway to the plasma membrane, where SUR2-containing channels are more rapidly and dynamically cycled to endosomal and lysosomal compartments. In isolated myocytes subjected to a hypoxia protocol, inhibitors of protein trafficking were shown to reduce the appearance of K$_{ATP}$ channels at the SL (3). It would be of interest to determine whether a similar

Fig. 6. Increase in sarcolemmal K$_{ATP}$ channel density in ventricular myocytes isolated from ischemic rat hearts. A and B: ventricular myocytes were isolated from nonischemic or ischemic rat hearts and subjected to whole cell patch clamping with a ramp protocol. A: representative current trace is depicted. The maximal K$_{ATP}$ channel current was estimated as the steady-state peak current after dinitrophenol (DNP) application. The dotted line indicates 0 current. B: current-voltage relationships of current densities before (circles) and after (squares) DNP application for cells in the nonischemic (filled symbols; n = 21) and ischemic (open symbols; n = 22) groups (left and right ventricular myocyte data were pooled). *P < 0.05. C: real-time RT-PCR analysis of indicted channel subunit mRNA expression in cardiac myocytes at 0 and 4 h after isolation from nonischemic (control) and ischemic hearts. Data (means ± SE) are expressed relative to housekeeping genes. Primer sequences are in the online supplement (Table S1).
Increased sarcolemmal $K_{ATP}$ channel density after myocardial ischemia. What might be the function of an intracellular pool of $K_{ATP}$ channels? One possibility is that the endosomal organelles might require $K_{ATP}$ channels to regulate their ionic milieu and/or function. Our cell fractionation and patch-clamp studies point to an additional function as a reservoir of channels available for recruitment to the SL after stress conditions, such as ischemia. A similar conclusion has been reached by others when describing sarcolemmal translocation after ischemia in Langendorff-perfused male rat hearts (9) and increased surface density of $K_{ATP}$ channels with “preconditioning” protocols in isolated cardiomyocytes (3). Of particular interest is our novel observation that the increased $K_{ATP}$ channel current protocols in isolated cardiomyocytes (3). Of particular interest is the postischemic period. Overall, mRNA expression of $K_{ATP}$ channel subunits occurred with cardiac remodeling during the weeks and months after an ischemic event (16). We did not observe such changes most likely because of the short (6 h) postischemic period. Overall, mRNA expression of $K_{ATP}$ channel subunits was remarkably stable, which suggests that the sarcolemmal translocation observed in our studies may be a short-term adaptive response to protect against the ischemic insult. It would be of interest to explore the time course of the sarcolemmal translocations and upregulation of sarcolemmal $K_{ATP}$ channel density to examine whether it may partly account for the sustained protection of ischemic preconditioning that is induced by short ischemic periods. The molecular mechanism(s) by which the $K_{ATP}$ channel translocates to the SL after an ischemic episode is also worth investigating. It is possible that the dynamics of endosomal/sarcolemmal cycling process are altered. However, it is also possible that the sarcolemmal stability of $K_{ATP}$ channels is increased. A potential candidate for mediating this process is ankyrin-B, a peripheral membrane protein that helps anchor plasma membrane proteins to the cytoskeleton and interacts with Kir6.2 (19). In any event, since the activity of sarcolemmal $K_{ATP}$ channels in cardiac myocytes is known to be important in protection from the effects of ischemia, the recruitment of $K_{ATP}$ channels from intracellular compartments is likely to play a significant contributory role in this process.

REFERENCES


