Plasticity of sarcolemmal $K_{\text{ATP}}$ channel surface expression: relevance during ischemia and ischemic preconditioning

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Submitted 19 February 2016; accepted in final form 30 March 2016

Yang HQ, Foster MN, Jana K, Ho J, Rindler MJ, Coetzee WA. Plasticity of sarcolemmal $K_{\text{ATP}}$ channel surface expression: relevance during ischemia and ischemic preconditioning. Am J Physiol Heart Circ Physiol 310: H1558–H1566, 2016. First published April 1, 2016; doi:10.1152/ajpheart.00158.2016.—Myocardial ischemia remains the primary cause of morbidity and mortality in the United States. Ischemic preconditioning (IPC) is a powerful form of endogenous protection against myocardial infarction. We studied alterations in $K_{\text{ATP}}$ channels surface density as a potential mechanism of the protection of IPC. Using cardiac-specific knockout of Kir6.2 subunits, we demonstrated an essential role for sarcolemmal $K_{\text{ATP}}$ channels in the infarct-limiting effect of IPC in the mouse heart. With biochemical membrane fractionation, we demonstrated that sarcolemmal $K_{\text{ATP}}$ channel subunits are distributed both to the sarcolemma and intracellular endosomal compartments. Global ischemia causes a loss of sarcolemmal $K_{\text{ATP}}$ channel subunit distribution and internalization to endosomal compartments. Ischemia-induced internalization of $K_{\text{ATP}}$ channels was prevented by CaMKII inhibition. $K_{\text{ATP}}$ channel subcellular redistribution was also observed with immunohistochemistry. Ischemic preconditioning before the index ischemia reduced not only the infarct size but also prevented $K_{\text{ATP}}$ channel internalization. Furthermore, not only did adenosine mimic IPC by preventing infarct size, but it also prevented ischemia-induced $K_{\text{ATP}}$ channel internalization via a PKC-mediated pathway. We show that preventing endocytosis with dynasore reduced both $K_{\text{ATP}}$ channel internalization and strongly mitigated infarct development. Our data demonstrate that plasticity of $K_{\text{ATP}}$ channel surface expression must be considered as a potentially important mechanism of the protective effects of IPC and adenosine.

$K_{\text{ATP}}$ channels; endocytic recycling; ischemia; ischemic preconditioning

NEW & NOTEWORTHY

Cardiac ischemia causes a loss of sarcolemmal $K_{\text{ATP}}$ channel density by a dynamin-mediated and CaMKII-dependent internalization mechanism. Ischemic preconditioning protects against the loss of surface $K_{\text{ATP}}$ channels in a PKC-dependent manner. Maintenance of surface $K_{\text{ATP}}$ channel expression must be considered as a potential protective mechanism of ischemic preconditioning.

AFTER MUCH RESEARCH AND DESPITE improvements in clinical outcomes, acute myocardial infarction secondary to ischemia is still the primary cause of morbidity and mortality in the United States. The major determinant of the long-term prognosis among patients who survive an infarct is the size of the infarct (the amount of myocardium that is destroyed as a result of ischemic injury). It is critical, therefore, to advance our understanding of the molecular, cellular, and physiological mechanisms involved in the development, progression, and treatment of coronary heart disease and to develop therapeutic strategies that will attenuate myocardial cell death and infarct size in patients.

In 1986 it was found that short bursts of ischemia/reperfusion (I/R) before an index ischemic episode significantly limit infarct size development (29). This phenomenon, termed ischemic preconditioning (IPC), is complex in nature and has a multifactorial mechanism. It is generally agreed that receptor signaling by adenosine and other endogenous autacoids are involved and that they act through intracellular signaling pathways that include PKC, PI3 kinase, Akt, and ERK (44), with multiple effectors including cytoskeleton, $K_{\text{ATP}}$ channel, connexin 43, and mitochondrial permeability transition pore (14).

Some of the earliest observations implicated $K_{\text{ATP}}$ channels as being important in the infarct-limiting benefits of IPC. For example, in dogs, the benefit of IPC was prevented by the $K_{\text{ATP}}$ channel blocker glibenclamide, whereas the $K_{\text{ATP}}$ channel opener RP-49356 (also named aprikalim) could substitute for IPC (12). A large number of studies with pharmacological approaches have now confirmed that $K_{\text{ATP}}$ channels have a key role in the cardioprotective mechanism(s) of IPC (9). The initial focus in the literature was on sarcolemmal $K_{\text{ATP}}$ channels, which consist of pore-forming Kir6.x subunits assembled with regulatory SURx subunits (9). Subsequently, $K_{\text{ATP}}$ channels were also found to be present in mitochondria (17), but their molecular composition is still unresolved. A large number of published studies have concluded that it is the mitochondrial $K_{\text{ATP}}$ channel that mediates cardioprotection, but these studies must be interpreted with some caution since they often rely on pharmacological approaches and the use of compounds such as diazoxide, which (at the concentration ranges used in these studies) lack the required specificity and selectivity (6). In contrast, genetic studies in mice and human patients have implicated the sarcolemmal $K_{\text{ATP}}$ channel subunits in cardiovascular events and protection (9). The focus of the current study is the sarcolemmal $K_{\text{ATP}}$ channel.
Thus endocytic recycling is a potentially powerful mechanism to regulate the surface density and the availability of ion channels (34). Cellular studies have demonstrated that K\textsubscript{ATP} channels can be endocytosed (4, 16, 23) and that they are recycled to the membrane (24). However, in hearts, K\textsubscript{ATP} channel endocytic recycling has not been studied during ischemia and IPC. The aim of this study was to investigate K\textsubscript{ATP} channel subcellular trafficking during ischemia and ischemic preconditioning. We found that K\textsubscript{ATP} channels are internalized during ischemia, that IPC and adenosine prevent this internalization, and that inhibition of K\textsubscript{ATP} channel internalization is cardioprotective.

**METHODS**

*Generation of cardiac-specific Kir6.2 knockout mice.* The mouse model was generated by inGenious Targeting Labs (iTL; Stony Brook, NY). In brief, a 13.27-kb region was subcloned from a positively identified C57BL/6 BAC clone (RPC1-23; 222N7). The region with a short homology arm extending ~2.55 kb 3' to exon 1 and a long 7.52 kb homology arm ending 5' to exon 1. A loxP/FRT-flanked Neo cassette was inserted on the 3' side of exon 1, and the single loxP site was inserted ~1.7 kb 5' of the start of exon 1. The target region was 3,198 bp and included exon 1 and ~1.7 kb of upstream sequence. The targeting construct was linearized with Ascl before electroporation into BA1 (C57BL/6 × 129/SvEv) hybrid embryonic stem cells. After G418 selection, surviving clones were expanded for PCR analysis to identify recombinant ES clones. Hybrid-positive ES cells were injected into C57BL/6 blastocysts. The Neo cassette was removed by breeding to FLP deleter mice (Jackson stock No. 005703), followed by backcrossing to a C57BL6 background for >10 generations to achieve congenicity. Animals were then crossed with αMHC-Mer-Cre-Mer mice (JAX No. 005650)(36) and bred to homozygosity to allow for tamoxifen-inducible Cre expression and cardiac-specific deletion of Kir6.2, which was performed by treatment for five consecutive days with tamoxifen (injected at 3 mg/g) to produce mice with deletion in the MHC promoter thereafter.

*AJP-Heart Circ Physiol* • doi:10.1152/ajpheart.00158.2016 • www.ajpheart.org
at least once a minute to evenly stain all sides. Once color development was achieved (TTC stains viable tissue brick red, whereas infarcted tissue is not stained and remained a pale tan/white color), the slices were washed and fixed in 10% formaldehyde for 20 min at room temperature before they were placed at 4°C overnight to improve contrast between the stained and unstained tissue. Slices were placed between two glass slides and imaged using a stereo microscope at 1.0× magnification and a charge-coupled device color camera (OptiXCam OCS-3.0). Infarct analysis was performed using a custom MATLAB program developed in-house. Using searching routines and contrast detection algorithms, the program automatically detects infarcted portions of heart in the red channel. This information is user verified and used to calculate the heart size, the infarct size, and the percentage of the infarct as a function of total heart size.

**Drugs used.** Dynasore hydrate was prepared as a 200-mM stock in DMSO and used at 80 μM by dissolving into KH solution. Chelerythrine chloride was prepared as a 380-mM stock in DMSO and KN-93 as a 1.6-mM stock in DMSO and respectively used at 3 μM and 0.5 μM by dissolving into KH solution. Adenosine (100 μM; Sigma-Aldrich, St. Louis, MO) was directly dissolved in KH solution.

**Statistical analysis.** When comparing two groups we used the Student’s t-test. For comparison of multiple groups we used one-way ANOVA. If overall significance was achieved, ad hoc pairwise comparison was performed using the Tukey t-test, or the Dunnett’s t-test. If comparison was performed using the Tukey t-test, or the Dunnett’s t-test. For comparison of multiple groups we used one-way ANOVA.

**RESULTS**

The infarct-limiting effect of IPC is absent in mouse hearts deficient in Kir6.2 expression. We verified the crucial role of sarcolemmal KATP channels in mediating the protective effects of IPC by using a conditional Kir6.2 knockout mouse model, which was crossed with αMHC-Mer-Cre-Mer mice (cardiac myocyte-specific Cre recombinase expression; JAX No. 005650) (36). Tamoxifen treatment of the heterozygous and homozygous Cre+ offspring, respectively, led to ~50% and near complete reduction of cardiac Kir6.2 protein expression (Fig. 1A). Brain Kir6.2 expression was unaffected. To assess effects on ischemia and IPC, Kir6.2-Cre+/flx mice were injected with tamoxifen, or with corn oil as a control, and used after 8 wk. Isolated hearts were subjected to 30-min global ischemia, either preceded by an IPC perfusion protocol (3 × 5 min ischemia) or a time-matched normoxic perfusion period. As expected, IPC strongly diminished the infarct size in the control group. In contrast, the infarct-reducing protection afforded by IPC was strongly mitigated in the tamoxifen-treated Kir6.2 knockout mouse hearts (Fig. 1B). These data demonstrate an essential role for Kir6.2-containing sarcolemmal KATP channels in mediating the protective effects of IPC.

Ischemia causes internalization of KATP channels, which is prevented by IPC. Because KATP channels undergo rapid endocytosis and recycling, we investigated the effects of ischemia on the subcellular distribution of KATP channels in hearts. We used density gradient ultracentrifugation with OptiPrep to separate sarcolemmal (SL; 0–10% fraction) and endosomal (E; 10–20% fraction) membranes from mouse hearts. In normoxic mouse hearts, on average ~60% of Kir6.2 was present in the sarcolemmal membrane fraction. After 30-min global ischemia, however, the averaged SL expression decreased to ~20% (Fig. 2, A and B). Ischemia-induced internalization of SUR2A was also observed (Fig. 2D). We next investigated the effects of IPC. Remarkably, not only did IPC limit the infarct development during ischemia but it also prevented the ischemia-induced internalization of Kir6.2 (Fig. 2, C). Another membrane protein, NCX1, showed only minor SL/E distribution changes (Fig. 2E). Thus ischemia causes a loss of surface KATP channel expression, whereas KATP channel surface expression is maintained by IPC.

Adenosine also prevents ischemia-induced KATP channel internalization. Several endogenous molecules, including adenosine, can mimic the protective effect of ischemic precon-
ischemia (IPC). Immunoblotting was performed with antibodies against Na+/H1005

from mouse hearts with normoxic perfusion (control), after 30-min global

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a deleterious role for CaMKII and a beneficial effect of CaMKII inhibition during ischemia (2). Moreover, CaMKII inhibition enhances the KATP current density (19), whereas CaMKII activation stimulates KATP channel endocytosis in cardiomyocytes (35). We therefore investigated the significance of CaMKII signaling during ischemia. When mouse hearts were perfused with the CaMKII inhibitor KN-93 before the index ischemia, Kir6.2 internalization did not occur (Fig. 4), suggesting that ischemia-induced KATP channel internalization occurs though a CaMKII-mediated mechanism.

**PKC signaling during IPC maintains the surface KATP channel density.** Adenosine and opioids mediate their cardioprotective effects through PKC activation. We therefore investigated the hypothesis that PKC activation positively regulates KATP channel surface expression during IPC. The PKC inhibitor chelerythrine did not prevent internalization of Kir6.2 during ischemia (Fig. 4). However, the protective effect of IPC on surface KATP channel expression was eliminated (Fig. 4). Thus we conclude that IPC causes re-expression of surface KATP channels in a PKC-dependent manner.

**Plasticity of KATP channel distribution can be directly observed with microscopy.** We next sought to investigate ischemia-induced KATP channel internalization using an independent assay by performing immunofluorescence microscopy of mouse hearts. Consistent with our previous report (15), we found KATP channel expression in punctate patterns in cardiomyocytes with enrichment at the intercalated disk (ICD) (Fig. 5A). For quantitative analysis, we focused on the ICD regions identified by connexin 43 (Cx43) expression (arrows). Consistent with the biochemical assays, the Kir6.2 staining at the ICD was significantly reduced by ischemia compared with nonischemic hearts. Moreover, IPC prevented this ischemia-induced

conditioning (14), a process also referred to as pharmacological preconditioning. We investigated the effects of pharmacological preconditioning with adenosine on the plasticity of KATP channel surface expression. As expected, perfusion of mouse hearts with 100 μM adenosine before a 30-min index ischemia effectively decreased infarct size (Fig. 3C). Interestingly, as we have observed with IPC, adenosine also fully prevented the ischemia-induced surface loss of KATP channel subunits (Fig. 3, A and B). Thus two forms of cardioprotection (IPC and adenosine) share the phenomenon of preventing the surface loss of KATP channels during ischemia.

**CaMKII signaling is involved in ischemia-induced KATP channel internalization.** We investigated some of the signaling mechanisms involved in modulating ischemia-induced KATP channel internalization. During ischemia, Ca**+/calmodulin-dependent protein kinase II (CaMKII) is activated (2), partly as a result of an elevated [Ca**+]). Considerable evidence indicates

FIG. 2. Ischemic preconditioning not only limits infarct size but also prevents ischemia-induced internalization of the Kir6.2 KATP channel subunit. Isolated mouse hearts were made globally ischemic for 30 min, either with (IPC) a 3 × 5 min IPC protocol or without (ischemia group). A: Western blot with an anti-Kir6.2 antibody of sarcolemmal (SL; 0–10% Optiprep) and endosomal (E; 10–20% Optiprep) membranes for the various groups. B: summary data of sarcolemmal Kir6.2 (as a fraction of the total amount; SL/[SL + E]) for the control (n = 9), ischemia (n = 9), and IPC (n = 7); *P < 0.05 vs. the control group. C: in a different set of hearts, infarct sizes were determined by TTC staining; control (n = 8), ischemia (n = 12), and IPC (n = 7). *P < 0.05 vs. the control group.

FIG. 3. Adenosine perfusion prevents ischemia-induced internalization of the Kir6.2 KATP channel subunit and limits infarct size. Isolated mouse hearts were made globally ischemic for 30 min. A time-matched control normoxic group was also included. A: Western blot with an anti-Kir6.2 antibody of sarcolemmal (0–10% Optiprep) and endosomal (10–20% Optiprep) membranes for the various groups. B: summary data of sarcolemmal Kir6.2 during ischemia was also ob-

served with microscopy. D: redistribution of Kir6.2 from sarcolemmal to endosomal membrane fractions with ischemia was also ob-

served with SUR2A. E: internalization of Kir6.2 during ischemia is not a general phenomenon. Sarcolemmal and endosomal membranes were prepared from mouse hearts with normoxic perfusion (control), after 30-min global ischemia (ischemia) or with 3 × 5 min ischemia before a 30-min index ischemia (IPC). Immunoblotting was performed with antibodies against Na+/Ca**+ exchanger (NCX1) and GAPDH. Data are representative of n = 3 experiments.

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FIG. 5. PKC signaling during IPC maintains the surface KATP channel density. Adenosine and opioids mediate their cardioprotective effects through PKC activation. We therefore investigated the hypothesis that PKC activation positively regulates KATP channel surface expression during IPC. The PKC inhibitor chelerythrine did not prevent internalization of Kir6.2 during ischemia (Fig. 4). However, the protective effect of IPC on surface KATP channel expression was eliminated (Fig. 4). Thus we conclude that IPC causes re-expression of surface KATP channels in a PKC-dependent manner.

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**CaMKII signaling is involved in ischemia-induced KATP channel internalization.** We investigated some of the signaling mechanisms involved in modulating ischemia-induced KATP channel internalization. During ischemia, Ca**+/calmodulin-independent protein kinase II (CaMKII) is activated (2), partly as a result of an elevated [Ca**+]). Considerable evidence indicates
redistribution of K<sub>ATP</sub> channels (Fig. 5, B and C). Although previous studies have described distribution changes of Cx43 with chronic ischemia and with ischemic cardiomyopathies (26), during acute ischemia and IPC, we found Cx43 redistribution to be minimal relative to that of K<sub>ATP</sub> channels (data not shown). Thus plasticity of K<sub>ATP</sub> channel expression and subcellular redistribution could be observed with two independent assays.

**Inhibition of endocytosis is cardioprotective.** Many membrane proteins, including K<sub>ATP</sub> channels (9), are internalized by clathrin- and dynamin-dependent endocytosis. To examine the role of endocytosis during ischemia, we used the dynamin inhibitor, dynasore (22). Consistent with a role for dynamin-dependent endocytosis during ischemia, we found that perfusing mouse hearts with 80 μM dynasore largely prevented ischemia-induced K<sub>ATP</sub> channel internalization, either when measuring K<sub>ATP</sub> channel subcellular distribution biochemically (Fig. 6A) or with microscopy (Fig. 7). Consistent with the hypothesis that ischemia-induced endocytosis is detrimental, we found dynasore to be powerfully cardioprotective in that it mimicked IPC by preventing infarct formation (Fig. 6B).

**DISCUSSION**

Using cardiac-specific K<sub>ATP</sub> channel knockout animals, we demonstrated an essential role for Kir6.2-containing sarcolemmal K<sub>ATP</sub> channels in the infarct-limiting effect of IPC in the mouse heart. A major finding of the current study is that ischemia causes a loss of surface K<sub>ATP</sub> channels and that IPC restores this defect. This plasticity of surface K<sub>ATP</sub> channel expression comes about from an internalization process during ischemia that depends on dynamin and CaMKII signaling (possibly as a result of intracellular Ca<sup>2+</sup> accumulation). The K<sub>ATP</sub> channel surface density is maintained by IPC and by adenosine, and this appears to occur as a result of a PKC-mediated re-expression of surface K<sub>ATP</sub> channels. Preventing endocytosis with dynasore mimics the cardioprotective infarct size-limiting effects of IPC. On balance, our data suggest that maintenance of the surface K<sub>ATP</sub> channel density represent a novel cardioprotective mechanism.

**Sarcolemmal K<sub>ATP</sub> channels are necessary for the protective effects of IPC.** Sarcolemmal K<sub>ATP</sub> channels contribute to cardioprotection and cardiovascular disease, as demonstrated by a multitude of animal studies using knockout mouse models of K<sub>ATP</sub> channel subunits (13, 38, 39, 43) and by the association of genetic variation in K<sub>ATP</sub> channel genes and human cardiovascular disease (3, 7, 30, 32, 33) and risk of myocardial infarction (27). There has been much controversy regarding the relative roles of mitochondrial and sarcolemmal K<sub>ATP</sub> channels in mediating the protective effects of IPC (9, 10). We used a novel mouse model in which cardiac myocytes are specifically deficient of the K<sub>ATP</sub> channel subunit, Kir6.2, which is thought to be a component of the sarcolemmal, but not the mitochondrial, K<sub>ATP</sub> channel (9). Interestingly, the infarct size in the hearts of these animals was not significantly larger during ischemia, which suggests that K<sub>ATP</sub> channel opening during ischemia might not directly contribute to cardioprotection. This finding is consistent with the observation made in several animal models that K<sub>ATP</sub> channel blockers by themselves generally have little effect on infarct size development (9). In striking contrast, we found that the protective effect of IPC on infarct development was completely absent in the hearts of mice that lack the sarcolemmal K<sub>ATP</sub> channel subunit, Kir6.2. This observation is consistent with a multitude of studies demonstrating that structurally diverse K<sub>ATP</sub> channel openers (including aprikalim, bimakalim, pinacidil, nicorandil, and diazoxide) are cardioprotective by reducing the size of the developing infarct when they are applied before index ischemia (instead of IPC) and that the protective effects of IPC can be prevented by K<sub>ATP</sub> channel blockers such as glibenclamide, 5-hydroxydecanoate, and HMR-1098 (9). Our data are also consistent with previous studies demonstrating an essential role for Kir6.2 in the genesis of diazoxide-induced preconditioning in mouse hearts (38, 43). Overall, our finding underscores the fact that the sarcolemmal K<sub>ATP</sub> channel has a crucial role in mediating the beneficial effects of IPC.

**Loss of surface K<sub>ATP</sub> channels during ischemia.** Sarcolemmal K<sub>ATP</sub> channel opening can be protective by conserving ATP consumption due to the negative inotropic effect caused by action potential shortening (the “energy sparing hypothesis”), by preventing Ca<sup>2+</sup>-induced mitochondrial dysfunction, and through other mechanisms (9, 37). Even though sarcolemmal K<sub>ATP</sub> channels are strongly linked to the cardioprotective effects of IPC (13, 19, 39, 43), the underlying molecular mechanisms have not been resolved. Our studies demonstrate that K<sub>ATP</sub> channels are internalized during ischemia, which would mitigate any protective roles of these channels.

In a previous study we reported that ischemia increased the SL K<sub>ATP</sub> channel subunit abundance in rat hearts harvested from dead rats (20 min after euthanasia) (1). We have now repeated the study with mouse hearts, by carefully controlling...
all experimental variables, including the temperature, optimization of the membrane preparation protocols, having time-matched controls, the duration of ischemia, and the presence of IPC. A main finding of the present study is that both Kir6.2 and SUR2A are internalized during ischemia (i.e., they translocate from SL to E membranes). Species differences are an unlikely explanation for the different result since we also observed Kir6.2 internalization with Langendorff-perfused rat hearts (not shown). The ischemia-induced internalization of K$_{\text{ATP}}$ channels is akin to the internalization of the Na$^+$/K$^+$ pump $\alpha$-subunit that occurs in cellular models of simulated ischemia (31). The K$_{\text{ATP}}$ channel internalization that we observed during ischemia is unlikely to reflect massive endocytosis (MEND), described in fibroblasts and in isolated cardiac myocytes subjected to anoxia (21). Hallmarks of MEND are that it is cargo-indiscriminate, it is dependent on palmitoylation, and it occurs without the involvement of classical endocytic proteins (18). In contrast, we show that internalization during ischemia discriminates between various cargos and can be blocked by inhibitors of endocytosis. The fact that dynasore blocks the process suggests that ischemia-induced internalization occurs through classical endocytosis described in other cells, namely cargo recognition mediated by binding of Kir6.2 to the $\mu$2 subunit of the AP-2 adaptor complex (23) and an internalization pathway mediated by clathrin and dynamin (23, 35).

The role of CaMKII. We showed that CaMKII inhibition with KN-93 prevents ischemia-induced K$_{\text{ATP}}$ channel internalization. A potential role for this Ca$^{2+}$-dependent signaling pathway in K$_{\text{ATP}}$ channel trafficking was previously suggested by the finding that CaMKII inhibition increases the K$_{\text{ATP}}$ channel density in membrane patches from cardiomyocytes (19). In a subsequent study, it was shown that CaMKII activation reduces K$_{\text{ATP}}$ channel surface density in isolated cardiac myocytes by stimulating endocytosis (35). Moreover, a Kir6.2

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**Fig. 5.** Immunohistochemistry (IHC) of mouse heart cryosections, demonstrating ischemia-induced K$_{\text{ATP}}$ channel subcellular redistribution and its prevention by IPC. A: representative images obtained with antibodies against Kir6.2 and Connexin 43 (Cx43) in the control, ischemia, and IPC groups. B: relationship between Kir6.2 fluorescence intensity and distance across the intercalated disk (ICD) in the control (black), ischemia (red), and IPC (blue) groups. C: quantification of the peak Kir6.2 fluorescence at the ICD in the control. For each group, data are from 3 hearts and 3 separate sections for each heart ($n = 9$). *$P < 0.05$ vs. the control group.
tyrosine-based internalization motif (330YSKF333) was necessary for CaMKII-induced endocytosis. Our finding provides further credence to the concept that internalization is mediated in part by CaMKII activation. A decreased number of sarcolemmal KATP channels during ischemia due to internalization would be expected to reduce the protective role of KATP channels. Rescuing KATP channel internalization with CaMKII inhibition (19) is therefore a potential mechanism for preventing cardiac damage.

Is trafficking inhibition the key to prevent ischemic damage? Our data show that a specific inhibitor of dynamin-dependent endocytosis (dynasore) mimics IPC by being powerfully protective against infarct size development. The interpretation of this result is not simple, since all forms of dynamin-dependent endocytosis are being inhibited by the drug. Given the positive regulation of endocytosis by CaMKII (19, 35), it is tempting to speculate that CaMKII inhibitors and internalization inhibitors (such as dynasore) share a common mechanism of action. The literature is not consistent in this regard, however, since it has been suggested that internalization of G protein-coupled receptor signaling complexes may serve as a mediator of IPC (40). It should be noted that endocytosis of GPCRs can occur via multiple mechanisms, including caveolae, clathrin-coated vesicles, or uncoated vesicles (5), and the potential deleterious or protective roles of specific endocytic pathways remain to be elucidated. Because dynasore improves the ischemic outcome, our data suggest that dynamin-dependent processes are deleterious to the ischemic heart. Further challenges remain, including the identities of the specific proteins involved (likely to be multifactorial) and their relative roles. The KATP channel appears to be one of these candidates, but it is unlikely to be the only relevant protein involved. Regardless, our data raise the tantalizing question whether trafficking inhibition is a key to
prevent ischemic damage and whether more targeted pharmacological or molecular approaches might be useful to mediate cardioprotection.

K\textsubscript{ATP} channel surface density is maintained by IPC and adenosine. A major finding of this study is that IPC not only limits infarct development, it also maintains the K\textsubscript{ATP} channel surface density by affecting subcellular distribution patterns. We verified this finding with two independent methods (biochemically and with immunohistochemistry). Given the key role of K\textsubscript{ATP} channels in mediating the protective effect of IPC on infarct size (9), also demonstrated with the conditional loss and restores the density of the sarcolemmal K\textsubscript{ATP} channels. The IPC protocol by itself, in the absence of index ischemia, did not cause a significant redistribution of K\textsubscript{ATP} channel subunits (not itself, in the absence of index ischemia, did not cause a significant redistribution of K\textsubscript{ATP} channel subunits (not shown), suggesting that IPC sets in motion a cascade of events that operate during a subsequent ischemic period. Indeed, we found that adenosine, an endogenous autacoid that mimics the infarct-limiting effects of IPC (8), similarly protects against ischemia-induced K\textsubscript{ATP} channel internalization. Our data are also in full support of the known cross-reaction between adenosine signaling and K\textsubscript{ATP} channels. For example, the protective effect of adenosine is prevented by K\textsubscript{ATP} channel blockers (20, 41) and is absent in K\textsubscript{ATP} channel-deficient Kir6.2\textsuperscript{-/-} mice (42). Moreover, our data are also in full support of the notion that the protective effects of IPC and adenosine occur through PKC activation (11). Indeed, PKC inhibition did not prevent ischemia-induced K\textsubscript{ATP} channel internalization, but disabled the protective effect of IPC to maintain the surface K\textsubscript{ATP} channel density. Thus, taken together, our data show that IPC, adenosine, and intracellular signaling through PKC converge on the preservation of the sarcolemmal K\textsubscript{ATP} channel density and that this response may be an important element in the protection afforded by these processes.

Conclusions

Our studies show that cardiac ischemia causes a loss of the sarcolemmal K\textsubscript{ATP} channel density by internalization through a pathway mediated by dynamin-dependent endocytosis and CaMKII-mediated signaling. Importantly, IPC counteracts this loss and restores the density of the sarcolemmal K\textsubscript{ATP} channels. Adenosine mimics IPC in this regard, and PKC signaling mediates the maintenance of surface K\textsubscript{ATP} density. Inhibition of endocytosis mimics IPC by protecting against infarct development. We propose that maintenance of the K\textsubscript{ATP} channel surface density must be considered as a protective mechanism of ischemic preconditioning (Fig. 8).

GrANTS

This work was supported by National Heart, Lung, and Blood Institute Grants HL-126905, HL-126905-S1, HL-093563, HL-085820, and HL-085820-S1 (to W. A. Coetzee).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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