Recombinant Cardiac ATP-Sensitive K⁺ Channel Subunits Confer Resistance To Chemical Hypoxia-Reoxygenation Injury

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Background—Opening of cardiac ATP-sensitive K⁺ (K\textsubscript{ATP}) channels has emerged as a promising but still controversial cardioprotective mechanism. Defining K\textsubscript{ATP} channel function at the level of recombinant channel proteins is a necessary step toward further evaluation of the cardioprotective significance of this ion conductance.

Methods and Results—K\textsubscript{ATP} channel–deficient COS-7 cells were found to be vulnerable to chemical hypoxia-reoxygenation injury that induced significant cytosolic Ca\textsuperscript{2+} loading (from 97±3 to 236±11 nmol/L). In these cells, the potassium channel opener pinacidil (10 μmol/L) did not prevent Ca\textsuperscript{2+} loading (from 96±3 nmol/L before to 233±12 nmol/L after reoxygenation) or evoked membrane current. Cotransfection with Kir6.2/SUR2A genes, which encode cardiac K\textsubscript{ATP} channel subunits, resulted in a cellular phenotype that, in the presence of pinacidil (10 μmol/L), expressed K⁺ current and gained resistance to hypoxia-reoxygenation (Ca\textsuperscript{2+} concentration from 99±7 to 127±11 nmol/L; P>0.05). Both properties were abolished by the K\textsubscript{ATP} channel blocker glyburide (1 μmol/L). In COS-7 cells transfected with individual channel subunits Kir6.2 or SUR2A, which alone do not form functional cardiac K\textsubscript{ATP} channels, pinacidil did not protect against hypoxia-reoxygenation.

Conclusions—The fact that transfer of cardiac K\textsubscript{ATP} channel subunits protected natively K\textsubscript{ATP} channel–deficient cells provides direct evidence that the cardiac K\textsubscript{ATP} channel protein complex harbors intrinsic cytoprotective properties. These findings validate the concept that targeting cardiac K\textsubscript{ATP} channels should be considered a valuable approach to protect the myocardium against injury. (Circulation. 1998;98:1548-1555.)

Key Words: ischemia ■ hypoxia ■ reperfusion ■ potassium channels ■ calcium

In recent years, targeting of cardiac ATP-sensitive K⁺ (K\textsubscript{ATP}) channels, a metabolism-sensitive ion conductance, has emerged as a promising therapeutic strategy against ischemic injury in the myocardium.¹-⁴ In particular, potassium channel openers, which promote opening of K\textsubscript{ATP} channels, have been found to decrease infarct size, mimic ischemic preconditioning, and improve functional and energetic recovery of cardiac muscle after ischemic and hypoxic insults.¹-⁸ Moreover, in the majority of studies, antagonists of K\textsubscript{ATP} channels, such as the sulfonylurea drugs, abolished the beneficial effect of potassium channel openers, further implying a cardioprotective role for K\textsubscript{ATP} channels.⁷,⁸ However, in the absence of more direct evidence that channel proteins themselves are responsible for cardioprotection, this concept has been continuously contested,⁹-¹¹ partly because of the complexity of regulation of K\textsubscript{ATP} channel behavior under ischemic conditions, as well as the difficulty in separating K\textsubscript{ATP} channel–dependent from K\textsubscript{ATP} channel–independent protective mechanisms that coexist within a cardiac cell.¹²-²⁰ In this regard, defining K\textsubscript{ATP} channel function at the level of recombinant channel proteins is a necessary step toward further evaluation of the cardioprotective significance of this ion channel. See p 1479

Proteins that constitute the K\textsubscript{ATP} channel complex have been cloned.²¹-²⁴ Cardiac K\textsubscript{ATP} channels are heteromultimers composed of at least 2 structurally distinct subunits. The pore-forming inwardly rectifying K⁺ channel core, Kir6.2, is primarily responsible for K⁺ permeance, whereas the regulatory subunit, also known as the sulfonylurea receptor, or SUR2A, has been implicated in ligand-dependent channel gating.²⁵ Coexpression of Kir6.2 with SUR2A in a cell line devoid of endogenous K\textsubscript{ATP} channels reconstitutes basic electrophysiological and pharmacological properties of the native cardiac channel.²³,²⁵ However, it is not known whether expression of recombinant cardiac K\textsubscript{ATP} channel proteins also confers protection against cell injury.

We therefore took advantage of the COS cell line, which lacks native K\textsubscript{ATP} channels²³,²⁵ and is vulnerable to oxidative stress, to deliver genes encoding the 2 cardiac K\textsubscript{ATP} channel subunits Kir6.2 and SUR2A and examined the outcome of activation of heterologously expressed channel proteins on the cellular susceptibility toward hypoxia-reoxygenation. We...
report that activation of recombinant cardiac Kir6.2 channels protects against hypoxia-reoxygenation–induced intracellular Ca\(^{2+}\) loading and present direct evidence that Kir6.2 and SUR2A are both necessary and sufficient to confer cellular protection.

Methods

Untransfected and Transfected COS-7 Cells

COS-7 cells (ATCC) were cultured in a tissue flask (at 5% CO\(_2\)) containing DMEM supplemented with 10% FCS and 2 mmol/L glutamine. Cells were then trypsinized (5 minutes, 37°C) and plated (2 \times 10^6 to 6 \times 10^6) on a 35 \times 10-mm or 60 \times 15-mm culture dish containing 12-mm or 25-mm glass coverslips. Cells were left untransfected or were transfected 24 hours later. At 40% to 60% confluence, cells were transfected by use of 8 to 24 \muL lipofectamine (Gibco), with 2 to 6 \muL of total plasmid DNA (full-length Kir6.2 and/or SUR2A cDNA subcloned into the mammalian expression vector pcDNA3.1\(^{+}\)) and with 0.2 to 0.6 \muL of the reporter green fluorescent protein (GFP) gene (Gibco).\(^{17}\) Kir6.2 was a gift from Dr S. Seino (Chiba University, Chiba, Japan).\(^{22,23}\) SUR2A was kindly provided by Dr Y. Kurachi (Osaka University, Osaka, Japan).\(^{24,25}\)

Digital Epifluorescent Imaging

In a chamber constructed with a coverslip as its base, COS-7 cells were superfused with Tyrode’s solution (in mmol/L: NaCl 136.5, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, glucose 5.5, and HEPES-NaOH 5.5; pH 7.4). Cells were loaded (for 30 minutes) with the esterified form of the Ca\(^{2+}\)-sensitive fluorescent probe fluo-3 (5 \mumol/L fluo-3-AM, dissolved in DMSO plus pluronic acid; Molecular Probes). Before loading with fluo-3-AM, transfected cells were preselected on the basis of GFP fluorescence, and GFP-dependent fluorescence was digitally subtracted. COS-7 cells were imaged with a digital epifluorescence imaging system coupled to an inverted microscope (Zeiss Axiosvert-135 TV) with a \(\times 40\) (numerical aperture, 1\(\times\)) oil-immersion objective lens. A 100-W mercury lamp served as a source of light to excite fluo-3 at 488 nm. An excitation dichroic mirror with a cutoff of 510 nm and a long-pass emission filter with a cutoff of 520 nm were used to detect fluo-3 fluorescence with an intensified charge-coupled device (CCD) camera. Detected fluorescence was digitized with imaging software (Attoflor RatioVision, Atto Instruments). An estimate of the cytosolic Ca\(^{2+}\) concentration as a function of fluo-3 fluorescence was calculated according to the equation [Ca\(^{2+}\)]\(_c\)=K\(_s\)(F−F\(_{\text{min}}\))/F−F\(_{\text{max}}\), where F\(_{\text{min}}\) and F\(_{\text{max}}\) are minimal and maximal fluorescence intensity, K\(_s\) is the dissociation constant of the fluo-3–Ca\(^{2+}\) complex (422 nmol/L), and F is the intensity of fluorescence. To obtain F\(_{\text{min}}\) and F\(_{\text{max}}\) values, cells were exposed to 100 \mumol/L ionomycin either in the absence of Ca\(^{2+}\) (extracellular Ca\(^{2+}\) was removed and 3 mmol/L EGTA added to the extracellular solution) or in the presence of saturating concentrations of Ca\(^{2+}\) (10 mmol/L CaCl\(_2\), respectively).\(^{26,27}\)

Electrophysiological Measurements

Fire-polished pipettes coated with Sylgard (resistance, 5 to 7 MΩ) were filled with “pipette solution” (in mmol/L: KCl 140, CaCl\(_2\) 1, MgCl\(_2\) 1, and HEPES-KOH 5; pH 7.3). COS-7 cells were superfused with Tyrode’s solution, and the patch-clamp technique was applied in the cell-attached configuration.\(^{28}\) Single-channel recordings were monitored on-line with a high-gain digital storage oscilloscope (VC-6025, Hitachi) and stored on tape with a PCM converter system (VR-10, Instrutech). Data were reproduced, low-pass filtered at 1 kHz (−3 dB) by a Bessel filter (Frequency Devices 902), sampled at

Figure 1. Hypoxia-reoxygenation induces Ca\(^{2+}\) loading in untransfected COS-7 cells. A, Epifluorescent digital images of fluo-3 loaded cells before (left) and after (right) hypoxia-reoxygenation. Hypoxia-reoxygenation was induced by application (3 minutes at 37°C) and removal of 2 mmol/L DNP. Bar=90 \muM. B, Time course of fluo-3 fluorescence in cells presented in A. AU indicates arbitrary units. C, Average concentration of intracellular Ca\(^{2+}\) at rest (open bar) and after hypoxia-reoxygenation (hatched bar). Bars represent mean±SEM (n=23); \(^*\)P<0.01.

Figure 2. Pinacidil fails to protect against hypoxia-reoxygenation–induced Ca\(^{2+}\) loading in untransfected COS-7 cells. A, Epifluorescent digital images before (left) and after (right) hypoxia-reoxygenation in fluo-3 loaded cells in presence of pinacidil. Same protocol as in Figure 1. Bar=90 \muM. B, Average concentration of intracellular Ca\(^{2+}\) at rest (open bar) and after hypoxia-reoxygenation (hatched bar) obtained in 10 \mumol/L pinacidil–treated cells. Bars represent mean±SEM (n=4); \(^*\)P<0.01. C, Channel record obtained in cell-attached configuration from pinacidil-treated untransfected COS-7 cell. Zero current level coincides with channel record.
a 50-μs rate, and analyzed with pClamp6 software (Axon Instruments). The threshold for judging the open state was set at half of the single-channel amplitude. Channel activity was expressed as \( N_P, \frac{P_o}{N}, \) number of channels in the patch; \( P_o, \) probability of each channel to be open.}

**Chemical Hypoxia-Reoxygenation Injury**

COS-7 cells superfused with Tyrode’s solution were exposed to 2 mmol/L 2,4-dinitrophenol (DNP), a metabolic poison that inhibits mitochondrial oxidative phosphorylation. After a 3-minute treatment, DNP was removed, and cells were reexposed to Tyrode’s solution. This chemical hypoxia-reoxygenation protocol was conducted in the absence or presence of the potassium channel opener pinacidil, with and without the potassium channel blocker glyburide. Both drugs were dissolved in DMSO, which in its final concentration did not exceed 0.1%. At this concentration, DMSO did not affect Ca\(^{2+}\) levels or channel activity.

**Single Ventricular Cardiomyocytes**

In a separate series of experiments, the same chemical hypoxia-reoxygenation protocol was applied to cardiomyocytes. Ventricular myocytes were dissociated from pentobarbital-anesthetized guinea pigs.\(^\text{18,19,28}\) The heart was perfused (at 37°C) with medium 199 for 3 minutes, followed by Ca\(^{2+}\)-EGTA–buffered low-Ca\(^{2+}\) medium (pCa=7) for 80 seconds and low-Ca\(^{2+}\) medium containing pronase E (8 mg/100 mL), proteinase K (1.7 mg/100 mL), BSA (0.1 g/100 mL), and 200 μmol/L Ca\(_\text{Cl}_2\). Ventricles were separated from atria and cut into small fragments in the low-Ca\(^{2+}\)-medium enriched with 200 μmol/L Ca\(_\text{Cl}_2\). Single cells were isolated by stirring the tissue (at 37°C) in a solution containing pronase E and proteinase K supplemented with collagenase (5 mg/10 mL). After 10 minutes, the first aliquot was removed, filtered through a nylon sieve, centrifuged for 60 seconds (at 300 rpm), and washed twice. Isolation continued for 2 to 3 such cycles. Rod-shaped cardiomyocytes with clear striations and smooth surfaces were imaged by digital epifluorescent microscopy as described above.

**Statistical Analysis**

Data are presented as mean \( \pm \) SEM, with \( n \) representing the number of imaged fields or patched cells. Mean values were compared by Student’s \( t \) test or by 1-way ANOVA. A value of \( P<0.05 \) was considered statistically significant.

**Results**

**Hypoxia-Reoxygenation Induces Ca\(^{2+}\) Loading in Untransfected COS-7 Cells**

At rest, COS-7 cells have a low cytosolic Ca\(^{2+}\) concentration (97.2 ± 3.1 nmol/L; \( n = 23 \); Figure 1A to 1C). A 3-minute-long exposure to chemical hypoxia induced by the mitochondrial uncoupler DNP, followed by reoxygenation evoked by removal of DNP, produced rapid and significant Ca\(^{2+}\) loading (236 ± 11 nmol/L, \( n = 23 \); \( P<0.01 \); Figure 1A to 1C).

**Pinacidil Does Not Protect Untransfected COS-7 Cells Against Hypoxia-Reoxygenation–Induced Ca\(^{2+}\) Loading**

The same hypoxia-reoxygenation insult also induced significant cytosolic Ca\(^{2+}\) loading (from 96.2 ± 3.0 nmol/L at rest to 233 ± 12 nmol/L after reoxygenation, \( n = 4 \); \( P<0.01 \)) in the presence of the K\(_{ATP}\) channel opener pinacidil (Figure 2A and 2B). In untransfected COS-7 cells, pinacidil did not evoke significant membrane current (Figure 2C; \( n = 14 \)).

**Hypoxia-Reoxygenation Induces Ca\(^{2+}\) Loading in COS-7 Cells Transfected With Recombinant Kir6.2 and SUR2A**

In COS-7 cells, transfection of Kir6.2 and SUR2A, in combination or alone, did not alter the resting cytosolic Ca\(^{2+}\) concentration (95.9 ± 2.0 nmol/L with Kir6.2/SUR2A combined, 96.4 ± 4.1 nmol/L with Kir6.2 alone, and 97.8 ± 3.5 nmol/L with SUR2A alone; \( n = 4 \) in each condition). In transfected COS-7 cells, hypoxia-reoxygenation induced significant Ca\(^{2+}\) loading (to 181 ± 10 nmol/L with Kir6.2/SUR2A, 236 ± 11 nmol/L with Kir6.2, and 232 ± 10 nmol/L with SUR2A; \( P<0.01 \); \( n = 4 \) in each condition; Figure 3). The
magnitude of increase in the cytosolic Ca\(^{2+}\) concentration induced by hypoxia-reoxygenation did not differ significantly between COS-7 cells transfected with Kir6.2 and SUR2A, alone or in combination (P>0.05), although cotransfection with Kir6.2/SUR2A was associated with a moderately lower degree of Ca\(^{2+}\) loading (Figure 3).

**Pinacidil Protects COS-7 Cells Cotransfected With Kir6.2 and SUR2A Against Hypoxia-Reoxygenation–Induced Ca\(^{2+}\) Loading**

Treatment with the K\(_{ATP}\) channel opener pinacidil (10 \(\mu\)mol/L) protected COS-7 cells cotransfected with Kir6.2/SUR2A from Ca\(^{2+}\) loading induced by hypoxia-reoxygenation (Figure 4A). Such a protective effect of pinacidil was not observed in cells transfected with Kir6.2 or SUR2A alone (Figure 4A). Accordingly, cytosolic Ca\(^{2+}\) levels after hypoxia-reoxygenation were significantly higher in cells transfected with Kir6.2 (237\(\pm\)11 nmol/L; n=4) or SUR2A (234\(\pm\)9 nmol/L; n=4) than in cells cotransfected with Kir6.2/SUR2A (127\(\pm\)11 nmol/L; n=4; P<0.01) (Figure 4B and 4C). In COS-7 cells cotransfected with Kir6.2/SUR2A and treated with pinacidil, Ca\(^{2+}\) levels were not significantly different before (98.7\(\pm\)6.8 nmol/L; n=4) and after (127\(\pm\)11 nmol/L; n=4; P>0.05) hypoxia-reoxygenation (Figure 4C).

The protective effect of pinacidil was abolished by the K\(_{ATP}\) channel antagonist glyburide (Figure 5A). After hypoxia-reoxygenation, in the presence of both pinacidil (10 \(\mu\)mol/L) and glyburide (1 \(\mu\)mol/L), cytosolic Ca\(^{2+}\) levels increased significantly, from 97.1\(\pm\)5.6 to 239\(\pm\)8 nmol/L in COS-7 cells cotransfected with Kir6.2/SUR2A (n=4; P<0.01) (Figure 5B). In cotransfected cells, 10 \(\mu\)mol/L pinacidil evoked opening of K\(^{+}\) channels, which was inhibited by 1 \(\mu\)mol/L glyburide (Figure 5C). Channel activity, expressed as NP\(_{o}\), was 0.1\(\pm\)0.3 at rest, 4.7\(\pm\)0.9 in the presence of pinacidil, and 0.8\(\pm\)0.5 in the presence of both pinacidil and glyburide (Figure 5D; n=4).

**Pinacidil Also Protects Cardiomyocytes Against Hypoxia-Reoxygenation–Induced Ca\(^{2+}\) Loading**

The same hypoxia-reoxygenation protocol also produced Ca\(^{2+}\) loading (from 142\(\pm\)17 to 3856\(\pm\)289 nmol/L; n=4; P<0.01) in cardiomyocytes, which natively express K\(_{ATP}\) channels (Figure 6A and 6B). Hypoxia-reoxygenation also induced cellular shortening (by 54\(\pm\)13%; n=4; Figure 6A to 6C). Treatment with pinacidil (100 \(\mu\)mol/L) inhibited Ca\(^{2+}\) loading (451\(\pm\)61 nmol/L in the presence compared with 3856\(\pm\)289 nmol/L in the absence of pinacidil; n=4; P<0.01; Figure 6A to 6B) and cellular shortening (n=4; Figure 6A to 6C). These effects of pinacidil were abolished by glyburide (10 \(\mu\)mol/L). On average, in cardiomyocytes treated with pinacidil plus glyburide, cytosolic Ca\(^{2+}\) was 139\(\pm\)18 nmol/L before and 4312\(\pm\)460 nmol/L after hypoxia-reoxygenation (n=4; P<0.01; Figure 6A and 6B), while cells concomitantly shortened by 43\(\pm\)12% (Figure 6A to 6C).
Discussion

In the present study, we show that coexpression of the recombinant cardiac $K_{ATP}$ channel subunits Kir6.2 and SUR2A confers resistance to intracellular Ca$^{2+}$ loading induced by hypoxia-reoxygenation. These findings indicate that the cardiac $K_{ATP}$ channel harbors cytoprotective properties inherent to the channel subunits themselves.

In recent years, use of recombinant channel proteins has proved to be a direct approach to determine intrinsic channel characteristics, independent of the cellular environment. Coexpression of Kir6.2/SUR2A has been found to reconstitute functional cardiac $K_{ATP}$ channels, in terms of single-channel conductance and gating properties. Here, we extended the use of recombinant channel proteins to determine the protective properties of cardiac $K_{ATP}$ channels intrinsic to constitutive channel subunits in the setting of hypoxia-reoxygenation injury.

Regardless of the cell type, it is well established that reoxygenation that follows a hypoxic insult initiates a series of cellular reactions leading to cell injury. Studies in a number of tissues have also established that hypoxia-reoxygenation induces intracellular Ca$^{2+}$ loading, which represents a major indicator of the degree of cell injury. In COS-7 cells, which were used here, basal levels of cytosolic Ca$^{2+}$, estimated by the intensity of fluo-3 fluorescence, were similar to those previously reported with other Ca$^{2+}$-sensitive fluorescence probes, such as fura-2. After hypoxia-reoxygenation, intracellular Ca$^{2+}$ concentration increased significantly, indicating that untransfected COS-7 cells are vulnerable to such an insult.

The potassium channel opener pinacidil was without effect on membrane ion currents or intracellular Ca$^{2+}$ loading in untransfected COS-7 cells, indicating that these cells lack a pinacidil-sensitive, $K_{ATP}$ channel–dependent protective mechanism. This is in accord with previous studies that have established COS-7 cells as a cell line devoid of endogenous $K_{ATP}$ channels and a reliable model to conduct studies on $K_{ATP}$ channel subunits. Transfection with Kir6.2 or SUR2A alone or cotransfection with Kir6.2/SUR2A did not protect, or protected only marginally, against hypoxia-reoxygenation. This is in line with previous findings that individual subunits do not form functional channels and that only modest opening of the cardiac $K_{ATP}$ channel complex occurs within the time frame of metabolic stress used here. Alternatively, this could be interpreted to mean that within a noncardiac environment, constitutive $K_{ATP}$ channel proteins are without cytoprotective properties. It is established that protection afforded through a $K_{ATP}$ channel–dependent mechanism commonly occurs after channel activation by ischemic preconditioning or by potassium channel openers. In the present study, we promoted opening of recombinant cardiac $K_{ATP}$ channels with pinacidil, which has been established to effectively activate the cardiac isoform of the channel. In COS-7 cells cotransfected with Kir6.2/SUR2A, pinacidil activated K$^+$ current and prevented intracellular Ca$^{2+}$ loading induced by hypoxia-reoxygenation. Both pinacidil-induced K$^+$ current and cellular protection were abolished by glyburide, an antagonist of $K_{ATP}$ channels, confirming that opening of recombinant $K_{ATP}$ channels is responsible for the observed cellular resistance to hypoxia-reoxygenation. Further investigation is necessary to determine the mechanisms underlying this protective effect.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Pinacidil-induced protection in COS-7 cells cotransfected with Kir6.2/SUR2A is glyburide-sensitive. A, Epifluorescent digital images before (left) and after (right) hypoxia-reoxygenation in fluo-3 loaded cells cotransfected with Kir6.2/SUR2A and exposed to pinacidil (10 μmol/L) plus glyburide (1 μmol/L). Same protocol as in Figure 1. Bar = 90 μm. B, Average changes in intracellular Ca$^{2+}$ concentration before (open bar) and after (hatched bar) hypoxia-reoxygenation in cells cotransfected with Kir6.2/SUR2A and treated with pinacidil (10 μmol/L) plus glyburide (1 μmol/L). Bars represent mean ± SEM (n = 4); *P < 0.01. C, Channel record obtained in the cell-attached configuration from a COS-7 cell transfected with Kir6.2/SUR2A and exposed first to pinacidil alone and then to pinacidil plus glyburide. Zero current level is indicated by dotted line. D, Channel activity expressed as NPo in COS-7 cells cotransfected with Kir6.2/SUR2A before (open bars) and after (hatched bars) exposure of 10 μmol/L pinacidil in absence and presence of 1 μmol/L glyburide.
Moreover, pinacidil was without effect in cells transfected with Kir6.2 or SUR2A alone, which are insufficient to form functional KATP channels.\(^{23}\) Taken together, the present finding provides direct evidence that the cardiac KATP channel complex is cytoprotective as a result of properties inherent to channel subunits themselves and that transfer of channel clones into a cell type lacking KATP channels confers resistance to chemical hypoxia-reoxygenation injury when opened by a KATP channel opener. Such a conclusion seems to be applicable to the myocardium itself, where Kir6.2 and SUR2A are naturally expressed, because we observed a similar response in cardiomyocytes exposed to the same experimental stress as cotransfected COS-7 cells.

The mechanism responsible for the K\(_{\text{ATP}}\) channel subunit-dependent reduction of chemical hypoxia-reoxygenation–induced Ca\(^{2+}\) loading is at present not known. In principle, several mechanisms underlying pinacidil-induced protection, including shortening of the action potential duration, hyperpolarization of the cell membrane, and targeting of mitochondrial K\(_{\text{ATP}}\) channels, have been proposed.\(^{1-4,11,36}\) Although the participation of such mechanisms remains to be established, a mechanism that would involve shortening of the action potential duration is rather unlikely under the present experimental conditions, because pinacidil-mediated protection was observed in cells that did not generate action potentials.
Recombinant $K_{\text{ATP}}$ Channels and Cytoprotection

The fact that expression of recombinant cardiac $K_{\text{ATP}}$ channel subunits could transform COS-7 cells from a reoxygenation-vulnerable to a reoxygenation-resistant cellular phenotype supports the notion that $K_{\text{ATP}}$ channels may have a ubiquitous cytoprotective role. Activation of natively expressed $K_{\text{ATP}}$ channels not only in cardiac cells but also in other tissues, including the brain and skeletal muscle, has been associated with a protective outcome. Conversely, natively occurring or engineered mutations of $K_{\text{ATP}}$ channel subunits impair channel activity or its regulation with consequences on cell function and cell survival. In pancreatic $\beta$-cells, in which $K_{\text{ATP}}$ channels are critical for glucose-mediated insulin secretion, disruption of native pancreatic $K_{\text{ATP}}$ channels in transgenic animals has been found to induce hypoglycemia with hyperinsulinemia in neonates and hyperglycemia with hypoinsulinemia in adults, with a decrease in viable $\beta$-cells. In humans, mutations in $K_{\text{ATP}}$ channel subunits have been linked to the syndrome of familial persistent hyperinsulinemic hypoglycemia of infancy, and mutations in SUR2 have been suggested as a candidate for a susceptibility gene in the clinical condition of brachydactyly with hypotension. Thus, in addition to previously established deleterious consequences of mutations or disruptions in $K_{\text{ATP}}$ channels, the present study provides evidence that expression of recombinant cardiac $K_{\text{ATP}}$ channel subunits induces gain of cytoprotective function in cells lacking the ability to withstand injury.

In conclusion, this study provides direct evidence that the cardiac $K_{\text{ATP}}$ channel is cytoprotective by virtue of properties intrinsic to proteins constituting the channel complex and validates the concept that targeting myocardial $K_{\text{ATP}}$ channels should be considered a valuable approach aimed at protecting the hypoxic myocardium. Furthermore, the demonstration that $K_{\text{ATP}}$ channel subunit-dependent protection can be transferable may provide the framework for future therapeutic strategies based on gene delivery of channel subunits in states associated with $K_{\text{ATP}}$ channel deficiency.

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