Amiodarone Inhibits Sarcolemmal but Not Mitochondrial KATP Channels in Guinea Pig Ventricular Cells

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ABSTRACT

ATP-sensitive K⁺ (KATP) channels are present on the sarcolemma (sarcKATP channels) and mitochondria (mitoKATP channels) of cardiac myocytes. Amiodarone, a class III antiarrhythmic drug, reduces sudden cardiac death in patients with organic heart disease. The objective of the present study was to investigate the effects of amiodarone on sarcKATP and mitoKATP channels. Single sarcKATP channel current and flavoprotein fluorescence were measured in guinea pig ventricular myocytes to assay sarcKATP and mitoKATP channel activity, respectively. Amiodarone inhibited the sarcKATP channel currents in a concentration-dependent manner without affecting its unitary amplitude. The IC50 values were 0.35 μM in the inside-out patch exposed to an ATP-free solution and 2.8 μM in the cell-attached patch under metabolic inhibition, respectively. Amiodarone (10 μM) alone did not oxidize the flavoprotein. In addition, the oxidative effect of the mitoKATP channel opener diazoxide (100 μM) was unaffected by amiodarone. Exposure to ouabain (1 mM) for 30 min produced mitochondrial Ca²⁺ overload, and the intensity of rhod-2 fluorescence increased to 246 ± 16% of baseline (n = 9). Amiodarone did not alter the ouabain-induced mitochondrial Ca²⁺ overload (236 ± 10% of baseline, n = 7). Treatment with diazoxide significantly reduced the ouabain–induced mitochondrial Ca²⁺ overload (158 ± 15% of baseline, n = 8, p < 0.05 versus ouabain); this effect was not abolished by amiodarone (154 ± 10% of baseline, n = 8, p < 0.05 versus ouabain). These results suggest that amiodarone inhibits sarcKATP but not mitoKATP channels in cardiac myocytes. Such an action of amiodarone may effectively prevent ischemic arrhythmias without causing ischemic damage.

From the Cardiac Arrhythmia Suppression Trial (CAST) (The CAST Investigators, 1989), strategies for the treatment of life-threatening ventricular tachyarrhythmias have changed markedly. Pure class III antiarrhythmic drugs such as d-sotalol and dofetilide have been developed as an alternative drug therapy to reduce sudden cardiac death (Singh et al., 1993). However, a large-scale clinical trial with native drug therapy to reduce sudden cardiac death (Singh et al., 1996). Amiodarone has been referred to as the prototype laboratory have shown that amiodarone inhibits the ligand-gated K⁺ channels, i.e., acetylcholine-sensitive muscarinic K⁺ channel and the Na⁺-activated K⁺ channel (Mori et al., 1996; Watanabe et al., 1996). So far, however, we have only limited knowledge about the effect of amiodarone on ATP-sensitive K⁺ (KATP) channel, a ligand-gated K⁺ channel. KATP channel is unique among K⁺ channels in being inhibited by cytoplasmic adenine nucleotides and thereby coupling metabolic events to cellular excitability (Noma, 1983). Recent studies have suggested that cardiac myocytes contain KATP channels not only in sarcolemmal plasma membrane (sarcKATP channels) but also in mitochondrial inner membrane (mitoKATP channels) (Garlid et al., 1996; Liu et al., 1998). It is acknowledged that the KATP channel is a hetero-

Abbreviations: KATP, ATP-sensitive potassium; sarcKATP, sarcolemmal KATP; mitoKATP, mitochondrial KATP; HMR 1883, 1-[[5-[(5-chloro-o-anisamido)ethyl]-2-methoxyphenyl][sulfonyl]-3-methylthiourea; SHD, 5-hydroxydecanoate; DNP, 2,4-dinitrophenol; Po, probability of opening; [Ca²⁺]₀, mitochondrial Ca²⁺ concentration.
octamer comprising two subunits: a pore-forming inwardly rectifying K+ channel subunit (Kir6.x) and a regulatory sulfonylurea receptor subunit (SURx) (for review, see Seino, 1999). From the analyses of Kir6.1- and Kir6.2-deficient mice, we have recently provided direct evidence that Kir6.2 form the pore region of cardiac sarcKATP channels, whereas the molecular identity of mitoKATP channels has not been established (Suzuki et al., 2001, 2002; Miki et al., 2002). Although the relative roles of the sarcKATP and mitoKATP channels remain elusive, it has been suggested that openings of the sarcKATP channels are arrhythmogenic (for review, see Wilde and Janse, 1994), whereas openings of the mitoKATP channels are cardioprotective (for review, see Sato and Marbán, 2000).

In this regard, inhibition of sarcKATP channel may prevent the ischemia-induced shortening of refractory period. In fact, the selective sarcKATP channel blocker HMR 1883 has been shown to prevent ischemia-induced ventricular fibrillation (Billman et al., 1998; Wirth et al., 1999). On the other hand, inhibition of mitoKATP channels may produce significant damage to the ischemic myocardium. Several recent studies have demonstrated that the mitoKATP channel activity. A recent electrophysiological study by Holmes et al. (2000) has shown that amiodarone inhibits the sarcKATP channel activity in rat ventricular myocytes. However, it still remains unclear whether amiodarone modulates the cardiac mitoKATP channel activity. Accordingly, the present study examined the effects of amiodarone on sarcKATP and mitoKATP channels in guinea pig ventricular myocytes. The results presented here show that amiodarone inhibits sarcKATP but not mitoKATP channels in cardiomyocytes.

Materials and Methods

The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23, revised 1985).

Cell Preparation. Single ventricular myocytes of the guinea pig hearts were obtained by enzymatic dissociation, as described previously (Tohse et al., 1992). The cells used in the present experiments had a regular shape with clear cross-striation.

Single SarcKATP Channel Recording. Single sarcKATP channel currents were performed by the inside-out and the cell-attached configurations of the patch-clamp techniques. Single myocytes were superfused with the HEPES-buffered Tyrode’s solution containing 143 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.33 mM NaH2PO4, 0.5 mM MgCl2, 5.5 mM glucose, and 5 mM HEPES (pH adjusted to 7.4 with NaOH). Patch electrodes were fabricated from glass capillaries (o.d. 1.5 mm) by a two-stage puller (PB-7, Narishige, Tokyo, Japan), and their tips coated with silicone and heat-polished. For inside-out patch recording, the internal solution contained 150 mM KCl, 1 mM EGTA, 5 mM HEPES, and 0.001 mM Na2ATP (pH adjusted to 7.4 with KOH) and the external (pipette) solution contained 150 mM KCl, 2 mM CaCl2, and 5 mM HEPES (pH adjusted to 7.4 with KOH). For cell-attached patch recording, the same pipette solution was used. After the gigaohm seal between the patch electrode and the cell membrane was formed, cells were exposed to a glucose-free HEPES-buffered Tyrode’s solution containing 0.2 mM 2,4-dinitrophenol (DNP), an uncoupler of oxidative phosphorylation.

When the openings of sarcKATP channels occurred, various concentrations of amiodarone were added to the solution. These experiments were performed at room temperature (≈22°C).

The single-channel currents were recorded by a patch-clamp amplifier (CEZ-2300; Nihon Kohden, Tokyo, Japan) and stored on videotapes through a pulse code modulator (VR-10B; InstruTECH Corporation, Port Washington, NY) for later analysis. The frequency response of the recording system was flat up to 37 kHz. The data were filtered at 2 kHz with a digital Gaussian filter and digitized at 10 kHz for data analysis with pClamp software (Axon Instruments, Union City, CA). Channel openings were identified by algorithm that used both amplitude and slope information, and measured with an interactive threshold for detecting events that was set at 50% of the expected amplitude. The probability of opening (Po) was calculated according to the following algorithm:

\[
P_o = \sum n!T_n/T
\]

where \(n = \) the number of simultaneously active channels, \(t_n = \) the length of time the channel is in state \(n\), \(T = \) the total time of the recording, and \(N = \) the maximum number of channels detected during the experiment. Relative channel activities in the presence of varying concentration of amiodarone (NP/NPC) were fitted to the Hill equation with a Marquardt-Levenberg algorithm:

\[
NP/NPC = (1 + [\text{amiodarone}]/IC_{50}^H)^{-1}
\]

where [amiodarone] is the concentration of amiodarone, IC_{50} is the half-maximum concentration for inhibition of amiodarone, and \(h\) is the Hill coefficient. Control channel currents were evaluated as an average of those from records before and after application of the drug because, in inside-out patches, the currents were gradually decreased and, in cell-attached patches, were gradually increased. The single-channel currents that did not recover after application of the drug were omitted from the data analysis to avoid contamination of the run-down of sarcKATP channels.

Flavoprotein Fluorescence Measurement. To stabilize the mitochondrial redox state, the cells were suspended in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum at room temperature until use. To index mitoKATP channel activity, flavoprotein fluorescence was measured by a modification of method described by Sato et al. (1998). Briefly, each cell was superfused with bath solution containing 143 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.33 mM NaH2PO4, 0.5 mM MgCl2, and 5 mM HEPES, adjusted to pH 7.4 with NaOH. Flavoprotein fluorescence was excited at 480 nm for 200 ms every 10 s and emitted at 550 nm. At the end of each experiment, cells were exposed to the mitochondrial uncoupler DNP (100 μM) to obtain maximal flavoprotein oxidation. These experiments were performed at room temperature (≈22°C). Emitted fluorescence was monitored with a cooled charge-coupled device digital camera (C4742-95; Hamamatsu Photonics, Hamamatsu, Japan). The imaging of flavoprotein was analyzed for average pixel intensities of regions of interest drawn to include whole cell and expressed as a percentage of the DNP-induced maximal oxidation, using an Aquacosmos image-processing system (Hamamatsu Photonics).

Mitochondrial Ca2+ Concentration ([Ca2+]m) Measurement. The Ca2+ fluorophore rhod-2 was used to measure changes of [Ca2+]m. For rhod-2 loading, cells were plated on uncoated 35-mm Falcon culture dishes with a medium based on a 1:1 mixture of Dulbecco’s modified Eagle’s medium and HEPES-buffered Tyrode’s solution, supplemented with 10% fetal calf serum. Then, cells were loaded with rhod-2 acetoxyethyl ester (10 μM) for 120 min at 4°C. After cold loading, cells were incubated for 30 min at 37°C. This two-step cold loading/warm incubation protocol achieves exclusive loading of rhod-2 into the mitochondria (Trollinger et al., 2000). Cells loaded with rhod-2 were perfused with a HEPES-buffered Tyrode’s solution containing 2.7 mM CaCl2 at 37°C. Rhod-2 fluorescence was excited at 540 nm (for 100 ms), with emission monitored through a 605-nm (55-nm bandpass) barrier filter. The imaging of rhod-2 was
analyzed for average pixel intensities of regions of interest drawn to include whole cell, after correction for background, using an Aquacosmos image-processing system (Hamamatsu Photonics).

**Chemicals.** Amiodarone was a kind gift from Taisho Pharmaceutical (Omiya, Japan). Diazoxide, 5HD, glibenclamide, and ouabain were purchased from Sigma-Aldrich (St. Louis, MO). DNP was purchased from Wako Pure Chemicals (Osaka, Japan). Rhod-2 acetoxymethyl ester was purchased from Molecular Probes (Eugene, OR). For electrophysiological experiments, amiodarone was dissolved in absolute ethanol at a concentration of 10 mM and then added to the bath solution containing bovine serum albumin (0.03–1.0%), as described by Honjo et al. (1991). Because bovine serum albumin alone enhanced the emitted fluorescence nonspecifically, stock solution of amiodarone was directly diluted in the perfusate for measurements of flavoprotein fluorescence. It was confirmed that the solvent of amiodarone affected neither the sarcKATP channel current nor the flavoprotein fluorescence. Glibenclamide was dissolved in dimethyl sulfoxide at a concentration of 100 mM. Ouabain, 5HD, and DNP were dissolved in the perfusate.

**Data Analysis.** Data are presented as mean ± S.E.M., and the number of cells or experiments is shown as n. Curve fits were performed with Origin 7J software (MicroCal Software, Northampton, MA). Intergroup comparisons are made by Student’s t test for two groups and by analysis of variance followed by Fisher’s post hoc test for multiple groups. A value of p < 0.05 was regarded as significant.

**Results**

**Effect of Amiodarone on SarcKATP Channel Current.** Effects of amiodarone on the sarcKATP channel activity induced by metabolic blockade were evaluated in guinea pig ventricular cells. Figure 1A shows a representative sarcKATP channel current recorded in the cell-attached mode from a cell exposed to DNP (0.2 mM)-containing, glucose-free solution. At the pipette potential of 0 mV, unitary currents through the inward rectifier K⁺ channel could be recorded using a K⁺-rich (150 mM) pipette solution, which was identified from its smaller slope conductance and longer open time. After the commencement of the superfusion of the cell with the ischemia-simulating Tyrode’s solution, opening the inward rectifier K⁺ channel decreased gradually, and single-channel currents having larger unitary amplitude occurred. The latter channel was identified as sarcKATP channel because the linear slope conductance obtained from the current-voltage relationship was 79.4 ± 1.0 pS (n = 3) and the channel opening was readily inhibited by the application of 1 to 10 μM glibenclamide. During the continuous recording of the sarcKATP channel activity, amiodarone was applied extracellularly. As shown in Fig. 1A, amiodarone effectively inhibited the channel openings. Amiodarone at a concentration of 10 μM significantly decreased Po by 84 ± 4% (from 0.25 ± 0.04 to 0.05 ± 0.03, n = 8, p < 0.05) without affecting the amplitude of unitary events.

Figure 1B illustrates the effect of amiodarone on the single sarcKATP channel current recorded form an inside-out patch of ventricular cell. A single channel current was recorded at a holding potential of −40 mV with an internal solution containing 1 μM ATP. The slope conductance of the unitary current from the current voltage relationship was 82 ± 2 pS (n = 3), and the current was reversibly inhibited by the application of 1 mM ATP to the internal solution, implying that the unitary current flowed through sarcKATP channels. As shown in Fig. 1B, amiodarone at a concentration of 1 μM significantly decreased Po by 79 ± 9% (from 0.37 ± 0.04 to 0.10 ± 0.05, n = 9, p < 0.05) without affecting the amplitude of unitary current. The sarcKATP channel slowly reverted toward the control on changing to drug-free solution.

Amiodarone-induced changes in relative open probability (NP/NPc) of the inward sarcKATP channel current recorded in the inside-out patch and in the cell-attached mode are summarized in Fig. 2. Amiodarone inhibited the sarcKATP channel current in a concentration-dependent manner, and the IC₅₀ values were 0.35 and 2.8 μM in the inside-out patch and cell-attached mode.
in the cell-attached mode, respectively. Furthermore, amiodarone (1 μM) inhibited the outward component of the sarcK<sub>ATP</sub> channel current at a holding potential of +40 mV in the inside-out patch membrane and decreased Po of the outward sarcK<sub>ATP</sub> channel current from 0.41 to 0.14 in two experiments.

**Effect of Amiodarone on Flavoprotein Fluorescence.** The effects of amiodarone on mitoK<sub>ATP</sub> channels were evaluated indirectly by measuring flavoprotein fluorescence. Figure 3, A and B, show the time course of flavoprotein fluorescence in a cell exposed to diazoxide and/or amiodarone. Diazoxide (100 μM), a mitoK<sub>ATP</sub> channel opener, reversibly oxidized flavoprotein (Fig. 3A). Exposure to amiodarone (10 μM) alone had no effects on flavoprotein fluorescence. Subsequent application of diazoxide (100 μM), in the continued presence of amiodarone, reversibly oxidized flavoprotein (Fig. 3B). As summarized in Fig. 3C, diazoxide (100 μM) alone increased flavoprotein oxidation to 32.4 ± 3.1% of the DNP value (n = 8). Amiodarone (10 μM) did not oxidize the flavoprotein (4.8 ± 1.8% of the DNP value, n = 7). In the presence of amiodarone, diazoxide increased flavoprotein oxidation to 35.0 ± 4.6% of the DNP value (n = 7). This degree of oxidation was comparable to that observed in the absence of amiodarone. The results indicate that amiodarone does not affect mitoK<sub>ATP</sub> channel function.

**Effect of Amiodarone on Mitochondrial Ca<sup>2+</sup> Overload.** We previously reported that the opening of mitoK<sub>ATP</sub> channels by diazoxide attenuates the mitochondrial Ca<sup>2+</sup> overload in rat ventricular myocytes (Ishida et al., 2001). We therefore examined the effect of amiodarone on mitochondrial Ca<sup>2+</sup> overload. As summarized in Fig. 4, treatment of myocytes with ouabain (1 mM) evoked the elevation of [Ca<sup>2+</sup>]<sub>m</sub>, and the intensity of rhod-2 fluorescence after 30 min significantly increased to 249.5 ± 16.2% of baseline (n = 9, p < 0.001). Diazoxide (100 μM) significantly attenuated the elevation of [Ca<sup>2+</sup>]<sub>m</sub> during exposure to ouabain (157.8 ± 15.5% of baseline, n = 8, p < 0.05 versus ouabain), and the effect was antagonized by the mitoK<sub>ATP</sub> channel blocker 5HD (500 μM, 258.1 ± 28.1% of baseline, n = 8). Amiodarone (10 μM) alone did not increase the [Ca<sup>2+</sup>]<sub>m</sub> (104.3 ± 1.1% of baseline, n = 8). Amiodarone per se did not attenuate the ouabain-induced increase in [Ca<sup>2+</sup>]<sub>m</sub> (236.5 ± 10.4% of baseline, n = 7). Furthermore, even in the presence of amiodarone, diazoxide could attenuate the elevation of [Ca<sup>2+</sup>]<sub>m</sub> during exposure to ouabain. These results indicate that amiodarone did not affect the mitoK<sub>ATP</sub> channel function.

**Discussion**

The first report of the effects of amiodarone on sarcK<sub>ATP</sub> channel came from Haworth et al. (1989) where amiodarone inhibited the <sup>86</sup>Rb uptake stimulated by rotenone plus p-trifluoromethoxy-phenylhydrazone with IC<sub>50</sub> value of 19.1 μM in rat heart cells. Because the <sup>86</sup>Rb uptake was potently inhibited by glibenclamide, they concluded that the stimulated <sup>86</sup>Rb uptake might reflect the sarcK<sub>ATP</sub> channel activity. A recent study by Holmes et al. (2000) has provided electrophysiological evidence that amiodarone inhibits the sarcK<sub>ATP</sub> channel activity in rat ventricular myocytes, with apparent IC<sub>50</sub> value of 0.14 to 2.3 μM. In agreement with previous reports, our present experiments demonstrated that amiodarone inhibits the sarcK<sub>ATP</sub> channel current in a concentration-dependent manner without affecting the unitary current both in the inside-out patches exposed to an ATP-deficient solution and in the intact cells under metabolic inhibition. We further found that there was a difference in the potency of sarcK<sub>ATP</sub> channel blockade by amiodarone between these two experimental conditions. In the inside-out patches, the sarcK<sub>ATP</sub> channel current could be inhibited by lower concentrations of amiodarone (IC<sub>50</sub> = 0.35 μM). In contrast, higher concentrations of amiodarone (IC<sub>50</sub> = 2.8 μM) inhibited the outward component of the sarcK<sub>ATP</sub> channel current at a holding potential of +40 mV in the inside-out patch membrane and decreased Po of the outward sarcK<sub>ATP</sub> channel current from 0.41 to 0.14 in two experiments.

![Fig. 3. Effects of diazoxide and amiodarone on flavoprotein fluorescence. A and B, time course of flavoprotein fluorescence in cell exposed to diazoxide (DZ, 100 μM) and amiodarone (AM, 10 μM). The flavoprotein fluorescence was calibrated by exposing the cells to DNP (100 μM) at the end of experiments. Bar indicates periods when the cells were exposed to each drug. C, summarized data for diazoxide and amiodarone on flavoprotein oxidation. Values are expressed as percent relative to those obtained with DNP. All data are presented as mean ± S.E.M.](image-url)

![Fig. 4. Summarized data for the relative changes in rhod-2 fluorescence measured after 30-min exposure to drugs. Each point indicates the mean ± S.E.M. *p < 0.001 versus ouabain (OUAB).](image-url)
μM) were needed to inhibit the sarcK \text{ATP} channel current recorded in the cell-attached mode under metabolic inhibition. One possible explanation for the difference of the potency may be that amiodarone could have easy access to the sarcK \text{ATP} channel in the inside-out membrane patch from the internal solution. In the experiments using the cell-attached mode recording, amiodarone had to diffuse across the cell membrane from the bath solution and then bind to the site of action, i.e., sarcK \text{ATP} channel. Therefore, higher concentration of amiodarone in the bath solution might be needed for the inhibition of the sarcK \text{ATP} channel activity recorded in the cell-attached mode. Another explanation may be that the sarcK \text{ATP} channel current activated by metabolic stress might be more resistant to amiodarone. It was demonstrated that sulfonylurea drugs such as glibenclamide and tolbutamide were no longer able to inhibit the opening of sarcK \text{ATP} channels during severe metabolic stress although the precise mechanisms have not been fully clarified (Venkatesh et al., 1991; Findlay, 1993). The effective concentration to inhibit the sarcK \text{ATP} channel current in this study was similar to or less than those needed to inhibit the other channels (Balsier et al., 1991; Sato et al., 1994; Mori et al., 1996; Watanabe et al., 1996). Moreover, acute and chronic administrations of amiodarone result in the plasma level of 0.1 to 6.5 μg/ml (Harris et al., 1983; Ikeda et al., 1984; Raeder et al., 1985), which correspond to approximately 0.16 to 10 μM. Therefore, the inhibitory effect of the drug on the sarcK \text{ATP} channel in ischemic myocardium would be expected in clinical settings, even with acute administration.

To determine whether amiodarone affects the mitoK \text{ATP} channel function, we measured flavoprotein fluorescence as an index of mitoK \text{ATP} channel activity (Liu et al., 1998). This approach enabled us to assay the function of mitoK \text{ATP} channels in intact cells, obviating the need for isolation of mitochondria or functional reconstitution. Hanley et al. (2002), however, reported that the mitoK \text{ATP} channel opener diazoxide could not increase flavoprotein fluorescence in guinea pig ventricular myocytes. This discrepancy may stem from the different experimental conditions. They used freshly isolated myocytes and measured flavoprotein fluorescence in the presence of glucose. In our experiments, to stabilize the mitochondrial redox state, the cells were kept in a culture medium until use. Because redox state of the FAD/FADH₂ is linked to that of mitochondrial NAD⁺/NADH (Chance et al., 1972), mitoK \text{ATP} channel-induced flavoprotein oxidation is detectable only if uncompensated by increased production of electron donor (such as NADH). For this reason, we used the glucose-free solution for measurement of flavoprotein fluorescence, and indeed, diazoxide oxidized flavoprotein in guinea pig ventricular myocytes (Fig. 3A). Using this experimental protocol, in the present study, we were unable to detect any significant effect of amiodarone (10 μM) on flavoprotein fluorescence, i.e., amiodarone alone did not oxidize the flavoprotein and the oxidative effect of diazoxide was unaffected by amiodarone (Fig. 3, B and C). These results suggest that amiodarone has no effect on the mitoK \text{ATP} channel function. Further support for this notion comes from the observation that, unlike diazoxide, amiodarone could not prevent the mitochondrial Ca²⁺ overload (Fig. 4). The experimental model of ouabain-induced mitochondrial Ca²⁺ overload was used in our earlier study, in which we showed that in rat cardiomyocytes the mitoK \text{ATP} channel opener diazoxide attenuated the mitochondrial Ca²⁺ overload and such effect associated with the depolarization of mitochondrial membrane potential (Ishida et al., 2001). The present study confirms that diazoxide prevents the mitochondrial Ca²⁺ overload in guinea pig ventricular myocytes. Furthermore, when amiodarone was tested at a higher concentration (10 μM), which is sufficient to block the sarcK \text{ATP} channels, it did not abrogate the cytoprotective effects of diazoxide. These results together indicate that amiodarone does not inhibit the opening of mitoK \text{ATP} channels, although further study is needed to define the chronic effects of amiodarone on mitoK \text{ATP} channels.

The activation of sarcK \text{ATP} channels shortens action potential duration and refractoriness, which may result in reentrant ventricular arrhythmias. Therefore, sarcK \text{ATP} channel blockers ought to be effective in ischemic arrhythmias, by preventing the action potential shortening. This concept is supported by the facts that the selective sarcK \text{ATP} channel blocker HMR 1883 (Sato et al., 2000) effectively prevented ischemia-induced ventricular fibrillation (Billman et al., 1998; Wirth et al., 1999). Blockade of sarcK \text{ATP} channels may predispose to aggravate ischemic injury by increasing the Ca²⁺ influx. Indeed, complete loss of sarcK \text{ATP} channel function in Kir6.2-deficient mice resulted in greater contractile dysfunction after ischemia/reperfusion (Suzuki et al., 2002). In rabbit hearts, however, HMR 1883 did not abolish the endogenous cardioprotective mechanism known as ischemic preconditioning (Jung et al., 2000). Thus, there may be species differences with regard to relative importance of sarcK \text{ATP} channels in cardioprotection. Alternatively, there is ample evidence suggesting that inhibition of mitoK \text{ATP} channel was detrimental to ischemic cardioprotection (O’Rourke, 2000). In this respect, the lack of effect of amiodarone on mitoK \text{ATP} channel is of advantage in management of ischemic arrhythmias compared with nonselective K \text{ATP} channel blockers such as glibenclamide, because the drug may effectively prevent arrhythmias without aggravating tissue damage. Such a salutary effect of amiodarone on ischemic myocardium may in part explain the reduction of cardiac death in patients with myocardial infarction, observed in several clinical trials such as Canadian Amiodarone Myocardial Infarction Arrhythmia Trial and European Myocardial Infarct Amiodarone Trial (Cairns et al., 1997, Julian et al., 1997).

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References


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