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High glucose protects single beating adult cardiomyocytes against hypoxia

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Abstract

In the heart, the opening of sarcolemmal ATP-sensitive K^+ (K_{ATP}) channels seems to be crucial for the cardiac protection against hypoxia/ischaemia. In the present study, we have exposed cardiomyocytes under hypoxia to high extracellular glucose (30 mM). Under these conditions, intracellular concentration of 1,3-bisphosphoglycerate has increased confirming stimulation of glycolysis. Perforated patch–clamp electrophysiology revealed that hypoxia induces whole-cell K^+ current in cardiomyocytes more efficiently in the presence than in the absence of high glucose. Glucose significantly promoted survival of cardiomyocytes exposed to hypoxia. HMR 1098, an antagonist of sarcolemmal K_{ATP} channels, inhibited glucose-induced activation of whole-cell K^+ current during hypoxia as well as glucose-mediated cytoprotection. An inhibitor of glyceraldehyde 3-phosphate dehydrogenase, iodoacetate, inhibited glycolysis in hypoxia and blocked the activation of sarcolemmal K_{ATP} channels. Based on the obtained results, we conclude that the activation of sarcolemmal K_{ATP} channels is involved in glucose-mediated cardioprotection.
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Keywords: Glucose; Glycolysis; ATP-sensitive K^+ channels; Hypoxia; Ischaemia

ATP-sensitive K^+ (K_{ATP}) channels belong to the group of intracellular ATP sensors and they couple the metabolic status of cell with the membrane excitability [1]. These channels are normally closed, but are activated during ischaemia to promote cellular survival. It has been suggested that the opening of sarcolemmal K_{ATP} channels protects against myocardial infarction, mediates ischaemic preconditioning, and promotes survival of cardiomyocytes exposed to different kinds of metabolic stresses [2–5]. These channels are heteromultimers in vivo comprised of pore-forming Kir6.2 subunit, regulatory SUR2A subunit, and at least four accessory proteins including adenylate kinase (AK), creatine kinase (CK), muscle form of lactate dehydrogenase (m-LDH), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). It has been suggested that these enzymes/accessory proteins control the level of ATP, ADP, 1,3-bisphosphoglycerate, and lactate in the microenvironment surrounding the channel [6–10]. ATP and ADP, products of AK and CK activity, are regulators of K_{ATP} channel activity and it is known for years that changes in the levels of these nucleotides are responsible for the opening of K_{ATP} channels during hypoxia [1,6,7,11,12]. Catalytic products of glycolytic enzymes, 1,3-bisphosphoglycerate and lactate, have been also shown to regulate the activity of K_{ATP} channels [8–10]. However, whether glycolysis per se plays any role in the activation of cardiac sarcolemmal K_{ATP} channels during hypoxia is yet unknown.

Therefore, in the present study, we have aimed to determine whether activation of glycolysis during hypoxia is associated with the opening of sarcolemmal K_{ATP} channels and, if it is so, whether this opening could regulate cardiac resistance to hypoxia. To address these aims, we have applied an experimental model of hypoxia that implements adult cardiomyocytes stimulated to beat. Hypoxia was induced solely by reducing partial oxygen tension (PO_2) without using additional means to metabolically challenge the cells, which, all together, makes this model closer to in vivo conditions than the majority of previously published cellular models of cardiac metabolic stress [13].

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To stimulate glycolysis we have exposed cardiomyocytes under hypoxia to high concentration of glucose in extracellular environment. We did not use insulin to stimulate glycolysis deliberately as this hormone has direct cardiac effects independent of glucose transport [14].

Thus, we have taken advantage of this experimental setting and examined whether stimulation of glycolysis regulates the activity of sarcolemmal K_{ATP} channels in hypoxia and whether it would influence cellular resistance under such conditions.

Materials and methods

Cardiac cell isolation. Ventricular cardiomyocytes were dissociated from guinea-pig hearts as described [7–10]. In brief, hearts were retrogradely perfused (at 37 °C) with medium 199, followed by Ca^{2+}-EGTA-buffered low-Ca^{2+} medium ($pCa = 7$), and finally low-Ca^{2+} medium containing pronase E (8 mg/100 ml), proteinase K (1.7 mg/100 ml), bovine albumin (0.1 g/100 ml, fraction V), and 200 μM $CaCl_2$. Ventricles were cut into fragments in the low-Ca^{2+} medium enriched with 200 μM $CaCl_2$. 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).

Experimental protocol of hypoxia. Hypoxia of isolated cardiomyocytes has been performed as described in Budas et al. [13] and Mora et al. [15]. Thus, cardiomyocytes were placed into Tyrode's solution (in mM: NaCl 136.5, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, glucose (0–30), and Hepes–NaOH 5.5, pH 7.4) and paced to beat by field stimulation (parameters of the stimulation: 5–20 mV depending on cellular threshold, 5 ms, 0.5 Hz). Beating cardiomyocytes were perfused with Tyrode's solution at a rate of 3 ml/min and, under these conditions, the partial pressure of O_2 (PO_2) in perfusate was 140 mmHg. To induce hypoxia, Tyrode's solution was bubbled with 100% argon ($PO_2 = 20$ mm Hg). Hypoxia was maintained until cell death or for 30 min. The moment of cell death was defined as a point when irreversible cellular rounding associated with the Ca^{2+} overload occurs. Some experiments were done in the presence of iodoacetate, 2-deoxyglucose (Sigma, Dorset, UK) or HMR 1098 (Aventis Pharma, Frankfurt, Germany).

Measurement of 1,3-bisphosphoglycerate. To determine whether high extracellular concentration of glucose would stimulate glycolysis in isolated cardiomyocytes, we have measured 1,3-bisphosphoglycerate, an intermediary product of glycolysis. Cells exposed to normoxic (for 10 min) or hypoxic Tyrode's solution (as described above) containing no glucose, glucose (30 mM) or glucose (30 mM) plus iodoacetate (1 mM) for 0, 5, 10, and 20 min were rapidly frozen in liquid nitrogen and homogenised using perchloric acid (6%, w/v). The homogenates were centrifuged at 24,000*g* to obtain the cytosolic fraction. 1,3-Bisphosphoglycerate was purified from the supernatant and measured using the procedure described by Negelein [16]. Briefly, 1,3-bisphosphoglycerate was determined in solution containing 1,3-bisphosphoglycerate separated from the other cytosolic components by precipitation with acetone at pH 2.1 (200 μl), pyrophosphate buffer (100 mM of $Na_4P_2O_7$ at pH 7.9; 830 μl; 100 mM), NADH solution (2.2 mM; 200 μl), and distilled water (1.24 ml). This solution was mixed and the absorbance was read at a wavelength of 340 nm using a spectrophotometer (WPA lightwave, Jencons, Forest Row, UK). Then, GAPDH solution (30 μl; 1 mg/ml of GAPDH protein) was added, mixed, and the absorbance was read again. The concentration of 1,3-bisphosphoglycerate was calculated based on the oxidation of NADH, measured by the change in absorbance at 340 nm, which is proportional to the 1, 3-bisphosphoglycerate concentration [16]. All reagents used to determine 1,3-bisphosphoglycerate concentration were from Sigma (Dorset, UK).

Laser confocal microscopy. Ventricular myocytes were loaded with 3.5 μM of the Ca^{2+}-selective fluorescent probe, Fluo-3 acetoxymethyl ester (Fluo-3AM), for 30 min at room temperature as previously described [17]. Myocytes loaded with Fluo-3 were plated on glass coverslips which were transferred to an experimental chamber mounted on the stage of a Zeiss LSM-510 laser-scanning confocal microscope (LSM-510, Zeiss, Göttingen, Germany) filled up with Tyrode's solution and paced to beat by field stimulation (parameters of the stimulation: 5–20 mV depending on cellular threshold, 5 ms, 0.5 Hz). Ca^{2+} levels and cell morphology were continuously imaged (a frame per 5 s) with a Zeiss LSM-510 laser-scanning confocal microscope (LSM-510, Zeiss, Göttingen, Germany) using Ar/UV laser to "excite" the dye at 488 nm. Emission light by photomultiplying tubes was detected at 520 nm. The parameters of image acquisition were similar for all examined cells (mid-cell section thickness was 1 μm and gain was set always at ~700 arbitrary units). Images were acquired, viewed, and analysed using Zeiss Image Examiner Software. Only beating rod-shaped cells with clear striations were used for experimentation. In experiments when hypoxia was fixed to last 30 min, the morphology of cells in the visual field was examined before and after 30-min long hypoxia. Rounded cells (defined as cells when the ratio between maximal and minimal diameter was <2) were considered irreversible injured and dead [13,15,18].

Patch–clamp electrophysiology. For perforated patch–clamp whole-cell electrophysiology, cells were superfused with Tyrode's solution (in mM: 136.5 NaCl; 5.4 KCl; 1.8 $CaCl_2$; 0.53 $MgCl_2$; glucose (0 or 30), 5.5 Hepes–NaOH; pH 7.4). Pipettes (resistance 3–5 MΩ) were filled with (in mM): KCl 140, $MgCl_2$ 1, amphotericin B (Sigma, 240 μg/ml), and Hepes–KOH 5 (pH 7.3). For all cells monitored, the membrane potential was normally held at −40 mV and the currents evoked by a series of 400 ms depolarising and hyperpolarising current steps (−100 mV to +80 mV in 20 mV steps) recorded directly to hard disc using an Axopatch-200B amplifier, Digidata-1321 interface, and pClamp8 software (Axon Instruments, Forster City, CA). The experiments were performed at room temperature. The capacitance compensation was adjusted to null the additional whole-cell capacitative current. The slow capacitance component measured by this procedure was used as an approximation of the cell surface area and allowed normalisation of current amplitude (i.e., current density).

Statistical analysis. Data are presented as means ± SEM, with n representing the number of patched/imaged cells or examined hearts. The difference between means was assessed using *t* test (duration of cell survival), χ^2 test/Fisher's exact test (percentage of survived cells) or by the two-way repeated-measures ANOVA followed by *t* tests (levels of 1, 3-bisphosphoglycerate and the effect of glucose on membrane current) when appropriate using SigmaStat program (Jandel Scientific, Chicago, Illinois). A value of $P < 0.05$ was considered statistically significant.

Results

High glucose stimulates glycolysis in cardiomyocytes exposed to hypoxia

To determine whether high extracellular glucose stimulates glycolysis, we have measured the levels of 1,3-bisphosphoglycerate in cardiac cells under normoxia/hypoxia in the absence and presence of 30 mM glucose. The levels of 1,3-bisophosphoglycerate in the absence of glucose and in normoxia were not measurable (Fig. 1). Under these conditions, exposure of cells to 30 mM of glucose induced significant increase in concentration of 1,3-bisophosphoglycerate (the estimated concentration was 0.06 ± 0.02 μM/ml under control conditions and 0.25 ± 0.09 μM/ml after 20 min hypoxia, $P = 0.03$, $n = 4$, Fig. 1). In cells exposed to hypoxia without glucose levels of 1,3-bisphosphoglycerate were barely measurable and they change a little over 20-min long hypoxia (the measured levels were 0.08 ± 0.02 μM/ml under control conditions and 0.09 ± 0.02 μM/ml after 20 min hypoxia, $P = 0.76$, $n = 4$, Fig. 1). In contrast, in the presence of high extracellular

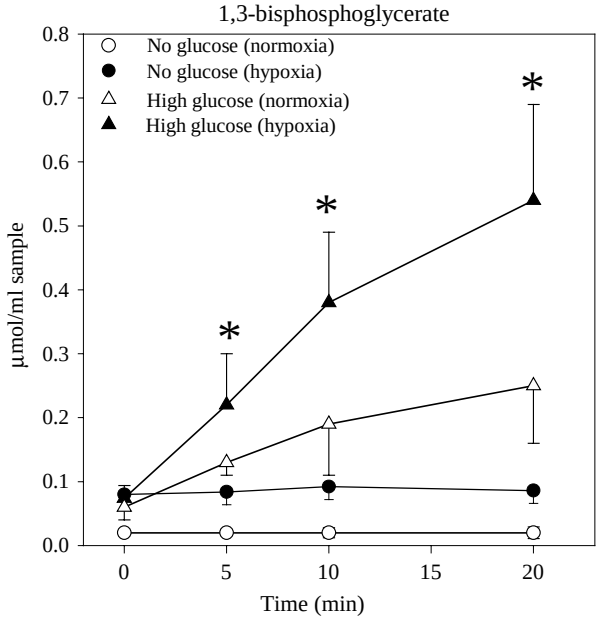

Fig. 1. Hypoxia increases 1,3-bisphosphoglycerate in cardiomyocytes exposed to hypoxia in the presence (30 mM), but not in the absence, of glucose. Time course of 1,3-bisphosphoglycerate concentration in cardiomyocytes exposed to normoxia or hypoxia in the absence and presence of glucose (30 mM). Each point represents mean ± SEM ($n = 4$ for each), and star indicates $P < 0.01$ when compared to any other point.

glucose (30 mM) intracellular levels of 1,3-bisphosphoglycerate rose significantly during hypoxia (the measured levels were 0.07 ± 0.01 µM/ml under control conditions and 0.54 ± 0.14 µM/ml after 20 min hypoxia, $P = 0.006$ when compared to the control and $P = 0.02$ when compared to 1,3-bisphosphoglycerate levels in high glucose during normoxia, $n = 4$, Fig. 1).

High glucose during hypoxia activates sarcolemmal K_{ATP} channels in cardiomyocytes

We have measured membrane currents from a guinea-pig ventricular myocyte in a perforated patch mode of whole-cell electrophysiology. In perforated patch–clamp mode intracellular milieu remains largely undisturbed as amphotericin B creates holes in the sarcolemma that are permeable to ions but impermeable to larger molecules [19]. Under these conditions the steady-state voltage–current (I–V) relationship was in an N shape due to the strong inward rectification of I_{k1} channels and absence of active K_{ATP} channels blocked by physiological levels of intracellular ATP (data not shown, see also [9]). Whole-cell K^+ current was largely steady during 20 min of recording (the current density was 3.2 ± 0.6 pA/pF when whole-cell configuration was established and 3.6 ± 0.4 pA/pF after 20 min, $P = 0.57$, $n = 5$; data not shown). Hypoxia is known to activate glucose transport in the heart, which is the main rate-limiting step in utilising glucose in myocardium. Under these conditions, extracellular concentration of glucose is the factor that strongly influences the activity of glycolytic pathway [20]. Therefore, to determine how glycolysis influences the activity of sarcolemmal K_{ATP} channels we have exposed cardiomyocytes to hypoxia in the absence of glucose, and in the presence of high concentration of extracellular glucose (30 mM). When cells were exposed to hypoxia, in the absence of glucose, whole-cell K^+ current increased significantly only after 12 min (the current density at 80 mV rose from 3.0 ± 0.4 pA/pF under control conditions to 4.9 ± 0.3 pA/pF after 12 min of hypoxia, $P = 0.008$, $n = 5$; Figs. 2A, B, and E). K^+ current was significantly increased at potentials more positive than −20 mV, and the inward rectification of the I–V relationship became much weaker, which is a typical finding when measuring whole-cell K^+ currents flow through K_{ATP} channels. When hypoxia was induced in the presence of high extracellular glucose (30 mM), whole-cell K^+ current increased significantly after only 3 min (the current density at 80 mV was from 2.9 ± 0.3 pA/pF under control conditions to 5.4 ± 0.6 pA/pF after 3 min of hypoxia, $P = 0.006$, $n = 5$; Figs. 2C, D, and E) and the magnitude of increase of hypoxia-induced K^+ current after 12 min was significantly higher in the presence than in the absence of glucose (the current density at 80 mV was to 4.9 ± 0.3 pA/pF without and 12.7 ± 1.2 pA/pF with glucose, $P = 0.0005$, $n = 5$; Fig. 2E).

High glucose protect cardiomyocytes against hypoxia

The activation of K^+ channels during hypoxia leads to the shortening of action membrane potential, decrease in Ca^{2+} influx, and protection of cardiac myocytes [17]. Single beating guinea-pig cardiomyocytes responded to hypoxia with intracellular Ca^{2+} loading and irreversible hypercontracture, which was indicative of cell death (Fig. 3A). The average time of survival in cells exposed to hypoxia in the absence of glucose was 13.5 ± 5.0 min ($n = 5$, Fig. 3C). When cells were exposed to hypoxia in the presence of high extracellular glucose (30 mM), the average time of survival was significantly increased to 80.0 ± 11.0 min ($P \ll 0.001$, $n = 5$, Fig. 3C). In the absence of glucose only 2.1% of cells survived 30-min long hypoxia while in the presence of glucose (30 mM) this number was significantly higher 87.5% (Fig. 3D, $n = 40$–47, $P \leqslant 0.001$).

Inhibition of sarcolemmal K_{ATP} channels opening abolishes glucose-induced cardioprotection

To determine a link between the glucose-induced activation of sarcolemmal K_{ATP} channels and cellular resistance to hypoxia, we have tested the effect of HMR 1098, a selective antagonist of sarcolemmal K_{ATP} channels [21], on cardiac cell resistance to hypoxia in the presence of glucose (30 mM). HMR 1098 (30 µM) abolished the protection against hypoxia afforded by glucose, as average time of cellular survival was 80.0 ± 11.0 min in the absence and 24.0 ± 2.0 min in the presence of HMR 1098 (30 µM)

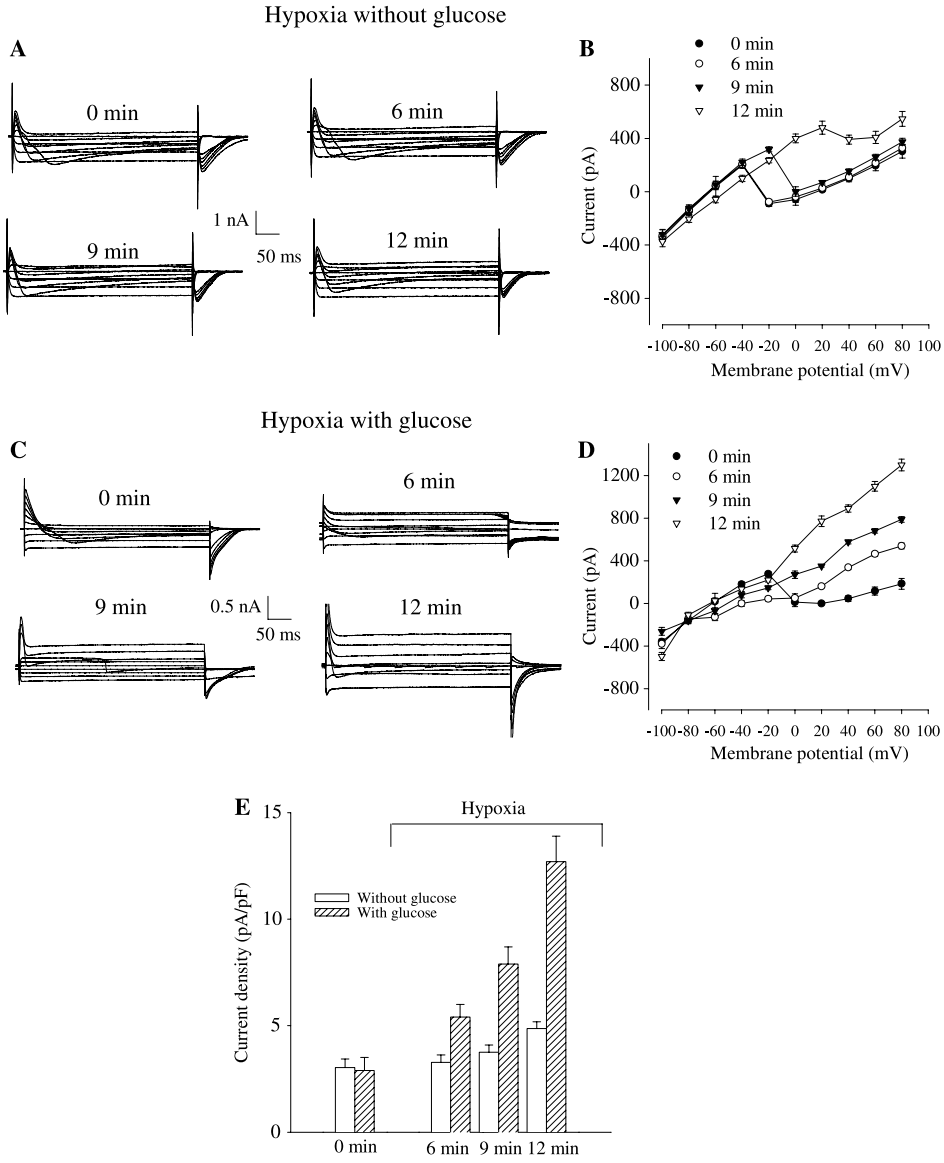

Fig. 2. Hypoxia induces whole-cell K^+ current more efficiently in the presence than in the absence of glucose. (A–D) Membrane currents recorded in response to 400-ms long pulses (from −100 to +80 mV) (A,C) and the corresponding *I–V* relationship (each point is mean ± SEM, $n = 5$, (B,D) in the absence (A,B) and presence of glucose (30 mM, C,D). Time point 0 min refers to the recording made immediately after the whole-cell configuration was established, i.e., when the serious resistance was low enough for a satisfactory voltage-clamp. (E) Bar graph shows current density at +80 mV in cells under depicted conditions and time points. Time points refer to the time from the moment of establishing the whole-cell configuration. Each bar represents mean ± SEM ($n = 5$). $P < 0.01$ when compared without and with glucose.

($P < 0.001$, $n = 5$; Figs. 4A and B). In addition, in the presence of both glucose (30 mM) and HMR 1098 (30 μM) only 21.6% of cells survived 30 min-long hypoxia which was significantly lower than 87.5% in the presence of glucose (30 mM) alone (Fig. 4C, $n = 14$–40, $P \leqslant 0.001$). At the same time, HMR 1098 (30 μM) blocked hypoxia-induced K^+ current in the presence of high glucose (Fig. 4C; the current density at 80 mV was from 1.6 ± 0.2 pA/pF under control conditions to 1.7 ± 0.6 pA/pF after 12 min of hypoxia, $P = 0.73$, $n = 4$; the current density was also significantly lower compared to those in the presence of glucose 12.7 ± 1.2 pA/pF, $n = 5$, $P < 0.001$).

Inhibition of glycolysis inhibits the activation of sarcolemmal K_{ATP} channels and cardioprotection afforded by high glucose

In the presence of iodoacetate (1 mM), which is an inhibitor of GAPDH [22], the levels of 1,3-bisphosphoglycerate did not rise during hypoxia regardless of the presence of 30 mM glucose (the measured levels were 0.09 ± 0.02 μM/ml under control conditions and 0.06 ± 0.02 μM/ml after 20 min hypoxia, $P = 0.17$, $n = 4$, Fig. 5A). In the presence of both high glucose (30 mM) and iodoacetate (1 mM) sarcolemmal K_{ATP} channels were not activated during hypoxia (the current density at 80 mV was from 1.9 ± 0.2 pA/pF under control conditions

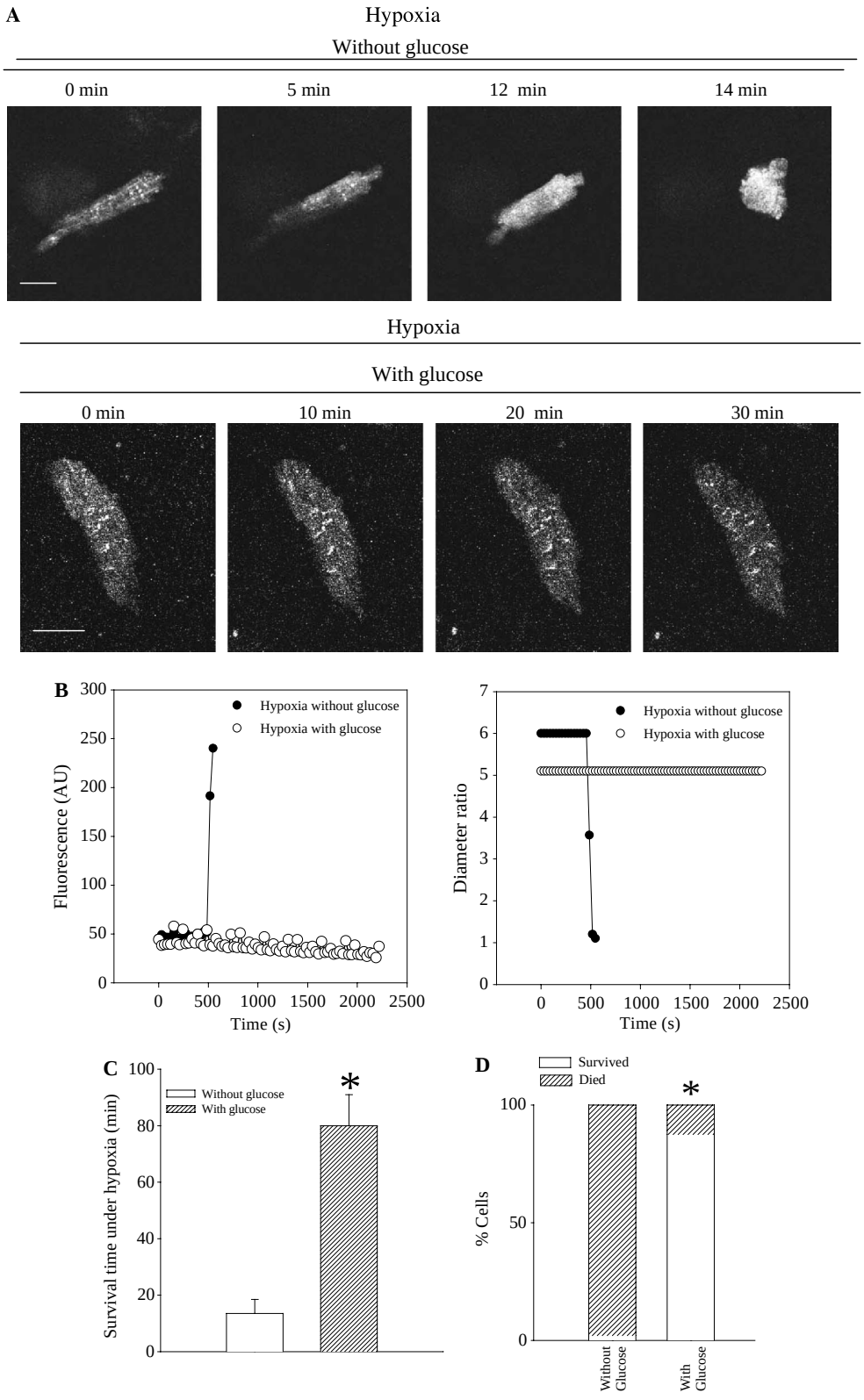

Fig. 3. High glucose protects cardiomyocytes against hypoxia. (A) Laser confocal images of Fluo 3-loaded cardiomyocytes exposed to hypoxia in the absence (without glucose) and presence of 30 mM glucose (with glucose). White horizontal bars correspond to 30 μm. (B) Time course of intensity of Fluo 3 fluorescence (AU = arbitrary units) and cell diameters corresponding to experiments in (A). (C) Average survival time of cardiomyocytes exposed to hypoxia in the absence and presence of 30 mM glucose. Bars represent means ± SEM ($n = 5$ for each), and star indicates $P < 0.01$. (D) Percentage of cells that survived/died in hypoxia in the absence and presence of glucose (30 mM), $n = 40$–47. Star indicates $P < 0.01$.

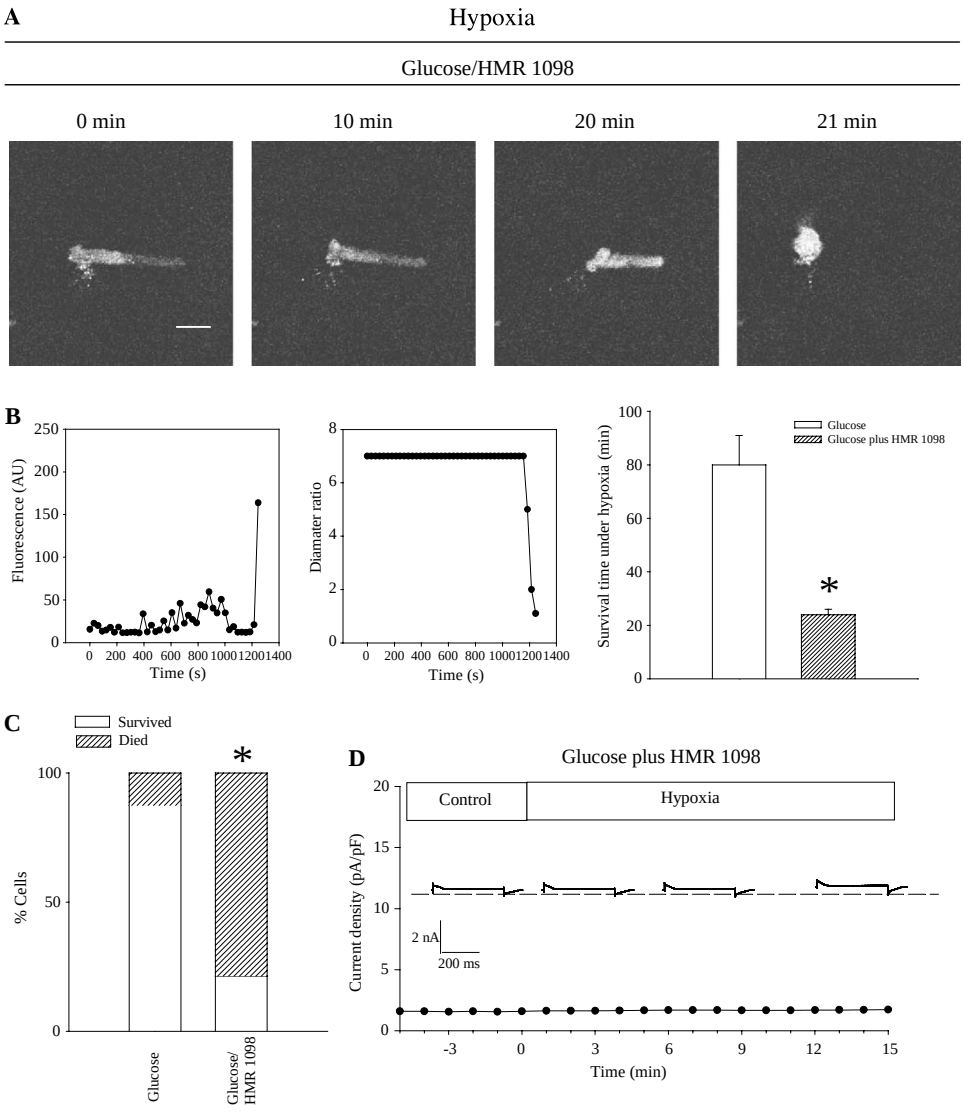

Fig. 4. Glucose-mediated cardioprotection is mediated via opening of sarcolemmal K_{ATP} channels. (A) Laser confocal images of Fluo 3-loaded cardiomyocytes exposed to hypoxia in the presence of glucose (30 mM) plus HMR 1098 (30 μM). White horizontal bar corresponds to 30 μm. (B) Left and middle graphs: time course of intensity of Fluo 3 fluorescence (AU = arbitrary units) and cell diameters corresponding to experiment in (A). Right graph: Average survival time of cardiomyocytes exposed to hypoxia in the presence of glucose (30 mM) or glucose (30 mM) plus HMR 1098 (30 μM). Bars represent means ± SEM ($n = 5$), and star indicates $P < 0.01$. (C) Percentage of cells that survived/died in hypoxia in the presence of glucose (30 mM) and glucose (30 mM) plus HMR 1098 (30 μM), $n = 14$–40. Star indicates $P < 0.01$. (D) Time course of whole-cell current density at a membrane potential of +80 mV in a cardiomyocyte under control conditions and during hypoxia in the presence of glucose (30 mM) plus HMR 1098 (30 μM). Insets: Typical whole-cell currents at membrane potential of +80 mV during different time points (corresponding to *y*-axis on the graph). The membrane potential was held at −40 mV and the current was evoked by a 400 ms current step (to +80 mV). Dotted line represents zero current line.

to 2.3 ± 0.6 pA/pF after 12 min of hypoxia, $P = 0.33$, $n = 4$; Fig. 5B) and the duration of cellular survival under these conditions was significantly decreased when compared to glucose alone (80.0 ± 11.0 min in the absence and 27.7 ± 3.7 min in the presence of 30 mM of glucose, $P = 0.002$, $n = 5$; Figs. 5C and D). In the presence of both glucose (30 mM) and iodoacetate (1 mM) only 23.1% of cells survived 30-min long hypoxia which was significantly lower than 87.5% in the presence of glucose (30 mM) alone (Fig. 5F, $n = 14$–40, $P \leqslant 0.001$). Similar results were obtained with 15-min long pretreatment with 2-deoxyglucose (50 mM), which is also an inhibitor of glycolysis [23], where only 28.4% of cells survived 30 min-long hypoxia (Fig. 5G, $n = 14$, $P \leqslant 0.001$ when compared with the glucose alone).

Discussion

In the present study, we have shown that the activation of glycolysis protects cardiomyocytes against hypoxia by activating sarcolemmal K_{ATP} channels.

It is well established that hypoxia stimulates glucose transport and glycolysis in the heart [24,25]. Our measurement of 1,3-bisphosphoglycerate in cardiomyocytes has confirmed that hypoxia stimulates transport of glucose/glycolysis and that levels of intermediary glycolytic products

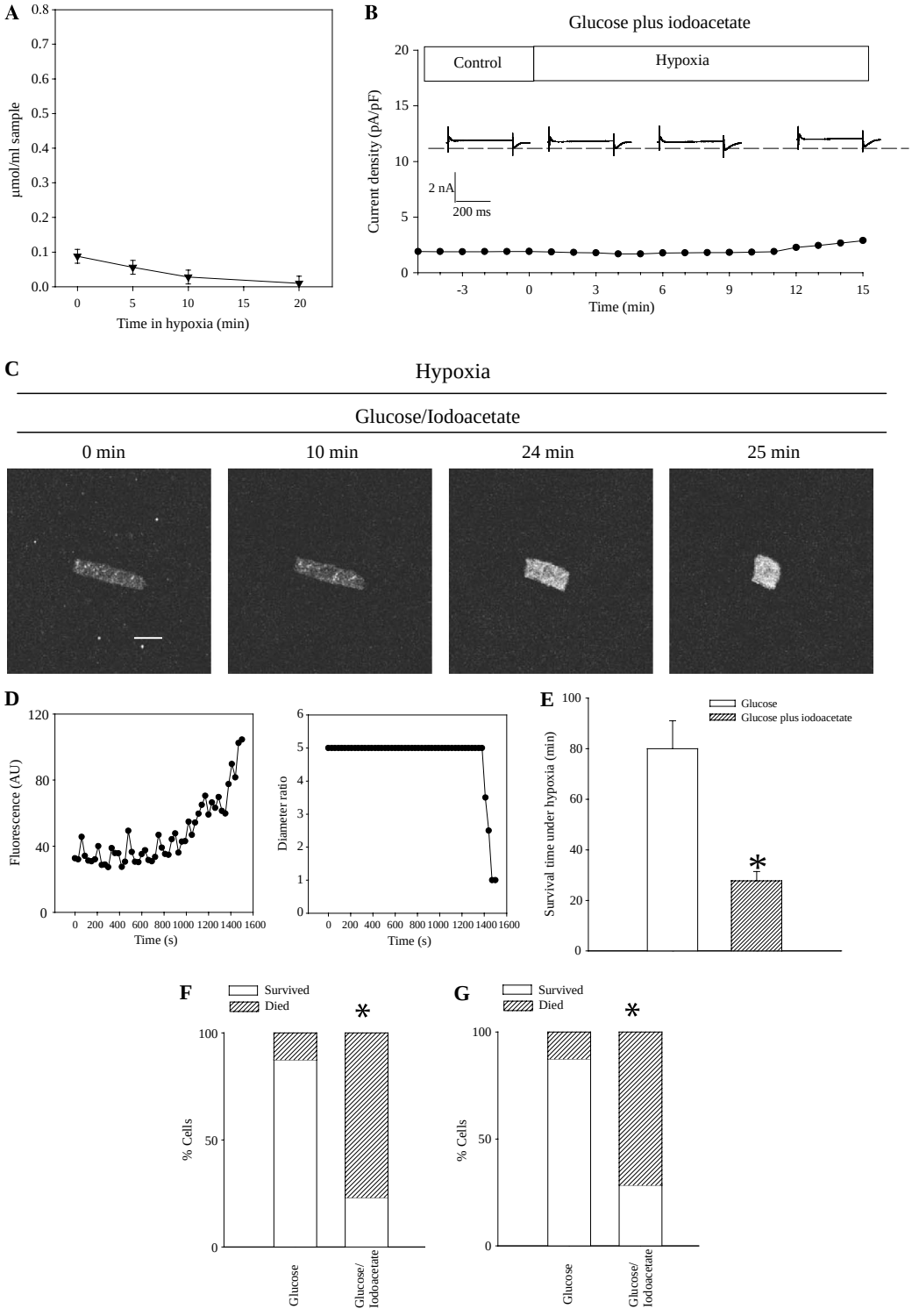

Fig. 5. Glucose-mediated cardioprotection and activation of sarcolemmal K_{ATP} channels requires intact glycolysis. (A) Time course of 1, 3-bisphosphoglycerate concentration in cardiomyocytes exposed to hypoxia in the presence of glucose (30 mM) plus iodoacetate (1 mM). Each point represents mean ± SEM ($n = 4$ for each). (B) Time course of whole-cell current density at a membrane potential of +80 mV in a cardiomyocyte under control conditions and during hypoxia in the presence of glucose (30 mM) plus iodoacetate (1 mM). Insets: Typical whole-cell currents at a membrane potential of +80 mV during different time points (corresponding to *y*-axis on the graph). The membrane potential was held at −40 mV and the current was evoked by a 400 ms current step (to +80 mV). Dotted line represents zero current line. (C) Laser confocal images of Fluo 3-loaded cardiomyocytes exposed to hypoxia in the presence of glucose (30 mM) plus iodoacetate (1 mM). White horizontal bar corresponds to 30 µm. (D) Left and right graphs: Time course of intensity of Fluo 3 fluorescence (AU = arbitrary units) and cell diameters corresponding to experiment in (A). (E) Average survival time of cardiomyocytes exposed to hypoxia in the presence of glucose (30 mM) or glucose (30 mM) plus iodoacetate (1 mM). Bars represent means ± SEM ($n = 5$), and star indicates $P < 0.01$. (F,G) Percentage of cells that survived/died in hypoxia in the presence of glucose (30 mM) and glucose (30 mM) plus iodoacetate (1 mM; F, $n = 14$–40) or of glucose (30 mM) and glucose (30 mM) plus 2-deoxyglucose (50 mM; G, $n = 14$–40). Stars indicate $P < 0.01$.

are increased in hypoxia. Here, we have applied a model suitable to study the behaviour of sarcolemmal K_{ATP} channels and cardiac responsiveness to stress that utilises adult beating cardiomyocytes. In principle, single cell models are used to study those mechanisms involved in cardioprotection that are independent of neuronal, vascular, and humoral factors. However, single cell systems developed so far have been restricted to the use of immature embryonic cardiac cells which beat spontaneously or non-beating quiescent adult cells. It has also been difficult to rigorously regulate oxygen tension around cardiomyocytes and therefore "ischaemia/hypoxia" have been usually mimicked by using metabolic inhibitors and oxygen scavengers [26]. To overcome these limitations, we have recently developed a model of single cell hypoxia implementing single adult cardiomyocytes that are stimulated to beat [13,15]. In this model, hypoxia is induced solely by reducing partial oxygen tension (PO_2) without using any additional means to metabolically challenge the cells. Thus, this model is probably closer to in vivo conditions than any other single cell model previously published.

Although it is well established that hypoxia activates sarcolemmal K_{ATP} channels the underlying mechanisms of this activation are still a matter of discussion. Originally, it has been proposed that a drop of intracellular levels of ATP is responsible for the activation of K_{ATP} channels during ischaemia [1]. However, later studies have suggested that this was not necessarily the case and that other intracellular signalling factors may be involved in the activation of sarcolemmal K_{ATP} channels by ischaemia/hypoxia [2–5,27]. Under anaerobic conditions glycolysis takes over fatty acid oxidation as the main source of energy in the heart [28]. As the glycolysis produces ATP, a K_{ATP} channel inhibitory ligand, on one side it is logical to expect that stimulated glycolysis could inhibit K_{ATP} channels from opening. On the other side, at least two products of anaerobic glycolysis have the ability to open K_{ATP} channels even in the presence of millimolar ATP [8–10]. Reports in early 1970s have suggested that elevating extracellular glucose attenuates action potential duration (APD) shortening during hypoxia in intact cardiac tissue and that APD shortening is even more rapid in the absence of glucose [29–31]. However, more recent studies have provided different results suggesting that hyperglycaemia do not inhibit ischaemia/hypoxia-induced shortening of APD [32,33]. A direct measurement of the ion channel activity in sarcolemma revealed that hyperglycaemia inhibits the activity of human ether-a-go-go-related gene (HERG) K^+ channel [34] but at the same time activates sarcolemmal K_{ATP} channels [9,10] which might explain the net nil effect on cardiac APD reported in del Valle et al. [33]. Under physiological levels of glucose, sarcolemmal K_{ATP} channels do not open in first 15 min of hypoxia, suggesting that nucleotide levels do not change sufficiently during this time period to affect the behaviour of K_{ATP} channels [13]. However, the fact that sarcolemmal K_{ATP} channels were opened much earlier in the presence of high glucose would support the idea that acute glycolysis activates sarcolemmal K_{ATP} channels.

Whether the activation of sarcolemmal K_{ATP} channels is cardioprotective is still a matter of discussion, but several lines of evidence would favour such hypothesis: (1) coexpression of Kir6.2 with SUR2A (in combination with K_{ATP} channel opener or muscle form of lactate dehydrogenase) confers resistance against metabolic stress in otherwise stress-sensitive cells [8,18], (2) in mice with genetically disrupted sarcolemmal K_{ATP} channel, the heart is more susceptible to metabolic stress [35], (3) K_{ATP} channel openers-mediated protection against ischaemia is associated with the effect on cardiac membrane potential and with measurable sarcolemmal K_{ATP} channels opening [17], (4) an increase in the number of sarcolemmal K_{ATP} channels increases cardiac resistance to metabolic stress [36–38]; (5) ischaemic preconditioning cannot be conferred in transgenic animals lacking sarcolemmal K_{ATP} channels and it is associated with sarcolemmal K_{ATP} channels opening and trafficking [13,39]. On the other hand, there are conflicting reports on the effect of high glucose on hearts exposed to ischaemia/hypoxia. Some studies have suggested that hyperglycaemia may have deleterious consequences on heart exposed to ischaemia [40,41] and that hyperglycaemia blocks cardioprotection afforded by ischaemic preconditioning [42]. In contrast, a number of other studies come up with the conclusion that hyperglycaemia may protect cardiac cells and the heart against ischaemia [43–46]. The reason for these differences is not yet known, but one offered explanation was that duration of exposure to hyperglycaemia plays a role in determining the effect of hyperglycaemia on ischaemic heart, i.e., it seems that acute hyperglycaemia is predominantly cardioprotective while chronic hyperglycaemia seems to be harmful to the heart [47]. There are numerous ways to determine the death of cardiomyocytes. Here, cell morphology was monitored to determine cellular viability as it has been established that irreversible hypercontracture (manifested as cellular rounding) is a very good indicator of cardiac cell death [15,18]. In the present study, we have shown that high glucose was associated with the activation of sarcolemmal K_{ATP} channels and consequent increase in cellular resistance to this type of metabolic stress. The fact that HMR1098, a selective antagonist of sarcolemmal K_{ATP} channels, inhibited both the channel opening and cardioprotection afforded by glucose would suggest that the opening of K_{ATP} channels mediates glucose-mediated cardioprotection.

To further assess the link between glycolysis, we have used iodoacetate and 2-deoxyglucose. These two agents are structurally unrelated and they block glycolysis in very different ways [22,23]. We have shown that the inhibition of glycolysis during hypoxia inhibits the channels opening and glucose-mediated cellular protection. All together, these results would suggest that there is a link between glycolysis and cardiac resistance to hypoxia mediated via activation of sarcolemmal K_{ATP} channels.

Recently, it has been shown that combined application of insulin and glucose protects the heart against myocardial infarction in a K_{ATP} channel-dependent manner [48]. As insulin does not affect cardiac sarcolemmal K_{ATP} channels directly [49,50], this study is quite compatible with our findings that a stimulation of glycolysis protects cardiac cells against hypoxia via activation of K_{ATP} channels. In the heart, opening of sarcolemmal K_{ATP} channels "on-time" seems to be crucial for the cardiac protection against hypoxia/ischaemia [8,18]. The exact time point when sarcolemmal K_{ATP} channels are activated during hypoxia depends on the balance between endogenous K_{ATP} channel inhibitors on the one and endogenous channel openers on the other side [2–5]. In these regards, an increase in intracellular concentration of channel openers, as lactate and 1,3-bisphosphoglycerate seem to be [8–10], would shift a balance towards the channel opening which, in turn, would promote cellular survival under hypoxia. Therefore, it seems that glycolysis could promote cardiac survival during hypoxia by activating sarcolemmal K_{ATP} channels in addition to other effects that were previously described [43–46].

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