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Hydrogen sulfide-mediated cardioprotection against ischemia reperfusion is linked to K_{ATP} channel for mitochondrial preservation but not for its distinct preference on interfibrillar mitochondria

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Abstract

Hydrogen sulfide has been shown to protect myocardium against ischemia-reperfusion injury by preserving interfibrillar mitochondria functional activities than subsarcolemmal mitochondria. In this study, the role of the K_{ATP} channel in modulating the mitochondrial subpopulations during the cardioprotection mediated by NaSH (H_2S donor) was investigated. Isolated rat hearts were treated with mitochondrial K_{ATP} channel closer glibenclamide ($10 \mu M$)/opener diazoxide (0.8 mM) via Langendorff perfusion apparatus before ischemia-reperfusion. The results showed that NaSH pre-conditioning in presence of glibenclamide significantly improved cardiac recovery without any significant difference between interfibrillar mitochondria and subsarcolemmal mitochondria. In conclusion, targeting K_{ATP} channel may not be good option to target interfibrillar mitochondria/subsarcolemmal mitochondria against ischemia-reperfusion injury.

Introduction

Ischemic heart diseases are the leading causes of mortality all over the world (Judith et al., 2013) and the strategies to restore blood flow in the ischemic areas of the heart often encounter ischemia reperfusion injury. Hydrogen sulfide, one of the well-known cardioprotective gasotransmitters (Wang, 2004), is reported to attenuate the ischemia reperfusion injury by preserving the mitochondrial functional activities. Mitochondrial ATP-sensitive potassium channel (mito K_{ATP}) channel play a crucial role in regulating the mitochondrial functional activity by changing the mitochondrial matrix volume (Kaasik et al., 2007), mitochondrial respiration rate and modulating membrane potential. Among the four potassium channels in the inner mitochondrial membrane, mito K_{ATP} and mito BK_{Ca} channels are known to interact

with hydrogen sulfide (Walewska et al., 2018). Evidences from the literature support a direct interaction of hydrogen sulfide on mito K_{ATP} , where it sulphhydrates the Cys43 in the Kir6.1 subunit (Mustafa et al., 2011).

Many drugs like nicorandil, bimakalin, pinacidil and levosimendan, that specifically target mito K_{ATP} are known to be effective in the management of cardiovascular diseases especially ischemia reperfusion (Pollesello and Mebazaa, 2004). Moreover, gas signaling molecules like H_2S , NO and CO are reported to provide cytoprotection via modulating the opening of mitochondrial potassium channels. This in turn regulates different cardioprotective signaling pathways, reported to have converging targets in mitochondria (O'Rourke, 2004).

But recent studies have reported the presence of



heterogeneous mitochondria in the myocardium that not only differ with respect to the spatial location but also with respect to biochemical activities and morphology (Kuznetsov and Margreiter, 2009). Our lab has shown that mitochondria present in the myofibril named interfibrillar mitochondria exhibits resistance to ischemia reperfusion injury (Banu et al., 2016) than those present near to the membrane named subsarcolemmal mitochondria. Moreover, there is distinct response of interfibrillar mitochondria and subsarcolemmal mitochondria toward diazoxide, an opener of ATP-sensitive K⁺ channels (Holmuhamedov et al., 2012).

Despite the higher preclinical success rate against ischemia reperfusion for hydrogen sulfide, it's potential to ameliorate revascularization injury in clinical subjects were moderate, indicating the existence of dearth in knowledge with regards to hydrogen sulfide mode of action. This brings the need to address the overlooked area of hydrogen sulfide research on ischemia reperfusion injury that focus on its effect on the mitochondrial sub population. In the present study, the role of K_{ATP} channel in interfibrillar mitochondria and subsarcolemmal mitochondria in determining the efficacy of hydrogen sulfide in the management of ischemia reperfusion injury was evaluated.

Materials and Methods

Animals and experimental design

The experiments were carried out with 250-300 g Wistar male rats. The rats were kept under a standard condition with regular diet and water *ad libitum*.

Perfusion protocol

The rats were anesthetized with 60 mg/kg sodium thiopentone and the hearts were excised and mounted in the Langendorff apparatus (AD Instruments, Australia) as per the previous procedure with slight modification (Chevion et al., 1993). The heart was stabilized for 20 min with Krebs-Henseleit buffer (NaCl 118.0 mM, KCl 4.7 mM, CaCl₂ 1.9 mM, MgSO₄ 1.2 mM, NaHCO₃ 25.0 mM, KH₂PO₄ 1.2 mM, glucose 10.1 mM) perfusion, maintained at 37°C and pH of 7.4. The buffer was oxygenated (95% O₂ + 5% CO₂) throughout the experiment. The perfusion was carried out according to the animal groups. Ischemia was given for 30 min by switching off the buffer flow, followed by reperfusion (reflowing the buffer into the heart) for 60 min. Preconditioning was given for 15 min in three cycles following 10 min stabilization. Each cycle consisted of 2 min ischemia followed by 3 min reperfusion.

Experimental groups

Rats were divided into 7 groups (n=6/group) randomly namely a) normal (to establish baseline parameters for the effects caused by subsequent manipulations, normal

group hearts were subjected to continuous perfusion for 115 min with Krebs-Henseleit buffer); b) ischemia-reperfusion (ischemia-reperfusion challenged isolated rat hearts were obtained by arresting the flow of Krebs-Henseleit buffer for 30 min followed by reperfusing the heart for 60 min after 25 min of stabilization with Krebs-Henseleit buffer); c) hydrogen sulfide preconditioning (hydrogen sulfide preconditioning hearts were perfused with NaSH (20 μM) after 10 min of stabilization for 15 min followed by ischemia for 30 min and reperfusion for 60 min); d) glibenclamide ischemia-reperfusion control (treated with glibenclamide (10 μM) prior to hydrogen sulfide preconditioning protocol); e) diazoxide ischemia-reperfusion control (treated with diazoxide (0.8 mM) respectively prior to hydrogen sulfide preconditioning protocol); f) glibenclamide plus hydrogen sulfide preconditioning (treated with glibenclamide and diazoxide respectively followed by ischemia-reperfusion protocol described above), g) diazoxide plus hydrogen sulfide preconditioning (treated with diazoxide followed by ischemia-reperfusion protocol described above).

The subsequent hemodynamic parameters like left ventricular end diastolic pressure in mmHg, developed pressure in mmHg, heart rate in beats per min, and rate pressure products were evaluated using LabChart pro of AD Instruments, Australia. Rate pressure product (RPP=HR*DP) was calculated using heart rate and developed pressure.

Mitochondrial isolation

Mitochondrial subpopulation were isolated from the heart by the method described previously (Palmer et al., 1977). Briefly, tissue homogenate was centrifuged at 800 x g for 5 min and the resulting supernatant was centrifuged at 9,000 x g for another 10 min. The resultant pellet was centrifuged at 8000 x g twice to yield the subsarcolemmal mitochondria fraction. The pellet obtained in the initial step (800 x g, 5 min) was treated with nagarase enzyme (0.5 mg/g tissue) and subjected to differential centrifugation procedure similar to subsarcolemmal mitochondria isolation to yield the interfibrillar mitochondria fraction. All procedures were carried out at 4°C.

Estimation of lactate dehydrogenase and creatine kinase

The cardiac injury markers lactate dehydrogenase and creatine kinase were estimated in the heart homogenate spectrophotometrically using the previously described method (Kurian et al., 2005).

Determination of infarct size

Myocardial infarct size was measured after staining the heart sections with 1.5% triphenyl tetrazolium chloride at 37°C for 10 min. Images were taken using zoom stereomicroscope (Nikon SMZ1270) having high-defini-

tion CCD camera (Nikon DSFi2) and NIS-elements documentation tool. Image J analysis tool (USA) was used to estimate the percentage of infarct tissue (Mensah et al., 2005).

Anti-oxidant enzymes

The activities of glutathione peroxidase and glutathione reductase were measured in the heart mitochondrial fraction as per the pre-described protocols (Blankenberg et al., 2003; Goldberg and Spooner, 1983). The level of reduced glutathione in the heart mitochondrial fraction was estimated to determine the oxidative stress by the method described elsewhere (Beutler and Kelly, 1963). Catalase activity was measured by following the rate of hydrogen peroxide consumption according to the method described elsewhere (Baudhuin et al., 1964). Total superoxide dismutase activity was measured by the pre-established procedure (Nandi and Chatterjee, 1988).

Mitochondrial electron transport chain and citric acid cycle enzyme activities

Electron transport chain enzyme activities were measured spectrophotometrically in both the mitochondrial subpopulations by using specific donor acceptors to evaluate the mitochondria's integrity. Complex I and II activities were assessed by rotenone-sensitive NADH-oxidoreductase (NQR) and succinate decylubiquinone DCPIP reductase (SQR) respectively. Ubiquinol-cytochrome-C reductase (QCR) was used to assess complex III and cytochrome c oxidase (Complex IV) activity was measured as per the protocol described (Frazier and Thorburn, 2012). The citric acid cycle enzymes malate dehydrogenase and succinate dehydrogenase, and NADH dehydrogenase activity, were measured spectrophotometrically.

Statistical analysis

Data were presented using \pm SD. For statistical analysis, GraphPad Prism 5.0 was used. One-way analysis of variance (ANOVA) followed by Dunnet's test was

carried out to know the difference between groups. $p < 0.05$ was considered as statistically significant.

Results

Effect of mito- K_{ATP} channel opener or closer on cardiac hemodynamics and injury

The cardiac physiological performance in ischemia reperfusion-treated rat heart was significantly different from the normal rat heart (Table I). The use of mitochondrial K_{ATP} channel closer (glibenclamide) and opener (diazoxide) in ischemia-reperfusion showed improved hemodynamics but prominent recovery was observed by diazoxide as measured by end diastolic pressure, developed pressure and rate pressure products respectively.

Cardiac injury was assessed via measuring triphenyl-tetrazolium chloride staining, where glibenclamide or diazoxide pretreatment exhibited 36 and 86% decline in cell death from ischemia reperfusion control respectively. This result was supported by significant decline of lactate dehydrogenase level (2.9 ± 0.4 and 1.8 ± 0.2 respectively) and creatine kinase level (0.4 ± 0.1 and 0.4 ± 0.2 respectively) activity in the myocardium (Figure 1).

Influence of NaSH on cardiac hemodynamics and injury in presence of mito- K_{ATP} channel opener or closer

After confirming the cardioprotective effect of hydrogen sulfide via hemodynamics (rate pressure products for hydrogen sulfide pretreated group, 83 ± 3 vs normal 95 ± 2), the protection in presence of mitochondrial K_{ATP} channel closer and opener were evaluated (Table I). The protective effect of hydrogen sulfide was not influenced by the presence of mitochondrial K_{ATP} channel closer or opener.

There was no significant difference in cardiac infarct size between the glibenclamide plus hydrogen sulfide (5.9 ± 0.5) and diazoxide plus hydrogen sulfide ($4.9 \pm$

Table I

Hemodynamic parameters

Pretreatment	Procedure		End diastolic pressure	Developed pressure	Rate pressure product (mmHg beats/min $\times 10^3$)
Buffer	-	-	4 ± 2	98 ± 4	95 ± 2
Buffer	Ischemia	Reperfusion	43 ± 5^a	42 ± 3^a	32 ± 3^a
Hydrogen sulfide	Ischemia	Reperfusion	23 ± 3	93 ± 3	83 ± 3
Glibenclamide	Ischemia	Reperfusion	50 ± 5^a	59 ± 3^a	57 ± 2^a
Glibenclamide plus hydrogen sulfide	Ischemia	Reperfusion	37 ± 4	93 ± 2	90 ± 3
Diazoxide	Ischemia	Reperfusion	25 ± 2	89 ± 2	87 ± 2
Diazoxide plus hydrogen sulfide	Ischemia	Reperfusion	39 ± 3^a	86 ± 1	84 ± 4

Ischemia for 30 min followed by reperfusion for 60 min; Values are expressed as mean \pm SD of $n = 6$ rats/group. ^aStatistically significant from normal perfusion ($p < 0.05$)

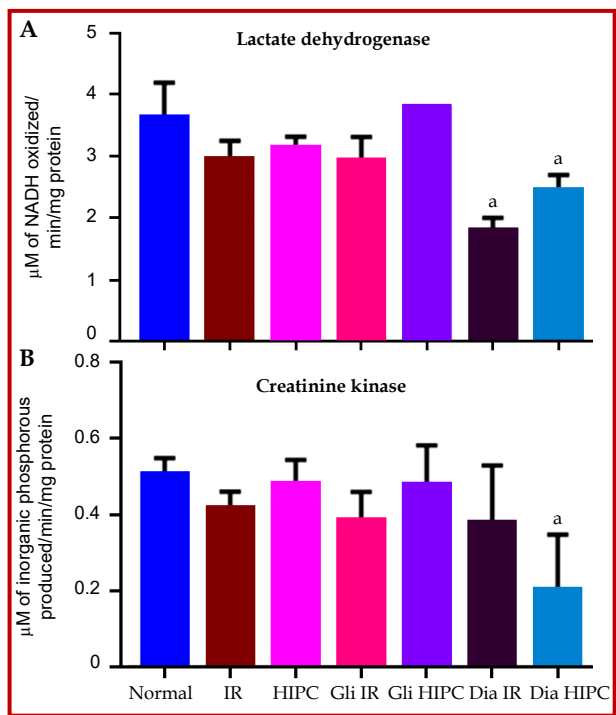


Figure 1: Effect of hydrogen sulfide on ischemia reperfusion-induced myocardial injury: hydrogen sulfide preconditioning effect on cardiac injury markers across the groups was represented by the activities of (A) lactate dehydrogenase and (B) creatine kinase. Values were expressed as mean ± SD of n=6 rats/group. *statistically significant from normal perfusion (p<0.05)

0.3) (Table II; Figure 2). This observations were further validated by level of cardiac markers in the myocardium and coronary perfusate and are in agreement with the above results.

Impact of mito-K_{ATP} channel modulators on the mitochondrial electron transport chain enzyme activities

Mitochondrial enzymes (NADH, SDH and MDH) and the electron transport chain enzyme activities (NQR, SQR, COX, and QCR) were measured in both the mitochondrial subpopulation namely interfibrillar

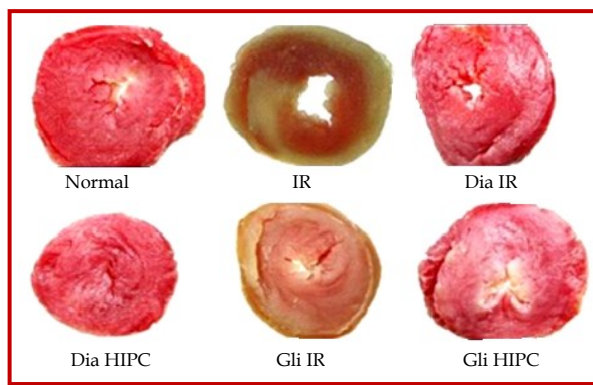


Figure 2: Infarct size measurement in heart using triphenyltetrazolium chloride (TTC) staining in different conditions

mitochondria and subsarcolemmal mitochondria from rat hearts subjected to ischemia-reperfusion (Figure 3). The efficiency of mitochondrial electron flux through respiratory enzymes were measured via NQR, SQR, COX and QCR activities in both sub-populations (Figure 3A, B, C and D) respectively. NQR activity representing the transfer of electrons through complex-I declined significantly in ischemia-reperfusion by 90% in subsarcolemmal mitochondria and 60% in interfibrillar mitochondria, in glibenclamide plus hydrogen sulfide-treated groups by 92% in subsarcolemmal mitochondria and 62% in interfibrillar mitochondria and diazoxide plus hydrogen sulfide-treated groups by 1.3% in subsarcolemmal mitochondria and 7.3% in interfibrillar mitochondria respectively when compared with the normal (Figure 3A). The electron transfer through complex-II represented by SQR activity decreased in Gli IR by 62 and 55% with no difference in their activity with respect to interfibrillar mitochondria and subsarcolemmal mitochondria (Figure 3B). But SQR activity was preserved in Dia IR group indicate the protective effect. The final electron acceptor in the electron transport chain (ETC), cytochrome c oxidase (COX) activity was found significantly low in both IR (63 and 60% in subsarcolemmal mitochondria and interfibrillar mitochondria respectively) and Gli IR groups (79 and 69% in subsarcolemmal mitochondria

Table II			
Infarct size measurement			
Pretreatment	Procedure		Infarct size (% of total heart)
Buffer	-	-	3.5 ± 0.5
Buffer	Ischemia	Reperfusion	32.2 ± 0.7 ^a
Glibenclamide	Ischemia	Reperfusion	20.5 ± 0.3 ^a
Glibenclamide plus hydrogen sulfide	Ischemia	Reperfusion	5.9 ± 0.5
Diazoxide	Ischemia	Reperfusion	4.5 ± 0.5
Diazoxide plus hydrogen sulfide	Ischemia	Reperfusion	4.9 ± 0.3

Data represent the heart infarct size calculated as percentage area affected, from TTC staining images. Values are expressed as mean ± SD of n = 4 rats/group. ^aStatistically significant from normal perfusion (p<0.05)

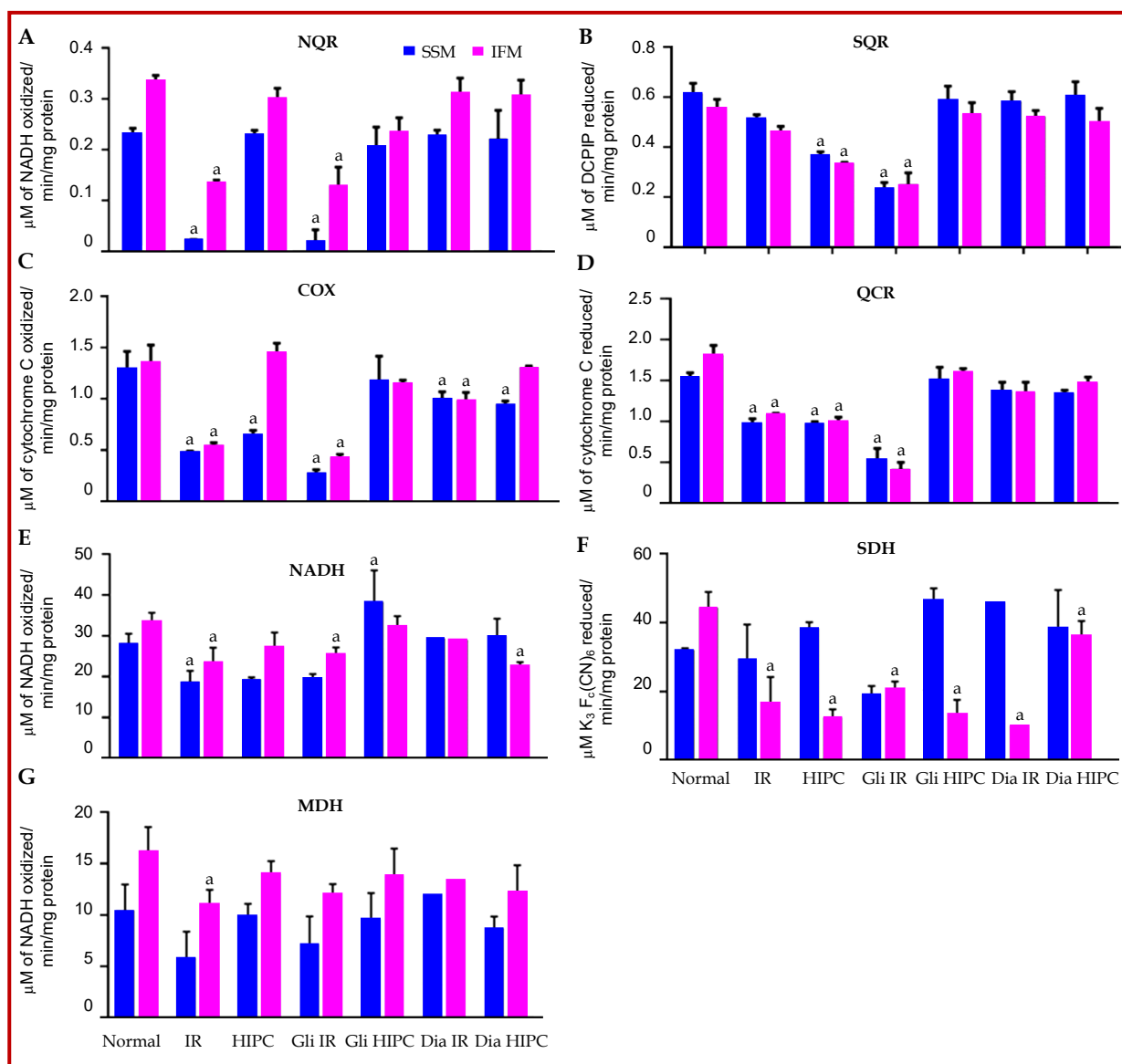


Figure 3: Effect of hydrogen sulfide and K_{ATP} modulators on cardiac mitochondrial enzyme activities: Effects of hydrogen sulfide on mitochondrial functional activities in the presence of K_{ATP} modulators were evaluated from the mitochondrial enzyme and electron transport chain enzyme activities: (A) NQR, (B) SQR, (C) COX and (D) QCR (E) NADH, (F) succinate dehydrogenase (SDH), (G) malate dehydrogenase (MDH). Results are expressed as mean \pm SD of $n=6$ rats/group. *Statistically significant from normal perfusion ($p < 0.05$) from the normal control

and interfibrillar mitochondria respectively). Similar to SQR activity, COX activity was found to be preserved in Dia IR groups.

Effect of hydrogen sulfide in the activities of electron transport chain enzymes in mitochondrial sub-population in presence of mito- K_{ATP} channel modulators

Previous publications from our lab confirmed the ability of hydrogen sulfide to preserve the ETC enzymes from interfibrillar mitochondria and subsarcolemmal mitochondria with a higher preference on interfibrillar mitochondria fraction. In the present study, we demarcated the effect of HIPC in presence of

mito- K_{ATP} channel modulators to find the rationale for the HIPC preference on interfibrillar mitochondria. The improved NQR activity by HIPC protocol on ischemia reperfusion challenged rat was maintained in both glibenclamide plus hydrogen sulfide and Dia HIPC, but only in subsarcolemmal mitochondria fraction. Even though glibenclamide plus hydrogen sulfide-induced heart showed improvement in interfibrillar mitochondria, the recovery was not similar as that of HIPC and Dia HIPC. However, an opposite effect was observed in SQR activity, where HIPC protocol was not effective to regain SQR activity from IR challenge, but in presence of mito- K_{ATP} channel modulators, the

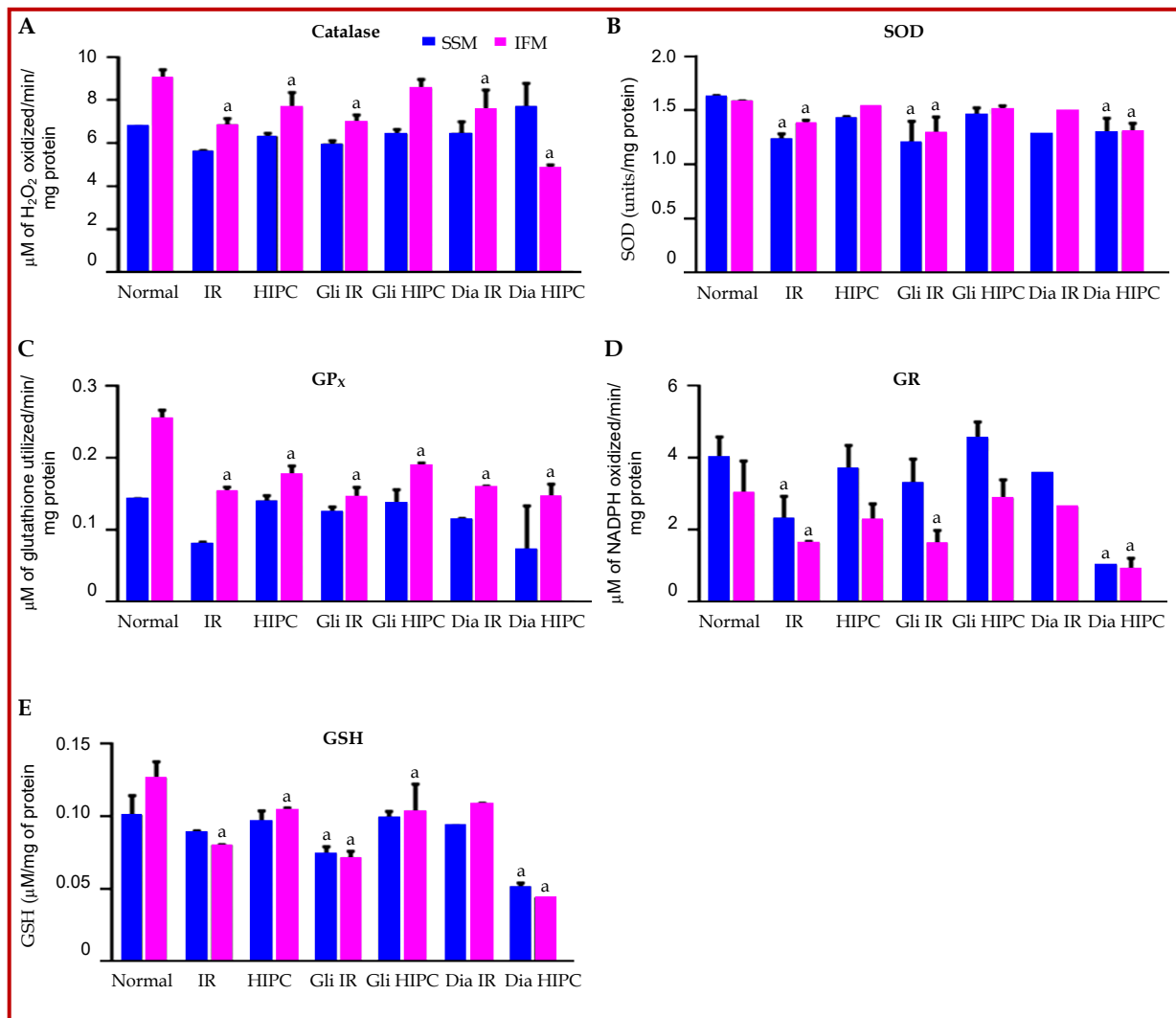


Figure 4: Effect of H₂S and K_{ATP} modulators on IR induced oxidative stress in cardiac mitochondrial subpopulation: Effect of H₂S and K_{ATP} modulators on IR induced oxidative stress in cardiac mitochondrial subpopulation were evaluated from the activities of (A) Catalase, (B) Superoxide Dismutase (SOD), (C) Glutathione Peroxidase (GPx) and (D) Glutathione Reductase (GR), (E) Glutathione level (GSH). Results are expressed as mean \pm SD of n = 6 rats/group. *Statistically significant from normal perfusion (p<0.05)

activity was significantly improved, without any prominent difference between the subpopulations. A similar pattern of changes was observed in COX activity as well.

Effect of hydrogen sulfide in the activities of NADH dehydrogenase, malate dehydrogenase and succinate dehydrogenase

Reperfusion injury induced a significant decline in the mitochondrial enzyme activities like NADH dehydrogenase (33.7% in subsarcolemmal mitochondria and 30% in interfibrillar mitochondria), SDH (9% in subsarcolemmal mitochondria and 62% in interfibrillar mitochondria) and MDH (45% in subsarcolemmal mitochondria and 32% in interfibrillar mitochondria) when compared with the normal. A similar pattern of

mitochondrial enzyme activities were found with GliIR groups as well (Figure 3E, F and G). However, these enzymes were significantly improved in both subpopulations in the heart from Gli HIPC.

Oxidative stress experienced by mitochondrial subpopulation: Role of mito-K_{ATP} channel modulators

The oxidative stress experienced by the mitochondrial subpopulation was evaluated through the activities of antioxidant enzymes (catalase, SOD, GPx and GR) and level of reduced glutathione, represented in Figure 4. Isolated mitochondria from ischemia reperfusion, glibenclamide or diazoxide ischemia-reperfusion groups experienced significant oxidative stress, evident from the activities of anti-oxidant enzymes. Reduced activity in catalase (17 and 24%, 12 and 23%, 5 and 6%

in subsarcolemmal mitochondria and interfibrillar mitochondria), SOD (24 and 13%, 26 and 18%, 20 and 18% in subsarcolemmal mitochondria and interfibrillar mitochondria), GPx (43 and 39%, 11 and 42%, 49 and 42% in subsarcolemmal mitochondria and interfibrillar mitochondria) and GR (1 and 47%, 16 and 47%, 70 and 74% in subsarcolemmal mitochondria and interfibrillar mitochondria) were found in ischemia reperfusion, glibenclamide or diazoxide ischemia-reperfusion groups respectively, when compared with the control group. Similarly anti-oxidant potential measured by reduced glutathione level showed significantly low concentration by 11 and 36%, 26 and 43%, 49 and 65% in subsarcolemmal mitochondria and interfibrillar mitochondria of ischemia reperfusion, glibenclamide or diazoxide ischemia-reperfusion groups respectively than normal control group. In fact, hydrogen sulfide preconditioning, glibenclamide or diazoxide ischemia-reperfusion reversed the negative effect of reperfusion injury on mitochondrial oxidative stress. Glibenclamide plus hydrogen sulfide preconditioning recovered the antioxidant enzymes level like catalase by 58 and 76% in subsarcolemmal mitochondria and interfibrillar mitochondria respectively, SOD by 61% in subsarcolemmal mitochondria and 73% in interfibrillar mitochondria respectively when compared with Gli IR.

Discussion

The present study had shown that hydrogen sulfide-mediated cardioprotection against ischemia-reperfusion injury was centered mainly on interfibrillar fraction than the subsarcolemmal mitochondria fraction of mitochondria (Banu et al., 2016). In the present study, it was aimed to evaluate whether hydrogen sulfide's preference for interfibrillar over subsarcolemmal mitochondria depends on the ATP-sensitive potassium channel, where the latter act as the therapeutic site for the hydrogen sulfide-mediated cardioprotection. The findings from the current study indicate that: 1) the action of hydrogen sulfide on interfibrillar and subsarcolemmal mitochondria fraction of mitochondria are similar in the presence of mitochondrial potassium ATP channel inhibitor. Hydrogen sulfide has protected the ischemic heart from the reperfusion injury by modulating the mitochondrial K_{ATP} channel present in both the interfibrillar and subsarcolemmal mitochondria. Thus, the effective preservation of interfibrillar fraction (the key player for the myocardial contractility) of mitochondria by hydrogen sulfide may be linked to its spatial location and associated effector molecule responsible for the physiological contractile recovery. 2) Persistent opening of K_{ATP} channel via diazoxide had an adverse effect on the hydrogen sulfide-linked cardioprotection.

Hydrogen sulfide preconditioning rendered the cardio-

protection against myocardial ischemia-reperfusion injury by reducing apoptosis, preserving mitochondria via stimulating different cardioprotective signalling like reperfusion injury salvage kinase pathway and survival activated factor enhancement pathway (Heng-Fei et al., 2012). From the literature, it is evident that mitochondrial preservation is one of the prime requirements for the intact myocardial contractile function (Kurian et al., 2012), which is governed by the electrogenic proton ejection via membrane potential that depends on K^+ entry through K_{ATP} channel into the organelle. The potassium entry improves the functional coupling between creatine kinase and adenine nucleotide translocase in mitochondria, thereby contribute to the protection (Oldenburg et al., 2002). Mitochondrial K_{ATP} channel opening mediates the protection via other mediators also like the low concentration of reactive oxygen species, the resistance of calcium overload and by stimulation of cardioprotective signalling molecules, thereby improving the ATP production as well. However, the mitochondria's capacity of oxidative phosphorylation, protein synthesis, protein and lipid composition and sensitivity to metabolic challenge differs in the two different cardiac mitochondrial fractions namely interfibrillar and subsarcolemmal mitochondria (Boengler et al., 2017). A recent study demonstrated an enhanced salvaging effect of hydrogen sulfide on interfibrillar over subsarcolemmal mitochondria in IR challenged rat heart (Ansari and Kurian, 2016). Similarly, another study suggests that the response of interfibrillar and subsarcolemmal mitochondria towards diazoxide, K_{ATP} channel opener are different and the effect was more prominent in the subsarcolemmal mitochondria (Holmuhamedov et al., 2012). But according to the present study, mitochondrial K_{ATP} channel modulators (inhibitor/opener) did not show distinct differences between the interfibrillar and subsarcolemmal mitochondria fraction in ischemia-reperfusion heart. These results underline the similarity of mitochondrial K_{ATP} channel in both the interfibrillar and subsarcolemmal mitochondria.

The interaction of hydrogen sulfide with K_{ATP} channel is already well established by using the patch-clamp technique and mutagenesis approach (Jiang et al., 2010). Both interfibrillar and subsarcolemmal mitochondria possess K_{ATP} channel and hydrogen sulfide action was found to be similar in interfibrillar and subsarcolemmal mitochondria fraction of mitochondria in the presence of mitochondrial potassium ATP channel inhibitor based on the present data. The insignificant difference observed between the interfibrillar and subsarcolemmal mitochondria's response towards mitochondrial K_{ATP} channel modulators in the heart makes it unable to explain the distinct impact of hydrogen sulfide on interfibrillar over subsarcolemmal mitochondria in the present study. It is evident from the literature that the cardiac K_{ATP} channel's pharmaco-

logy, molecular composition and properties varies between the different cardiovascular components like ventricle, atrium, conducting system, endothelium and cell organelles like mitochondria and sarcoplasmic reticulum (Foster and Coetzee, 2016). However, the properties and pharmacological regulation of the K_{ATP} channel in different cardiac cell types are not well established. The similar response of interfibrillar and subsarcolemmal mitochondria in the p interfibrillar presence of potassium ATP channel inhibitors from this study may be attributed to the non-specific modulation of potassium ATP channel by the chemical (glibenclamide, diazoxide) in different cardiac cell types (Foster and Coetzee, 2016), that may influence the distinct preservation of interfibrillar by hydrogen sulfide during ischemia-reperfusion.

By using diazoxide (K_{ATP} channel openers), the present study demonstrated the reversal of hydrogen sulfide-mediated cardioprotection and the observed adverse effect was associated with the persistence opening of the channel. This was well in agreement with previous work by Pomerantz et al, who showed transient opening of mitochondrial K_{ATP} channel with diazoxide provided protection to human atrial trabeculae against ischemia-reperfusion injury and a loss of cardioprotection was observed when diazoxide treatment was clubbed with ischemic preconditioning, due to the continuous opening of the potassium channel (Pomerantz et al., 2000). K_{ATP} channel modulates the mitochondrial function by changing the matrix volume, membrane potential and oxygen consumption that resulted in the release of varying concentration of reactive oxygen species and calcium ions. The concentration of diazoxide/glibenclamide plays a key role in determining the transient or permanent opening of the K_{ATP} channel. For instinct, for the pharmacological action, glibenclamide of concentration 1.2 nM is required in atrial K_{ATP} channel, whereas 1-2 μ M is required to induce the effect on K_{ATP} channel in mitochondria (Foster and Coetzee, 2016). Thus, the sensitivity of the chemicals will change depending on the metabolic activity of the cardiac cell type that may be varied in different diseased conditions. The hydrogen sulfide being a potassium channel opener works similar to diazoxide, it is highly recommended to determine a threshold dose for the transient opening of the mK_{ATP} channel, which is essential for its clinical success.

Based on the above observations, we emphasized the importance of mitochondrial subpopulations in understanding the underlying mechanism of cardioprotective drug or procedure that works via stimulating cardiac mitochondria. Previously, investigators were more concerned about the healthy mitochondrial population for the drug action. But now, scientists are forced to think the number, composition and the unique contribution

of the subpopulations in the healthy mitochondrial population in ischemia-reperfusion challenged the heart. The biggest challenge ahead in this area of research is to develop unique reliable marker to distinguish the mitochondrial subpopulations and its utilization in bedside via identifying its signature in blood samples.

Conclusion

Targeting K_{ATP} channel may not be good option to target interfibrillar mitochondria/subsarcolemmal mitochondria against ischemia-reperfusion injury.

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Ethical Issue

All the animal experimental protocols were approved by Institutional Animal Ethical Committee of SASIRA University, Thanjavur, Tamilnadu. Experiments were done in accordance with the guidelines of Control and Supervision of Experiments on Animals, Chennai, India (CPCSEA Approval No.: 347/SASIRA/IAEC/RPP).

Conflict of Interest

Authors declare no conflicts of interest

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