

Liver ischemia preconditions the heart against ischemia-reperfusion arrhythmias

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ABSTRACT

Objective(s): This study aimed to examine the hypothesis that an antiarrhythmic effect might be obtained by ischemic preconditioning of the liver, and also to characterize the potential underlying mechanisms.

Materials and Methods: Male Wistar rats were anesthetized by thiopental sodium (50 mg/kg, IP) followed by IV injection of heparin (250 IU). Remote ischemic preconditioning (RIPC) was induced by 3 cycles of 5 min liver ischemia followed by 5 min of reperfusion. The hearts were excised within 5 min after the final cycle of preconditioning and perfused using Langendorff's system. The isolated perfused hearts were subjected to 30 min global ischemia followed by 90 min reperfusion. The myocardial arrhythmias induced by ischemia-reperfusion (I/R) were determined in accordance with the guidelines of Lambeth Conventions. The potential role of K_{ATP} channels on RIPC was assessed by injection of glibenclamide (nonselective K_{ATP} blocker) or 5-hydroxydecanoate (mitochondrial K_{ATP} blocker) on rats 30 and 15 min before induction of RIPC in the liver, respectively.

Results: Hepatic remote preconditioning of the heart significantly ($P<0.0001$) prevented the incidence of myocardial arrhythmias induced by I/R in the perfused hearts (5.33 ± 1.54 vs. 32.33 ± 6.44). However, the protective effects of remote preconditioning was significantly ($P<0.01$) abolished by the K_{ATP} blocker, glibenclamide (25.5 ± 4.9 vs. 5.33 ± 1.54).

Conclusion: Hepatic RIPC may prevent the arrhythmias induced by I/R in the isolated perfused hearts via K_{ATP} channels.

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Introduction

Severe ventricular arrhythmias represent a major challenge for therapeutic intervention due to complexity of pathophysiological mechanisms initiating arrhythmias in the ischemic heart disease (1), so new strategies are urgently needed to prevent and manage the condition. In the ischemia-reperfusion (I/R) condition, the imbalance between the production of reactive oxygen species (ROS) and the availability of endogenous antioxidants plays an important role in the genesis of myocardial injury (2-4). This can result in malignant I/R-induced arrhythmias (5, 6). Deleterious effect of an oxidative load has been shown by the ability of exogenous free radical scavengers to improve functional recovery of the post-ischemic reperfused heart (7-9). Different approaches have been developed to prevent myocardial arrhythmias induced by I/R in the heart. It has also been shown that myocardial I/R injury can be dramatically reduced by subjecting the heart

to one or more episodes of non-lethal myocardial I/R prior to the sustained coronary artery occlusion (10). This endogenous cardioprotective phenomenon termed as ischemic preconditioning (IPC) exerts different beneficial effects including decreased myocardial damage (11-14), improvement of functional recovery (15), and antiarrhythmic effects (16-18). However, the IPC is an invasive procedure being applied directly to the heart tissues in order to obtain myocardial protection. This treatment in some clinical settings can be impractical and may cause harmful effects. An alternative and more compliant strategy is to apply the cardioprotective stimulus to an organ or tissue far from the heart (19). The classical preconditioning at a distance in the heart itself is induced by a brief occlusion of one coronary artery followed by prolonged occlusion of other coronary artery (20). This approach entitled as remote ischemic preconditioning (RIPC) can dramatically prevent the myocardial injury induced by I/R (21, 22).

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Although the exact molecular mechanisms responsible for cardioprotection afforded by IPC are unclear, the results of some studies suggest that ATP-sensitive K^+ (K_{ATP}) channels can be the key players in this process (23-25). Opening of sarcolemmal K_{ATP} channels has been initially proposed as an end-effector mechanism in the IPC cascade. Glibenclamide, a K_{ATP} channel blocker, has been able to abolish the IPC protection in dogs (26). Moreover, it has been reported that K_{ATP} openers can also mimic the IPC-induced protection (23, 27). However, there is evidence that K_{ATP} channel modulators may exert both anti- and pro-arrhythmic effects (28), though other reports suggested that the cardioprotection may occur independent of any alteration in the action potential duration as the main determinant of arrhythmogenesis and a target for sarcolemmal K_{ATP} openers (29). It has been shown that 5-hydroxydecanoate (5-HD), a selective mitochondrial K_{ATP} (mito K_{ATP}) inhibitor, abolished protection against contractile dysfunction in guinea pig papillary muscle conferred by hypoxic PC (30). However, a cardioprotection has been induced by IPC (31) in rabbits and rats without affecting the shortened action potential (32). Garlid *et al* reported that mitochondrial (mito) K_{ATP} channel is 2000-fold more sensitive than the sarcolemmal one to the K_{ATP} opener diazoxide, which is the most likely end-effector involving in IPC (33). It seems that the opening of K_{ATP} channel not only plays as the end-effector in the preconditioning cascade, but also induces an upstream mechanism of protein kinase activation (34). It has been suggested that some underlying pathways and subsequent signal transductions activated in remotely preconditioned cardiomyocytes may be similar to those recruited in IPC procedure (35). Steen *et al* have reported that the cardioprotection induced by RIPC is exerted via mito K_{ATP} channels (36), but the mechanism of the antiarrhythmic protection induced by mito K_{ATP} opening remains still unclear.

The cardioprotective effect of RIPC is under intensive investigations and many unsolved problems remains to be examined. There is no evidence whether a protection against the myocardial arrhythmias induced by I/R could be obtained by preceding previous brief ischemic episodes of the liver as RIPC for the heart. Therefore, the present study was conducted to examine the hypothesis that hepatic RIPC can induce antiarrhythmic effects on I/R-exposed isolated hearts through the opening of K_{ATP} channels.

Materials and Methods

Animals

Male Wistar rats (250 to 300 g) were purchased and housed in standard conditions (12 hr light/day cycle with 20 to 22°C temperature and 40 to 50% humidity). The animals had access to commercial

chow and water *ad libitum*. The project had prior approval from the Institutional Animal Care and Use Committee, and all procedure conducted on the animal was in accordance with the guidelines described by the Helsinki Declaration, as revised in Edinburgh 2000.

The surgery for hepatic ischemic preconditioning and isolated rat heart preparations

The animals were anesthetized by thiopental sodium (50 mg/kg, IP) and then they received heparin (250 IU) from femoral artery (37). The ventral midline abdomen of the animals was excised, and the liver was exposed by disconnecting its connective ligaments to the abdominal wall. The hepatic RIPC was induced by intermittent portal triad clamping for 5 min episodes (38). The heart was isolated immediately after the final episode of hepatic ischemia, and then perfused using Langendorff's setup. To achieve this, the heart was removed; the aorta was cannulated for retrograde perfusion at a constant flow rate of 10 ml/min with a non-recirculating Krebs-Henseleit buffer solution (KHB). The KHB solution contained (mmol/l) NaCl 118.0, NaHCO_3 25.0, KCl 4.7, KH_2PO_4 1.2, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1.2, CaCl_2 1.25, and glucose 11.0, which was saturated with a mixture of 95% O_2 and 5% CO_2 and kept at pH =7.4 and 37°C. A global ischemia was induced by occlusion of the KHB inflow (30 min) followed by reperfusion for 90 min (37). To monitor the occurrences of I/R induced arrhythmias, two stainless steel electrodes were connected to the apex and right atrium of the heart (39). The coronary perfusion pressure (CPP) was measured through a three-way stop cock using a pressure transducer (MLT844 Physiological Pressure Transducer, ADInstruments). Left ventricular pressure was measured by an elastic water-filled balloon inserted into the left ventricle via the left atrium (adjusted to obtain end-diastolic pressure of 10 mmHg) and connected to a pressure transducer (MLT844 Physiological Pressure Transducer, AD Instruments) (40).

Experimental protocol

Rats were randomly divided into eight experimental groups of six rats in each. In group I chosen as sham-operated, the isolated heart was perfused for a period of 140 min. In group II (I/R), the isolated heart was exposed to 30 min ischemia followed by 90 min reperfusion. The procedure in group III (control) was the same as I/R group except that a sham operation was performed on the liver for RIPC. Group IV (treatment) underwent a hepatic RIPC by 3 cycles of 5 min occlusion of the portal triad prior to myocardial I/R. The rats in group V were treated the same as the group III but received 0.3 mg/kg glibenclamide (Gliben) through IV injection (36) 30 min prior to the sham operation on the liver. The procedure in group VI designated as 5-

hydroxydecanoate (5-HD) treated control group, was the same as in group III but the rats were treated with 5 mg/kg of IV 5-HD (41) 15 min before sham operation on the liver. Group VII (Gliben treated treatment group) received 0.3 mg/kg Gliben through IV 30 min before induction of RIPC in the liver, and

the remaining protocol was the same as described in group IV. In group VIII labeled as 5-HD treated treatment group, the rats were administered 5-HD (5 mg/kg, IV) 15 min before induction of RIPC, and then the procedure was performed the same as described in group IV.

Table 1. Pre-ischemic and post-ischemic values of hemodynamic parameters in different groups of isolated perfused rat hearts. The ischemia-reperfusion was induced in the isolated heart by 30 min ischemia followed by 90 min reperfusion, the remote ischemic preconditioning was induced by 3 cycles of 5 min liver ischemia followed by 5 min of reperfusion, and the K_{ATP} channel blocker was injected into the animal 30 and 15 min before induction of remote ischemic preconditioning

	Preischemia	End of ischemia	Reperfusion time (min)				
			2	15	30	60	90
HR							
Sham	216.8±4.1	209.7±11.9	211.5±8.1	212.2±7.5	208.3±9.3	197.7±10.6	193±13.4
Ischemia/Reperfusion	247.5±11.4	^a 0	246.2±13.4	242.2±12.5	235.6±16.6	214.47±7.2	214.5±6.3
Control	238.9±11.3	^a 0	236.6±17.8	217.19±13.7	210.8±17.4	214.7±5.9	203.2±4.87
Treatment	249.3±5.8	^a 0	260.7±17.04	232.4±16.2	241.1±10.1	232.2±7.3	226.7±11.3
Control (Gliben)	240.46±11.4	^a 0	239.21±13.4	235.2±12.5	228.55±16.6	207.41±7.2	207.51±6.3
Control (5-HD)	249.00±13.4	^a 0	226.63±12.6	225.93±9.8	227.6±12.6	220.96±14.7	205±4.8
Treatment (Gliben)	235.80±12.5	^a 0	239.06±11.8	224.21±15.8	209.60±9.5	211.00±9.6	207.35±9.5
Treatment (5-HD)	254.6±8.6	^a 0	239.14±15.7	228.80±12.2	215.16±7.1	216.18±9.9	215.33±11.4
CPP							
Sham	90.5±5	88.75±6.5	90.96±6.1	82.09±9.8	89.02±7.8	86.72±7.8	85.22±7.9
Ischemia/Reperfusion	94.66±1.8	^a 0	71.02±4.5	77.09±6.7	77.71±4.7	71.56±3.5	65.4±1.2
control	76.42±4.3	^a 0	61.47±7.6	54.47±4.2	55.64±6.7	50.94±8.1	47.42±6.2
Treatment	92.69±3.6	^a 0	87.66±3.4	98.08±6.5	97.68±2.7	94.03±3.3	91.62±5.2
Control (Glibenclamide)	51.38±2.6	^a 0	50.36±2.3	48.95±1.9	53.12±3.3	50.61±3.1	50.03±3.0
Control (5-HD)	58.62±2.5	^a 0	55.33±1.3	53.49±1.8	55.95±3.8	53.93±2.0	50.95±2.9
Treatment (Glibenclamide)	49.97±2.7	^a 0	50.61±1.75	47.7±1.62	46.16±1.8	48.9±2.5	48.98±2.5
Treatment (5-HD)	53.17±4.0	^a 0	48.25±1.8	46.10±2.7	45.17±2.1	47.58±2.8	49.47±1.4
Max dp/dt							
Sham	2620±213	2707±186	2676± 238	2527±303	2567±270	2553±316	2476±366
Ischemia/Reperfusion	2835±88	^a 25±0.6	^a 1917±41	^b 2022±94	^b 2111±131	^{ab} 1843±71	^{ab} 1825±79
Control	2254±157	^a 26±0.1	^a 1976±171	^{ab} 1863±204	^b 1983±169	^{ab} 1651±153	^{ab} 1653±178
Treatment	2806±127	^a 26±0.1	2353±71	3125±185	3286±30	3373±81	3204±158
Control (Glibenclamide)	3161±157	^a 26±0.6	2147±41	^b 2252±94	^b 2341±131	2040±66b	^b 1988±42
Control (5-HD)	2667±199	^a 26±0.2	2007±76	^b 2140±36	^b 2096±146	^b 1982±85	^b 1938±120
Treatment (Glibenclamide)	2390±206	^a 26±0.1	1783±170	^b 2097±253	^b 2057±173	^b 1976±235	^b 1908±162
Treatment (5-HD)	2851±147	^a 26±0.05	1971±99	^b 2364±79	^b 2383±45	^b 2146±98	^b 2138±51

Data are means±SEM, n = 6 in each group, HR – heart rate (beats/min), CPP– coronary perfusion pressure (ml min⁻¹), Max dp/dt (mmHg/s), a=P<0.0001vs. sham, b=P<0.05 vs. test

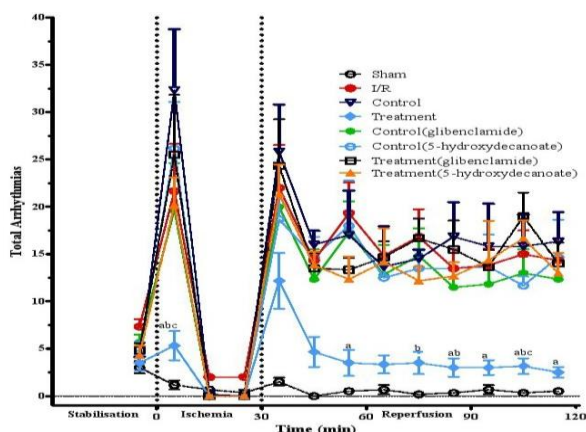


Figure 1. Effects of hepatic remote ischemic preconditioning and K_{ATP} channel blockers on the ischemia-reperfusion (I/R) -induced total arrhythmias in the isolated perfused rat heart. Values are expressed as means \pm S.E.M. of 6 isolated hearts per group in which a= $P<0.05$ vs. control, b= $P<0.05$ vs. treatment (glibenclamide), c= $P<0.05$ vs. treatment (5-hydroxydecanoate)

Measurement and classification of ventricular arrhythmias

Ventricular arrhythmias induced by I/R in the isolated perfused rat hearts were monitored and analyzed using the data obtained from ECG recordings. Identification and classification of the I/R-induced arrhythmias in the experimental groups was based on the Lambeth Convention (15). Ventricular premature beats (VPBs) were characterized by the presence of QRS complexes not preceded by P waves. Single isolated VPBs were defined as singles, whereas two or three consecutive VPBs were considered as salvos, and four or more consecutive VPBs were defined as ventricular tachycardia. Consistent with the Lambeth Convention, ventricular fibrillation was defined as a signal for which individual QRS complexes cannot be distinguished from each other.

Statistical analysis

Data analysis and drawing of the graphs were performed using GraphPad Prism Software v5.0 (GraphPad Software, USA). Data were expressed as mean \pm SEM obtained from at least six isolated perfused rat hearts in each experimental group. Statistical comparisons between different experimental groups were performed using analysis of variance (ANOVA) followed by Bonferroni's *Post-hoc* test to compare the differences of means. A *P*-value less than 0.05 were considered as significant.

Results

Characteristics of isolated hearts

The values for different hemodynamic parameters in terms of heart rate, coronary perfusion pressure and max dp/dt in different experimental groups are shown in Table 1. As the table shows, there were no significant

differences among the groups in the hemodynamic values before the induction of ischemia.

Effects of hepatic RIPC on post-ischemic recovery of myocardial contractile dysfunction

There was a significant post-ischemic contractile dysfunction in the isolated perfused hearts exposed to I/R as determined by changes in the level of max dp/dt. However, the RIPC markedly ($P<0.0001$) attenuated post-ischemic contractile dysfunction by increasing max dp/dt recovery from 1653 ± 178 (in the control) to 3204 ± 158 (in the treatment group). Pretreatment of test animals with glibenclamide and 5-HD did not affect max dp/dt recovery in non-preconditioned hearts, but reversed the cardioprotective effect induced by RIPC in the Gliben and 5-HD treated treatment groups. As table 1 shows, the levels of max dp/dt at 90 min of reperfusion was significantly ($P<0.05$) declined from 3204 ± 158 (in the treatment group) to 1908 ± 162 and 2138 ± 51 in the Gliben and 5-HD treated groups, respectively.

Effect of remote ischemic preconditioning, glibenand 5-HD on susceptibility to ventricular arrhythmias

The myocardial injury induced by I/R significantly ($P<0.05$) caused incidence of arrhythmia in the control and I/R groups, and the maximal occurrences was shown during 0 and 10 min of ischemia and at the beginning of reperfusion (Figure 1). The rates of single arrhythmias were also significantly ($P<0.0001$) higher in the I/R and control groups compared to the sham-operated group. However, there was no significant difference between the treatment and the sham groups. The application of RIPC significantly ($P<0.0001$) reduced single arrhythmias from 14.67 ± 2.9 in the control group to 2.33 ± 0.61 in the treatment group. Glibenclamide and

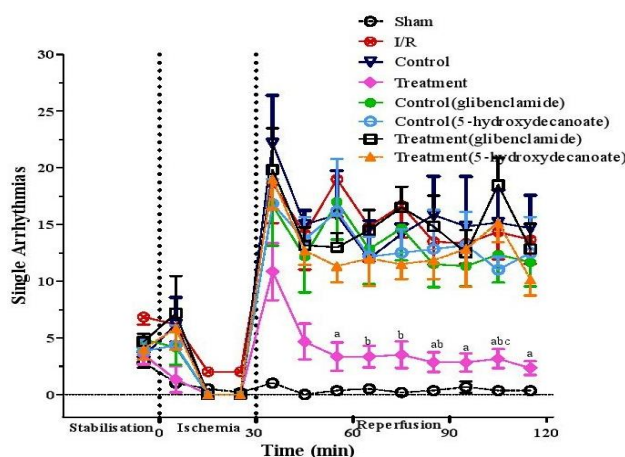


Figure 2. Effects of hepatic remote ischemic preconditioning and K_{ATP} channel blockers on ischemia-reperfusion (I/R) -induced single arrhythmias in the isolated perfused rat heart. Values are expressed as means \pm SEM of 6 hearts per group in which a= $P<0.05$ vs. control, b= $P<0.05$ vs. treatment (glibenclamide), c= $P<0.05$ vs. treatment (5-hydroxydecanoate)

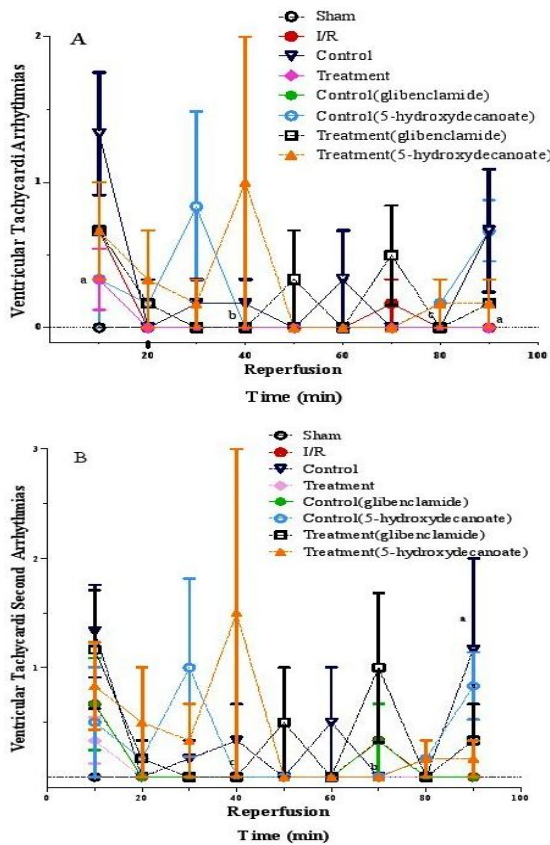


Figure 3. Effects of hepatic remote ischemic preconditioning and K_{ATP} channel blockers on ischemia-reperfusion (I/R)-induced ventricular arrhythmias in the isolated perfused rat heart. A: Number of episodes of ventricular tachycardia. B: Duration of ventricular tachycardia. VT-ventricular tachycardia. Values are expressed as means \pm S.E.M. of 6 hearts per group in which a= $P < 0.05$ vs. control, b= $P < 0.05$ vs. treatment (glibenclamide), c= $P < 0.05$ vs. treatment(5-hydroxydecanoate)

5-HD significantly ($P < 0.01$) abolished the antiarrhythmic effect induced by hepatic RIPC (18.5 ± 2.40 and 15.17 ± 1.72 vs. 3.16 ± 0.83 in treatment). However, administration of these K_{ATP} channel blockers in the non-preconditioned hearts exposed to I/R had no effect on the incidence of single arrhythmias (Figure 2). Gliben or 5-HD in the control groups did not significantly change the incidence of salvo in explanted hearts.

Ventricular tachyarrhythmia (tachycardia and fibrillation) was the most severe form of arrhythmia that occurred in all isolated hearts exposed to I/R. Marked attenuation of arrhythmias was observed in the preconditioned hearts. However, pretreatment with Gliben or 5-HD in preconditioned hearts reversed the improvement of ventricular tachyarrhythmia induced by hepatic RIPC (Figures 3, 4). The hepatic RIPC significantly ($P < 0.01$) decreased the mean number of the episodes of VT from 1.33 ± 0.42 to 0.33 ± 0.21 , and also shortened the total duration of VT (0.64 ± 0.33 vs. 2.3 ± 0.4 s in the control, Figure 3). As Figures 4A and 4B show, the exposure of rat to RIPC significantly ($P < 0.0001$) reduced the number of episodes of VF and its total duration

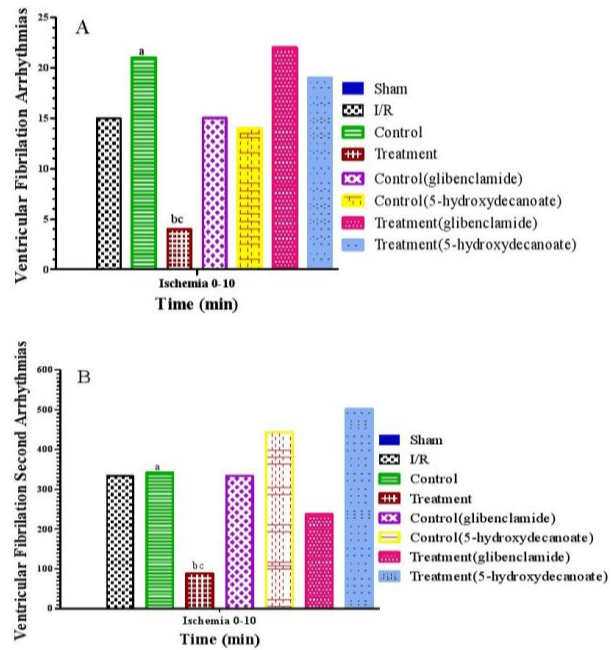


Figure 4. Effects of hepatic remote ischemic preconditioning and K_{ATP} channel blockers on ischemia-reperfusion (I/R)-induced ventricular arrhythmias in the isolated perfused rat heart. A: Number of episodes of ventricular fibrillation. B: Duration of ventricular fibrillation. VF-ventricular fibrillation. Values are expressed as means \pm S.E.M. of 6 hearts per group in which a= $P < 0.0001$ vs. treatment, b= $P < 0.001$ vs. treatment (glibenclamide), c= $P < 0.001$ vs. treatment (5-hydroxydecanoate)

compared to the control groups. The maximal effect of RIPC was shown between 0 and 10 min of ischemia. The total numbers of arrhythmias was also significantly ($P < 0.0001$) reduced in the isolated perfused hearts previously preconditioned by liver ischemia (5.33 ± 1.54 vs. 32.33 ± 6.44 in the control, Figure 1). Although pretreatment with glibenclamide or 5-HD in non-preconditioned hearts did not change the total number of arrhythmias, blockade of K_{ATP} channels before preconditioning exacerbated arrhythmias and partially attenuated the effect of RIPC. This has been shown by increased incidence of VT in treated hearts (81%) compared to the non-treated preconditioned hearts ($P < 0.01$, Figure 3). In addition, blockade of non-selective K_{ATP} and mito K_{ATP} channels significantly ($P < 0.0001$) increased the number of episodes of VF from 4.1 ± 0.40 to 18.5 ± 4.53 and 14.33 ± 3.40 in gliben and 5-HD treatment groups, respectively (Figure 4A).

Discussion

The present study was conducted to examine the hypothesis that brief episodes of ischemia in the liver as a remote preconditioning might cause an antiarrhythmic effect against myocardial I/R injury in isolated perfused hearts and also to characterize the potential mechanism of this protective procedure. The common definition of IPC is used for a procedure in which a cardioprotective effect can be

obtained by induction of one or several episodes of ischemia in the heart against the damage induced by a subsequent prolonged period of cardiac ischemia (10). It is known that an episode (usually 3 to 5 min) of regional ischemia induced by the coronary ligation either in the intact animal or in the isolated heart is protective against the deleterious electrophysiological, biochemical, and mechanical effects of a longer ischemia within the same region of the heart (35, 13). The induction of ischemia at a distance in the heart itself by ligation of a different coronary artery has been reported to be cardioprotective (42). Such a protective effect of RIPC in general may be mediated by different endogenous substances including adenosine and calcitonin-gene related peptide released from tissues into the blood that subsequently affect the organ in danger at a distance (43-45). The antiarrhythmic effect is one of the cardioprotective outcomes of ischemic preconditioning that is well documented in various species using different experimental models (16, 18). Tatyana *et al* reported that brief ischemia of an extremity as a non-invasive preconditioning of the heart can be protective against reperfusion-tachyarrhythmia (46). Consistently, Heidi *et al* demonstrated that induction of RIPC during coronary occlusion increased the ventricular arrhythmia threshold in conscious rats (47). Thus, in the present work, we used a model of the explanted heart in which protection is manifested by attenuation of I/R-induced arrhythmias to explore the possibility of obtaining the antiarrhythmic effect using RIPC of the liver.

In the present study, we found that RIPC of liver can improve the profile of arrhythmias as it significantly reduced total incidences of arrhythmias and the occurrence and the total duration of VT. In addition, cardiac performance including Max dp/dt was improved by hepatic RIPC. There is evidence that different factors including the size of the occluded zone and the changes in the heart rate may influence the occurrences of arrhythmias (48, 49). However, in the method used in the present study, the impact of the influencing factors have been excluded by exposing the experimental groups to a similar method of global ischemia, and there were no differences in the size of ischemic area between the groups correlating with the reduction of coronary flow or the heart rate (50). These findings are in agreement with the findings of other investigators (24, 46) who demonstrated an arrhythmias reduction in the preconditioned rats and rabbits.

The attenuation of spatial dispersion of repolarization between the epi- and endocardial layers of the myocardium as a substrate for reentry arrhythmias induced by ischemia has been suggested as the physiological mechanisms underlying antiarrhythmic effect induced by RIPC (51). However, the role of K_{ATP} channels in the

antiarrhythmic effects is still a matter of dispute. In the present study, we showed the involvement of K_{ATP} channels in the protection against I/R-induced arrhythmias by application of glibenclamide as a non-selective K_{ATP} blocker and 5-HD as a selective mito K_{ATP} blocker. It was found that the use of these K_{ATP} channel inhibitors before the preconditioning phase, attenuated the antiarrhythmic effect induced by RIPC and increased the incidence of ventricular tachycardia. These results are consistent with those of Munch-Ellingsen *et al* and Véggh and Parratt studies who found that 5-HD reduced the cardioprotection in the preconditioned rats and dogs (25, 32).

K_{ATP} channel activation may be attributed to the release of reactive oxygen species (ROS) during the preconditioning (52). It has been proposed that Superoxide (O_2^-) ROS generated during IPC may activate mito K_{ATP} channels through direct action on the sulfhydryl groups of the channel proteins subsequently leading to the opening of K_{ATP} channels (53). Kazuaki *et al* have reported that hepatocyte protection was mediated through ROS generation by Kupffer cells after IPC (54). This is in agreement with the study of Matejikova *et al* who reported that myocardial IPC were associated with a temporal moderate increase in generation of ROS prior to sustained ischemia (17). There is also evidence that cardioprotective effect of IPC and diazoxide may be manifested during the phase of prolonged ischemia by reduction of ROS generation and mobilization of antioxidant reserves (54-56). The use of diazoxide and preconditioning is found to be associated with an improved mitochondrial recovery after I/R injury (58), and that myocardial IPC attenuated ROS production at the end of sustained ischemia (17). There is also evidence that hepatic ischemic preconditioning is able to prevent excessive ROS generation and subsequent injury induced by IR in the liver and lung (59, 60). It seems that opening of the mito K_{ATP} channel occurs upstream of the mitochondrial activities and the generation of ROS in the protective pathway. Since this protection mechanism is abolished by two free radical scavengers, 2-mercapto-propionylglycine and n-acetyl cysteine (53, 61-63).

The activation of K_{ATP} channels as a final step in the cardioprotective signaling mechanisms has been supported by the findings that activated protein kinase C (PKC) phosphorylates sarcolemmal K_{ATP} channels (64), and that NO, PKC and mitogen-activated protein kinase (MAPK)-mediated mechanisms facilitate the opening of mito K_{ATP} channels (65, 66). It is possible that the opening of a K_{ATP} channels may act as both a trigger and a mediator of preconditioning (67, 36). Different mechanisms that may be involved in this process include depolarization of the mitochondrial inner

membrane in conjunction with limitation of calcium uptake by mitochondria (68), regulation of mitochondrial volume and rate of respiration (69) and modulation of ROS production (67). The regulation of antiapoptotic proteins in the mitochondria has been proposed as another potential mechanism of attenuation of cell death induced by opening of mito K_{ATP} channels (70).

Conclusion

This study showed that short episodes of liver ischemia followed by reperfusion caused significant cardioprotective effect against the I/R- induced arrhythmias in the isolated rat heart similar to the preconditioning of the heart with regional ischemia in the heart itself. In an attempt to characterize the potential mechanism of this endogenous protective procedure, it was found that the protection process is dependent on the mito K_{ATP} channels activities. These findings provide evidence that the short episodes of liver ischemia can precondition the heart against the I/R- induced arrhythmias.

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