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Ischemic preconditioning decreases the reperfusion-related formation of hydroxyl radicals in a rabbit model of regional myocardial ischemia and reperfusion: The role of K_{ATP} channels

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Abstract

The objective of this study was to assess the effects of ischemic preconditioning (IP) on hydroxyl free radical production in an *in vivo* rabbit model of regional ischemia and reperfusion. Another goal was to determine whether K_{ATP} channels are involved in these effects.

The hearts of anesthetized and mechanically ventilated New Zealand White rabbits were exposed through a left thoracotomy. After IV salicylate (100 mg/kg) administration, all animals underwent a 30-min stabilization period followed by 40 min of regional ischemia and 2 h of reperfusion. In the IP group, IP was elicited by 5 min of ischemia followed by 10 min of reperfusion (prior to the 40-min ischemia period). Glibenclamide, a K_{ATP} channel blocker, was administered prior to the preconditioning stimulus. Infarct size was measured by 2,3,5-triphenyl tetrazolium chloride (TTC) staining. We quantified the hydroxyl-mediated conversion of salicylate to its 2,3 and 2,5-dihydroxybenzoate derivatives during reperfusion by high performance liquid chromatography coupled with electro-chemical detection.

IP was evidenced by reduced infarct size compared to control animals: 22% vs. 58%, respectively. Glibenclamide inhibited this cardioprotective effect and infarct size was 53%. IP limited the increase in 2,3 and 2,5-dihydroxybenzoic acid to 24.3 and 23.8% above baseline, respectively. Glibenclamide abrogated this effect and the increase in 2,3 and 2,5-dihydroxybenzoic acid was 94.3 and 85% above baseline levels, respectively, similar to the increase in the control group. We demonstrated that IP decreased the formation of hydroxyl radicals during reperfusion. The fact that glibenclamide inhibited this effect, indicates that K_{ATP} channels play a key role in this cardioprotective effect of IP.

Keywords: *Heart, myocardium, ischemic preconditioning, oxygen free radicals, ATP sensitive potassium channels*

Introduction

Repeated brief episodes of myocardial ischemia protect the myocardium against subsequent prolonged ischemic insults. This phenomenon has been termed ischemic preconditioning (IP), and has been described in various animal models, as well as humans [1–3]. However, the mechanism that activates the intrinsic effectors which protect intracellular structures following preconditioning is not yet completely understood.

Occlusion of a major branch of a coronary artery is followed by physiological and metabolic changes that appear within seconds after cessation of coronary flow mainly characterized by a shift of energy metabolism from aerobic to anaerobic glycolysis [4].

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Because the demand of the myocytes for energy exceeds the supply provided by anaerobic glycolysis and the reserves of high-energy phosphates, tissue adenosine triphosphate (ATP) decreases and adenosine diphosphate (ADP) begins to accumulate. Restoration of blood flow to ischemic myocytes results in restoration of aerobic metabolism and salvage of the ischemic myocardium [5]. However, restoring blood flow to the ischemic myocardium may also cause tissue damage, thus further injuring the affected myocardium [6]. Bolli showed that 50–70% of the reperfusion injury is caused by a burst of oxygen-derived free radicals liberated during the first few minutes of reperfusion [7]. These free radicals damage the myocyte and thereby cause much of the "stunning" effect. "Stunned" myocardium is a myocardium which has been rendered ischemic, but not irreversibly damaged [8]. It appears as a prolonged depression of regional myocardial function, which continues long after restoration of blood flow and reappearance of a normal electrocardiogram.

The exact changes that lead to myocyte dysfunction during reperfusion are unknown. At present, alterations in calcium homeostasis, and the release of oxygen free radicals are considered key events in the pathogenesis of reperfusion injury [6]. Upon reperfusion, as a consequence of mitochondrial electron transport chain dysfunction [9], molecular oxygen undergoes sequential reduction to form reactive species of oxygen, including hydrogen peroxide, superoxide and the hydroxyl radical. Hydroxyl radicals (·OH) are extremely reactive and can oxidize and damage many cellular components [10]. At high concentrations, these highly reactive oxidants can induce peroxidation of membranes and membrane proteins involved in ion transport, thus altering their integrity, affecting cellular ionic homeostasis and leading to depressed cardiac function and myocyte death [11]. Vanden Hoek *et al.* [12] demonstrated that IP in ventricular embryonic chick cardiomyocytes is cardioprotective by attenuating oxidant stress at reperfusion. Zhai *et al.* [13] and Zhou *et al.* [14] used rat cardiomyocytes and demonstrated that IP might confer protection 24 h after preconditioning by decreasing superoxide levels.

There is evidence that oxygen free radicals play a crucial role in the triggering mechanism of myocardial preconditioning because myocardial protective effects were abolished when reactive oxygen species scavengers were administered during preconditioning pulses of ischemia [15,16]. On the other hand, attenuation of oxidant stress during reperfusion may decrease the damage caused by ischemia and reperfusion [17]. However, to our knowledge, there are no reports of *in-vivo* evaluation of the effect of IP on oxygen free radical production during reperfusion using an intact heart.

The initial objective of this study was to determine whether IP would limit hydroxyl oxygen free radical production and release during reperfusion in an *in situ* rabbit model. We used an experimental model, in which ·OH radicals react with salicylate and generate 2,3 and 2,5-dihydroxybenzoic acids (DHBAs). These can be measured by high performance liquid chromatography-electrochemical detector [18]. The formation of DHBAs after systemic administration of salicylate is used as an index of ·OH generation in the heart.

The ATP sensitive potassium (K_{ATP}) channels have been shown to be an important mediator in the process of IP [19]. Kersten *et al.* [20] and Gross *et al.* [21] have shown that the protective effect of myocardial preconditioning is mediated via activation of the K_{ATP} channels. Therefore, the second aim of this study was to examine whether attenuation of hydroxyl radicals generation by IP is also a K_{ATP} channel-mediated process. To elucidate the possible role of K_{ATP} channels in the cardioprotective effect of IP, the effect of the sulfonylurea K_{ATP} channel blocker, glibenclamide, on hydroxyl radical production was examined.

Materials and methods

All experiments were conducted following the approval of the Institutional Committee for Animal Care and Laboratory Use, and in compliance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institute of Health (NIH publication No. 85–23, revised 1996).

General preparation

New Zealand white rabbits weighing 2.5–3.5 kg were initially premedicated with an intramuscular injection of a ketamine (50 mg/ml)/xylasine (10 mg/ml) solution at a volume of 0.6 ml/kg body weight. They were then anesthetized with intravenous thiopental (10 mg/kg) administered via a 20-g catheter in a marginal ear vein. Anesthesia was maintained during the experiment by 10 mg thiopental supplements as needed (according to the eyelash reflex). The neck was opened with a ventral midline incision and a tracheostomy performed. The rabbits' lungs were mechanically ventilated with positive pressure ventilation using 100% oxygen. Ventilation was adjusted to maintain the blood pH within the physiologic range. End tidal carbon dioxide tension was continuously monitored. A 22-g catheter filled with heparinized saline was placed in a carotid artery for blood pressure monitoring and blood sampling. Core body temperature was measured via a rectal temperature probe and maintained at 38.5°C (normothermic for rabbits) with radiant heat and a warming blanket. Three lead electrocardiogram was continuously recorded. A left thoracotomy was performed in the fourth intercostal space to expose the heart, and the pericardium

was opened. A 4-0 silk suture was passed around a prominent branch of the left coronary artery with a tapered needle and the ends of the suture were threaded through a small vinyl tube to form a snare. Coronary artery occlusion was achieved by tightening the snare around the coronary artery. Myocardial ischemia was confirmed by regional epicardial cyanosis and ST-segment elevations in the electrocardiogram. Reperfusion was achieved by releasing the snare and was confirmed by visual observation of reactive hyperemia.

Experimental protocol

All animals were given intravenously salicylate (100 mg/kg) at the beginning of the experiment. Salicylate is a highly effective hydroxyl free radical scavenger, which upon scavenging ·OH, forms 2,3 and 2,5-DHBA by hydroxylation. After 30 min of stabilization, all animals underwent 40 min of regional ischemia followed by 2 h of reperfusion. Preconditioning was elicited by 5 min of coronary occlusion followed by 10 min of reperfusion, beginning 15 min prior to the period of prolonged coronary occlusion. Rabbits were randomly assigned to one of the following groups: Control group (ischemia and reperfusion without further intervention) (C, $n = 10$); ischemic preconditioning (IP, $n = 10$); IP + 0.05 mg/kg of intravenous glibenclamide (IP + G, $n = 10$). In order to rule out the possibility that glibenclamide itself may influence the generation of reactive oxygen species during reperfusion, another control group in which glibenclamide was administered prior to ischemia and reperfusion but without preconditioning treatment was also included (C + G, $n = 6$). Glibenclamide was administered 10 min prior to the 5 min of coronary occlusion or 40 min of coronary occlusion in groups IP + G and C + G, respectively (Figure 1). In a third control group ($n = 6$) glibenclamide was given to sham-operated rabbits to rule out any possible direct effect of this agent on the baseline levels and production of reactive oxygen species in non-ischemic hearts (data not shown). Blood pressure, heart rate and temperature were recorded continuously. Blood samples for salicylate and hydroxyl free radical measurements and blood glucose levels were obtained as follows: At the beginning of the experiment (baseline), every 2 min for the first 10 min of the prolonged reperfusion and thereafter every 10 min (up to 30 min of reperfusion). The samples were then kept at −80°C until analyzed. In addition, in groups IP and IP + G blood samples for salicylate and hydroxyl radical levels were also collected at the end of the first coronary occlusion and after 5 and 10 min during the first reperfusion period. The yield of DHBA derivatives depends on both the flux of the generated hydroxyl radicals and the concentration of salicylate. The values of DHBA derivatives were normalized to

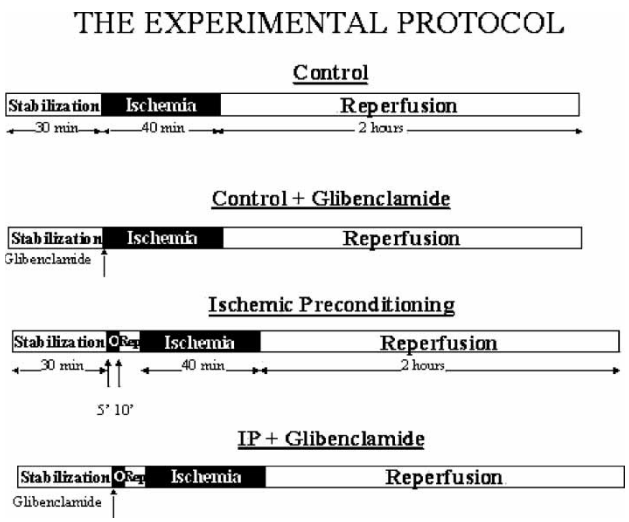

Figure 1. Diagram of the experimental protocol. *O*—occlusion; Rep—reperfusion. Glibenclamide (0.05 mg/kg) was administered 10 min prior to preconditioning.

account for the difference in salicylate concentration at different time points. Variations in salicylate concentration are attributed to its metabolism and renal clearance. The concentrations of hydroxyl radicals generated in the heart are expressed as the ratio between DHBA (ng) and salicylate (μg).

Determination of infarct size and area at risk

At the end of the experimental protocol, hearts were excised, mounted on a Langendorff apparatus, and perfused with phosphate buffered saline at 100 cm H_2O for 1 min in order to wash out intravascular blood. The coronary artery was re-occluded and 0.1% methylene blue was infused into the aortic root to label the normally perfused zone with deep blue color, thereby delineating the risk zone as a non-stained area. The hearts were then removed from the Langendorff apparatus, trimmed of atria and great vessels, weighed, and frozen (in a cold chamber with a temperature of −18°C). Hearts were then cut into 2 mm transverse slices. The slices were incubated in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) in pH 7.4 buffer for 20 min at 37°C. The slices were then placed in 10% neutral buffered formalin for 10 min to increase the contrast between stained and non-stained tissue. Since TTC stains viable tissue a deep red color, nonstained tissue was presumed to be infarcted. Slices were then photographed, and risk and infarct areas in each slice were measured by computed planimetry. The mass-weighted average of the ratio of infarct area to the area at risk of the ventricle from each slice was determined (percent infarction).

Quantification of hydroxyl radicals by salicylate

DHBA levels were identified and measured by high performance liquid chromatography coupled with

electrochemical detection using a Varian 5000 liquid chromatograph (Varian Medical Systems, Palo Alto, CA), equipped with a Rheodyne 7125 sample injector (20 µl loop) (Rheodyne LLC, Rohnert Park, CA). The column used for separation of salicylate and DHBA was a 25 cm × 4 mm Li Chrospher 100 RP-18, 5 µm (E-Merck, Darmstadt, Germany). The mobile phase contained 0.03 M citric acid, 0.03 M acetic acid, 0.2 g/l sodium azide and 2 × methanol. The mobile phase was titrated with solid NaOH to pH 3, followed by titration with CH_3COONa to a final pH of 3.6. The flow rate was 1 ml/min. The system was equipped with two detectors in series. Salicylate was identified and measured fluorimetrically using a FD-300 model fluorescence detector (Spectrovision, Chelmsford, MA) employing excitation and emission wave lengths of 300 and 412 nm, respectively. DHBA derivatives were quantified using an electrochemical amperometric detector (Model 4A, Bioanalytical Systems, West Lafayette, IN), with a plastic cell equipped with a glass carbon electrode operated at +0.80 V, using an Ag/AgCl reference electrode. The signals from the detector were acquired on an EZ Chrome data acquisition and handling system and subsequently processed.

Data analysis and statistics

Data are expressed as mean ± SEM. Hemodynamics over time within each group were analyzed using analysis of variance (ANOVA) with Dunnett's *post hoc* testing for multiple comparisons. Differences in hemodynamics and infarct size between groups were analyzed using ANOVA with the Tukey *post hoc* test. Incidence of ventricular fibrillation was analyzed with Kruskal-Wallis test. Differences in DHBA concentrations between groups were assessed by ANOVA with Student-Newman-Keuls *post hoc* testing. Significance level was set at $p < 0.05$.

Results

A total of 42 rabbits were studied. The results from 35 animals contributed to the final data set. The remaining seven animals were excluded because of technical failures: Accidental release of the snare during the ischemia period ($n = 2$) or ventricular fibrillation ($n = 5$). The incidence of refractory ventricular fibrillation was not significantly different among groups (2/10 C, 1/10 IP, 2/10 IP + G; 0/6 C + G, 0/6 sham operated animals $p = 0.35$ with Kruskal-Wallis test).

Mean arterial pressure was not significantly different among groups at any time point. There was a trend for mean arterial pressure to decrease over time within all groups. Heart rate was similar in all groups, as was the rate–pressure product, thus differences in myocardial infarct size between groups could not be attributed to decrease in blood pressure or differences in heart rates. Hemodynamic data are presented in Figure 2. No hypoglycemia was noted and the differences in blood glucose concentrations between the groups were not significant.

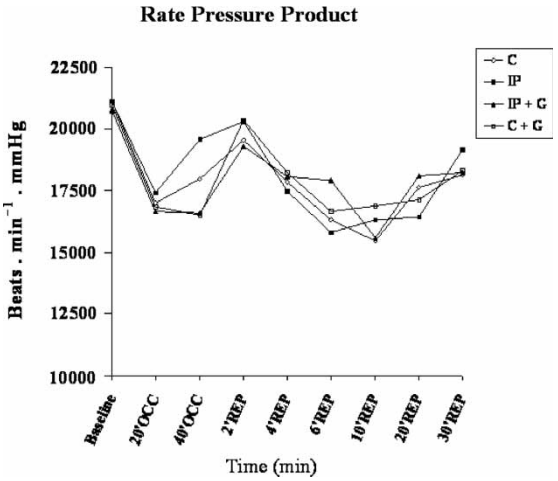

Figure 2. Rate-pressure product. There was no significant difference between the groups for any time point. Data are the means. SE bars were omitted to improve clarity. *C*—control; IP—ischemic preconditioning; IP + *G*—ischemic preconditioning + glibenclamide; *C* + *G*—control + glibenclamide.

Inhibition of the cardioprotective effect of IP on infarct size by pretreatment with glibenclamide

The area at risk to left ventricular mass ratio (AR/LV) did not differ significantly among the groups (51 ± 2% in the control group, 54 ± 3% in the IP group, 47 ± 4% in the IP + G group, 48 ± 3% in the C + G group, p = NS) (Figure 3). These data suggest that changes in the infarct sizes observed in the various experimental groups can not be related to the percentage of the left ventricular myocardium that was occluded.

In the control group (C) the measured infarct size was 58 ± 4% of the area at risk. IP had a cardioprotective effect demonstrated by a reduction of the infarct size to 22 ± 5% of the area at risk ($P < 0.01$). Administration of glibenclamide prior to IP abolished the protective effect of IP in regard to infarct size reduction. The measured infarct size in group IP + G was 53 ± 5% of the area at risk ($P < 0.01$, compared to the IP group and not significantly different compared to group C) (Figure 3). The measured infarct size in group C + G was 55 ± 3% of the area at risk (not significant compared to group C), thus indicating that glibenclamide itself has no direct influence on infarct size (Figure 3).

Changes in the generation of hydroxyl radicals during IP and after administration of glibenclamide

In the IP and IP + G groups the level of 2,3 and 2,5-DHBA did not increase significantly after 5 min

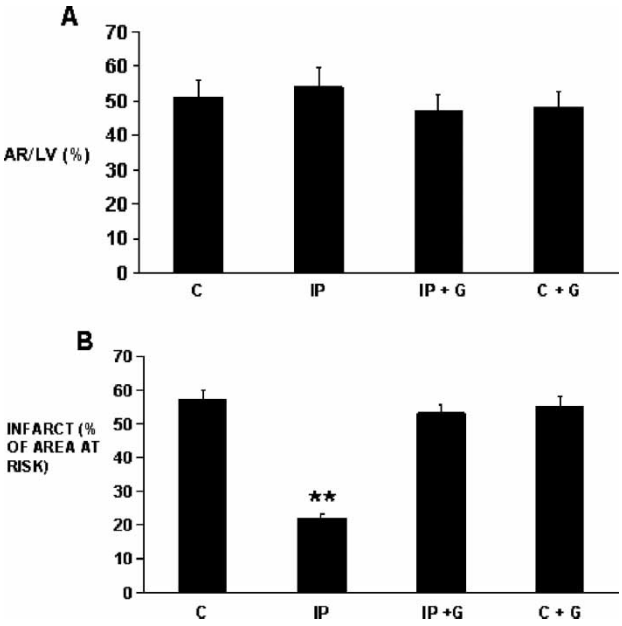

Figure 3. Bar graphs showing the area at risk as percentage of the left ventricle mass (AR/LV; A) and infarct size as percentage of the area at risk (B). Ischemic preconditioning significantly reduced infarct size as compared to control animals, whereas the administration of glibenclamide abolished this protective effect and infarct size is similar to that of the controls. Data are mean ± SEM. *C*—control; IP—ischemic preconditioning; IP + *G*—ischemic preconditioning + glibenclamide; *C* + *G*—control + glibenclamide $^{**}P < 0.01$.

of index ischemia during the preconditioning period (12 and 9%, respectively compared to baseline values in group IP, [P = NS] and 8 and 5% in group IP + G, respectively [P = NS]). After 5 min of reperfusion following the preconditioning stimulus there was an increase of 29% ($P < 0.05$) above baseline in 2,3-DHBA. A further 36% increase ($P < 0.05$) above baseline levels was observed after 10 min, at the end of the first reperfusion period. The level of 2,5-DHBA increased by 26% compared to baseline values ($P < 0.05$) at 5 min of reperfusion and increased further to 32% ($P < 0.05$) above baseline values after 10 min. Administration of glibenclamide attenuated the increase in hydroxyl radical levels in group IP + G during the preconditioning period: The measured normalized values of 2,3-DHBA were 11% (P = NS) and 14%(P = NS) above baseline levels, after 5 and 10 min of the first reperfusion period, respectively. Furthermore, as was observed with 2,3-DHBA, glibenclamide attenuated the increase in the concentration of 2,5-DHBA during preconditioning: At 5 min of reperfusion the measured value of 2,5-DHBA was only 11% (P = NS) above baseline levels and at 10 min 12% (P = NS) above the baseline values.

An acute increase of 50% compared to baseline values ($P < 0.05$) in normalized 2,3-DHBA in the control group was observed after 2 min of reperfusion. Following 10 min of reperfusion, the peak value was measured (81.3% increase compared to baseline levels; $P < 0.01$) (Figure 4). After 10 min of reperfusion there was only a 24.3% increase in the concentration of 2,3-DHBA in the IP group ($P < 0.01$, compared to control group). However, the addition of glibenclamide, prior to IP, reversed this effect and the hydroxyl radical production increased to a degree similar to that seen in the control group (increase of 94.3%, P = NS compared to group C). In group C + G there was an increase of 87.9% in the concentration of the 2,3 derivative (P = NS compared to group C), thus ruling out any possible effect of glibenclamide itself on the generation of hydroxyl radicals. Maximal production of 2,5-DHBA occurred in the control group after 4 min of reperfusion: A 74.5% increase compared to baseline values, ($P < 0.01$) (Figure 5). IP, however, significantly attenuated this increase to only 23.8% above baseline values ($P < 0.01$, compared to group C). Glibenclamide inhibited the preconditioning effect and the increase in 2,5-DHBA (81.7%) was comparable to that of the control group (P = NS). As with 2,3-DHBA, glibenclamide itself had no effect on the production of 2,5-DHBA. After 4 min of reperfusion there was an increase of 78.2% in the level of this derivative compared to baseline levels. This increase was similar to that of the control group (P = NS).

At all time points during reperfusion (up to 30 min), there was a significant difference between the control group and the preconditioning group (Figures 4 and 5). At 60 min of reperfusion and afterwards, values returned to baseline in all groups. In the sham operated

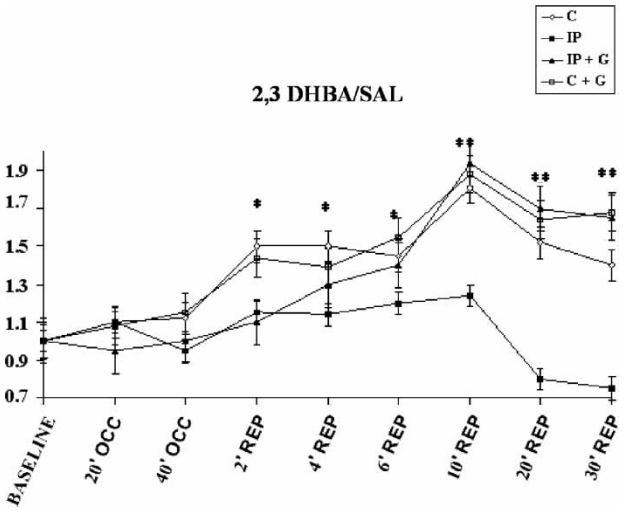

Figure 4. Mean normalized concentrations of 2,3-dihydroxybenzoic acids (DHBA) (ng DHBA/μg salicylate) in the blood of rabbits exposed to 40 min of regional ischemia (occlusion of a prominent branch of the left coronary artery) and 120 min of reperfusion. An asterisk denotes a significant difference between control (*C*, $n = 8$), ischemic preconditioning (IP, $n = 9$), ischemic preconditioning + glibenclamide (IP + *G*, $n = 8$), or control + glibenclamide (*C* + *G*, $n = 6$) groups. Data are presented as mean ± SEM $^{**}P < 0.01$ $^{*}P < 0.05$.

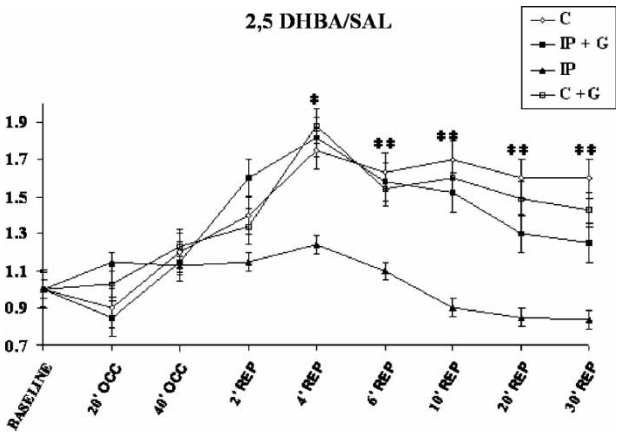

Figure 5. Mean normalized concentrations of 2,5-dihydroxybenzoic acids (DHBA) (ng DHBA/μg salicylate) in the blood of rabbits exposed to 40 min of regional ischemia (occlusion of a prominent branch of the left coronary artery) and 120 min of reperfusion. An asterisk denotes a significant difference between control (C, $n = 8$), ischemic preconditioning (IP, $n = 9$), ischemic preconditioning + glibenclamide (IP + G, $n = 8$), or control + glibenclamide ($C + G$, $n = 6$) groups. Data are presented as mean ± SEM $^{**}P < 0.01$ $^{*}P < 0.05$.

animals there was no significant difference in the concentration of the DHBA derivatives compared to baseline levels in all time points (data not shown).

Discussion

Using an *in-vivo* rabbit model of regional myocardial ischemia and reperfusion, IP significantly decreased the production of hydroxyl radicals during reperfusion and decreased myocardial infarction size. Interestingly, IP induced a short transient increase in the generation of the hydroxyl radicals during reperfusion at the preconditioning period. This data is in agreement with other investigations that demonstrated an increase in reactive oxygen species during the preconditioning period [12,16]. Administration of glibenclamide attenuated the increase in the hydroxyl radicals level at preconditioning, suggesting that opening of the K_{ATP} channels is an upstream event thus releasing free radicals during preconditioning [22]. Furthermore, glibenclamide abolished the cardioprotective effect of IP as indicated by the increased infarct size in group IP + G.

These findings are in agreement with several reports that suggested that IP protects the myocardium by decreasing oxidative stress. Vanden Hoek and co-workers [12] in their isolated cardiomyocyte model, showed that oxygen free radicals caused increased fluorescence of 2′,7′-dichlorofluorescin diacetate with a peak value after 10 min of reperfusion. Others demonstrated that IP activated magnesium superoxide dismutase activity during the early phase of protection [13,14]. However, these studies were performed in cardiomyocytes because it is difficult to monitor transient changes in hydroxyl radical marker production in intact organs. In the present study, the method to monitor free radical-related events *in vivo* involves chemical trapping of oxygen free radicals as they are formed in the tissues [23]. The formation of DHBA after systemic administration of salicylate is used as an index of ·OH generation in the heart [18].

Similar results were recently presented by Kevin and colleagues [24]. Using spectrophotoflurometry at the left ventricle wall of guinea pig isolated hearts the intracellular production of superoxide was continuously measured and the findings demonstrated that IP reduced the generation of superoxide compared to nonpreconditioned animals. This reduction in oxidant stress reduced post-ischemic myocardial dysfunction and limited infarct size in the preconditioning group. Our results confirm and further extend the observations of Kevin et al. We used an *in-vivo* model of regional myocardial ischemia and reperfusion and not an isolated perfused heart model to demonstrate that the reduction of the post-ischemic formation and release of hydroxyl radicals is cardioprotective. Furthermore, our data indicate that this effect on the generation of free radicals is mediated by K_{ATP} channels.

There is a time difference between the peak concentration of the two DHBA derivatives (10 and 4 min after reperfusion in 2,3 and 2,5-DHBA, respectively). One possible explanation for this difference could be the way these derivatives are formed: 2,3-DHBA is released from myocytes after activation of the complement system and the inflammatory cascade, whereas 2,5 DHBA is generated mainly via activation of the cytochrome P450 system [25,26].

Free radicals disrupt membranes and cause calcium overload with resultant structural damage both in the myocytes and coronary endothelium leading to decreased myocardial contractility and a markedly increased coronary vascular resistance [6]. Therefore, the early myocardial protection provided by IP may be due in part to its ability to attenuate ·OH formation and release.

The adenosine triphosphate potassium (K_{ATP}) channels have been shown to be involved in the mechanism of IP [19,27]. Recent publications emphasize the pivotal role of the mitochondrial, rather than the membranal K_{ATP} channels in mediating preconditioning [27,28].

In the present study, glibenclamide (a K_{ATP} channel blocker) completely inhibited the beneficial effect of IP on infarct size reduction and on hydroxyl radical production during reperfusion. These findings suggest that K_{ATP} channels are involved in IP-mediated attenuation of hydroxyl radical release and the decrease in the extent of myocardial infarction. Glibenclamide, a sulfonylurea compound, is known to inhibit K_{ATP} channels both in the sarcolemmal membrane of cardiomyocytes [29,30]

and the inner membrane of mitochondria [31]. Jung and coworkers [32] showed that HMR1883, a selective sarcolemmal membrane K_{ATP} channels blocker, did not abolish the beneficial effect of IP on myocardial infarct size, supporting the hypothesis that mitochondrial K_{ATP} channels are involved in IP. However, Sanada et al [33] demonstrated in a canine model that both sarcolemmal and mitochondrial K_{ATP} channels contributed to the cardioprotective effect of IP. It seems that the K_{ATP} channel is not the end effector of IP, but rather functions as a trigger of the preconditioned state [34]. Several investigators showed that opening of mitochondrial K_{ATP} channels generated minute amounts of oxygen free radicals that triggered a preconditioned state and activation of kinases if the heart becomes ischemic again [35,36]. IP seems to have a dual effect on free radical production: Short ischemia causes opening of mitochondrial K_{ATP} channels and generates low levels of oxygen free radicals. The latter in turn initiates the rest of the IP sequence [24]. Therefore, the generation of small amounts of free radicals during a short ischemic episode is not sufficient to cause cell necrosis but enough to activate cellular mechanisms that induce preconditioning effects [34]. It is possible that glibenclamide prevented the cardioprotective effect of IP by blocking the K_{ATP} channels, thus attenuating the transient increase in free radicals during the preconditioning period and inhibiting the following preconditioning cascade. In contrast to their beneficial effect in triggering preconditioning, numerous studies indicate that free radicals play a detrimental and major role in the pathogenesis of reperfusion injury [6–9].

Not all studies on free radicals are in agreement with respect to a reduction in infarct size, nor do they all support the concept of reperfusion injury. Uraizee and colleagues [37] and Gallagher and coworkers [38], were unable to demonstrate a reduction in myocardial infarct size with superoxide dismutase and catalase in the canine heart subjected to regional myocardial ischemia and reperfusion. Reasons for the divergent outcomes in the canine heart are not readily apparent, but have been attributed to undocumented differences in collateral blood flow between control and treatment groups, imprecise determinations of myocardial infarction and, most importantly, the wide variation of collateral blood flow in the canine heart.

In the present study, IP significantly decreased post-ischemic production of hydroxyl radicals and attenuated the extent of myocardial infarction. This effect was inhibited by a K_{ATP} channel blocker indicating that it was due to opening of this channel. Our results are supported by several studies that demonstrated that reduction in the generation or release of oxygen free radicals during reperfusion had a protective effect on the heart regarding postischemic contractile dysfunction [39] and myocardial infarction [26,40].

The current results must be interpreted within the constraints of a potential limitation: Myocardial infarct size is determined primarily by the size of the area at risk and extent of coronary collateral perfusion. The area at risk, expressed as a percentage of total LV mass, was similar between groups in the current investigation. Rabbits have also been shown to possess little if any coronary collateral blood flow [41,42], thus, it appears unlikely that differences in collateral perfusion between groups account for the observed results. However, coronary collateral blood flow was not specifically quantified in the current investigation.

In conclusion, this study demonstrates that IP reduces the formation of post-ischemic hydroxyl radicals and protects the heart against ischemia. Furthermore, K_{ATP} channels are involved in triggering the beneficial effects of IP. The present results may have clinical implications, however, further investigations are essential to safely apply these findings.

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