Effects of Ischemic and Sevoflurane-induced Preconditioning on Myocardial Infarction and Arrhythmias in Rabbits In Vivo

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Abstract

Objectives: The present study aimed to investigate whether ischemic or sevoflurane-induced preconditioning exerts infarct size limiting effects and depresses ischemia-reperfusion arrhythmias through opening of mitochondrial KATP channels in rabbits in vivo.

Methods: Rabbits anesthetized with ketamine and xylazine given intramuscularly underwent 30 min of left anterior descending coronary artery (LAD) occlusion followed by 3 hrs of reperfusion. Before this, rabbits were randomized into one of five groups. Control rabbits received no intervention before 30 min LAD occlusion and 3 h reperfusion (Group-C). The ischemia-preconditioned (IP) rabbits underwent 5 min LAD occlusion followed by 10 min of reperfusion before prolonged ischemia-reperfusion (Group-IP). In the sevoflurane (S)-preconditioned group, 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout before prolonged ischemia-reperfusion (Group-S). The selective mitochondrial K_{ATP} channel blocker, 5-hydroxy-decanoate (5-HD, 5 mg/kg) was given intravenously 10 min before ischemic preconditioning and sevoflurane exposure, respectively (Group-5-HD-IP, Group-5-HD-S). An electrocardiogram was recorded throughout the experiment via lead 2 of the standard electrocardiogram. At the end of the 3 hrs reperfusion period, area at risk (R) and infarct size (I) were measured.

Results: RPP decreased in Group-S-HD-IP and Group-S-HD-S compared with Group-S at 30 min after ischemia. The ratio of R to left ventricular mass showed no significant difference among all groups. I/R values of each group were 51.6 ± 3.0% in Group-C, 33.3 ± 4.7% in Group-IP, 36.6 ± 4.8% in Group-S, 48.9 ± 5.2% in Group-5-HD-IP, 54.8 ± 4.2% in Group-5-HD-S. There was no significant difference in duration of arrhythmias during myocardial ischemia and reperfusion among 5 groups.

Conclusions: Ischemic preconditioning and sevoflurane-induced preconditioning exert infarct size limiting effects through opening of mitochondrial K_{ATP} channels. However, ischemic preconditioning and sevoflurane-induced preconditioning do not have antiarrhythmic effects. This suggests that the opening of mitochondrial K_{ATP} channels does not cause antiarrhythmic effects.

Keywords: Arrhythmia; Ischemia/reperfusion injury; 5-Hydroxy-decanoate; Preconditioning

Introduction

Lethal injury to the heart can be dramatically blunted by brief conditioning periods of ischemia and reperfusion: a phenomenon called ischemic preconditioning [1,2]. And brief exposure to a volatile anesthetic agent such as isoflurane [3] and sevoflurane [4,5] also protects the heart against subsequent ischemia–reperfusion injury. This phenomenon has been recognized as anesthetic preconditioning [6]. To date, numerous studies have investigated the mechanism of cardiac preconditioning, and many crucial components underlying cardioprotection have been identified. Adenosine triphosphate-sensitive potassium (K_{ATP}) channels have long been considered as essential components of cardioprotection by both ischemic and anesthetic preconditioning [7,8]. There are two populations of K_{ATP} channels in cardiac myocytes: the mitochondrial (mitoK_{ATP}) channel located in the inner mitochondrial membrane and the sarcosomal (sarc K_{ATP}) channel located in the plasma membrane [9]. A role for mitochondrial K_{ATP} channels in ischemic preconditioning has been suggested [10]. As for the role for mitochondrial K_{ATP} channels in anesthetic preconditioning, our laboratory demonstrated that the protective effects by sevoflurane as well as brief ischemia may be mediated by mitochondrial rather than sarcosomal K_{ATP} channel opening [4].

Although the term “preconditioning” is currently employed primarily to describe a limitation of infarct size, subsequent studies [6,11] have demonstrated that preconditioning can in some models, afford protection against ischemia/reperfusion- induced arrhythmias. It remains to be determined, however, if antiarrhythmic protection is a direct consequence of anti-ischemic protection and if the mechanisms involved are the same as those involved in protection against infarction. Also, it is little known whether ischemic preconditioning and anesthetic preconditioning have antiarrhythmic effects or not, and whether K_{ATP} channels are related to arrhythmias during acute myocardial ischemia and reperfusion especially in rabbit hearts.

The present study, therefore, aimed to investigate whether ischemic or sevoflurane-induced preconditioning exerts infarct size limiting effects and depresses ischemia-reperfusion arrhythmias through opening of mitochondrial K_{ATP} channels in rabbits in vivo.
Materials and Methods

The present study was performed in accordance with the Guidelines of Animal Care and Use Committee of Kanagawa Dental University.

General surgical preparation

Male New Zealand White rabbits weighing 2.7-3.2 kg were allowed ad libitum access to standard laboratory stock diet and water. Animals were initially anesthetized with ketamine (35 mg/kg) and xylazine (5 mg/kg) given intramuscularly. Tracheotomy was performed and rabbits were intubated with an uncuffed endotracheal tube (ID 3.5 mm). The animals were ventilated with room air supplemented with additional oxygen.Inspired and expired anesthetic concentration, inspiratory O₂ percentage and end-tidal CO₂ partial pressures were continuously monitored using a multigas anesthetic monitor (Datex, Capnomac, Helsinki, Finland). The respiratory rate was frequently adjusted to maintain P_O₂ greater than 100 mm Hg, P_CO₂ at 35-45 mm Hg, pH 7.35-7.45, and Base Excess between -3 and +3. After the left jugular vein was exposed and cannulated with a polyethylene catheter, 0.9% sodium chloride (0.15 ml/min) was continually administered during the experiments. The carotid artery was dissected out and fluid-filled polyethylene tube was placed in it and connected immediately to an electrocardiogram monitor (Nihon-kohden Co, Life scope 11, Tokyo, Japan) via pressure transducer (Nihon-kohden Co, TP-400T, Tokyo, Japan) for arterial pressure recording. An electrocardiogram was recorded throughout the experiment via lead II of the standard electrocardiogram. Ischemia or reperfusion-induced arrhythmias included premature ventricular contraction (PVC) and ventricular tachycardia (VT). Left thoracotomy was performed and the pericardium was opened to expose the heart. A silk thread (K-890H, Ethicon, Somerville, NJ) with taper C-1 needle was passed around the left anterior descending artery (LAD) and the end of the tie were occluded by pulling the snare, which was then fixed by clamping the thread through a small vinyl tube to form a snare. The LAD was occluded by pulling the snare, which was then fixed by clamping the tube with a mosquito hemostat. Myocardial ischemia was confirmed by regional cyanosis, ST segment elevation and decreased blood pressure.

Study groups and experimental protocol

Figure 1 presents study groups and experimental protocol. Anesthesia was maintained with ketamine and xylazine solution (ketamine 35 mg/kg/hr, xylazine 5 mg/kg/hr I.m.; k/x) with room air supplemented with additional pure oxygen. After all the surgical procedures had been performed, a 15 min period was allowed for stabilization. All anesthetized open-chest rabbits underwent 30 min of left anterior descending coronary artery (LAD) occlusion followed by 3 hrs of reperfusion. Before this, the animals were randomized into one of the following experimental protocols:

Control rabbits received no intervention before 30 min LAD occlusion and 3 hrs reperfusion (Group-C; n=9). The ischemia preconditioned (IP) rabbits underwent 5 min LAD occlusion followed by 10 min of reperfusion before prolonged ischemia-reperfusion (Group-IP; n=10). In the sevoflurane (S)-preconditioned group, 30 min of sevoflurane exposure at a 1.5% end-tidal concentration was followed by 15 min of washout before prolonged ischemia-reperfusion (Group-S, n=6). The selective mitochondrial K⁺ channel blocker, 5-hydroxy-decanoate (5-HD, 5 mg/kg) was given intravenously 10 min before ischemic preconditioning and sevoflurane exposure, respectively (Group-5-HD-IP, n=6, Group-5-HD-S, n=6). At the end of the 3 hrs reperfusion period, area at risk and infarct size were measured.

Hemodynamic measurements

Hemodynamic measurements included systolic, diastolic, mean arterial blood pressures and heart rate. Rate pressure product was calculated as the product of heart rate and peak arterial pressure. Baseline hemodynamic measurements were taken prior to any experimental manipulations. Subsequently, the measurements were taken at 29 min of ischemia and 30, 60 and 180 min of reperfusion.

Determination of area at risk and infarct size

Following completion of experimental protocol, the in vivo visualization of the myocardium at risk was accomplished with reocclusion of the coronary artery and injection of 10% Evans blue into the venous cannula until the eyes turned blue. The Evans blue was allowed to circulate for about 30 sec to demarcate the risk and non-risk regions. The hearts were quickly excised under deep anesthesia and frozen. The frozen hearts were then cut into six transverse slices of equal thickness. The area at risk was determined by negative staining with Evans blue. The slices were stained by incubation for 15 min in 1% triphenyl tetrazolium chloride (TTC) in isotonic pH 7.4 phosphate buffer. After staining, the sections were placed in formalin for preservation, and measurements of risk area, infarct area and left ventricle were made with computer aided morphometry. From each section, the ischemic area at risk (unstained by blue dye) and the infarcted area (unstained by TTC) were outlined and measured by planimetry. The area from each region was averaged from the slices. Infarct size was expressed both as a percentage of total left ventricular mass (L) and as a percentage of the ischemic risk area. This method has generally been found to be an accurate measure of ultimate infarct size at 2 to 48 hr of reperfusion when compared with subsequent histologic analysis [12,13].

Rabbits which could not be survived until the end of experiments with lethal arrhythmias such as ventricular fibrillation (VF) have excluded from the data analysis and not considered further.

Statistical Analysis

Statistical comparisons of individual hemodynamic parameters between groups were made using one-way ANOVA followed by Fisher’s protected least significant difference. Bartlett’s test for equality of variances was used to ensure the validity of statistical comparison using the one-way ANOVA. Statistical comparisons of infarct size/area
Results

Rate pressure product (RPP) is shown in Table 1. RPP decreased in Group-5-HD-IP and Group-5-HD-S compared with Group-S at 30 min after ischemia. The ratio of areas at risk (R) to left ventricular mass (L) ranged from 54.0 ± 4.9% to 66.1 ± 5.3% with no significant difference among all the groups (Figure 2). Figure 3 shows the infarct size (I) expressed as percentage of area at risk (R) in five groups. I/R values of each group were 51.6 ± 3.0% in Group-C, 33.3 ± 4.7% in Group-IP, 48.9 ± 5.2% in Group-5-HD-IP, 54.8 ± 4.2% in Group-5-HD-S. The mean infarct sizes in Group-IP and Group-S were significantly smaller than that of Group-C. Infarct size in Group-IP was significantly smaller compared with that of Group-5-HD-IP. The mean infarct sizes in Group-IP and Group-S were significantly smaller than that of Group-5-HD-S. The duration of arrhythmias during myocardial ischemia were 91.4 ± 20.4 sec in Group-IP, 151.0 ± 41.4 sec in Group-5-HD-S (Figure 4). The duration of arrhythmias during reperfusion were 200.5 ± 39.1 sec in Group-C, 127.4 ± 41.4 sec in Group-IP, 150.1 ± 35.7 sec in Group-S, 153.0 ± 94.9 sec in Group-5-HD-IP, 80.5 ± 38.3 sec in Group-5-HD-S (Figure 5). There was no significant difference in duration of arrhythmias during myocardial ischemia and reperfusion among 5 groups.

Discussion

In the present study, both ischemic and sevoflurane-induced preconditioning also reduced infarct size, and 5-HD, an effective blocker of mitochondrial K_{ATP} channels abolished infarct size limiting effect. Our previous study [14] in the same experimental system and other studies [15-17] have demonstrated that 5-HD alone caused no significant changes in myocardial infarct size and hemodynamics. These results and observations suggest that both ischemic and sevoflurane-induced preconditioning exerted infarct size limiting effect through opening of mitochondrial K_{ATP} channels. Thus, the cardioprotection by ischemic and sevoflurane-induced preconditioning may be mediated, at least in part, by mitochondrial K_{ATP} channel. However, ischemic preconditioning and sevoflurane-induced preconditioning did not have anti-arrhythmic effects. In this experiment, ischemia may result in increased potassium ion (K') efflux and accumulation of extracellular K', and leads to produce arrhythmias. The mechanisms underlying reperfusion-induced arrhythmias seem to be different from those responsible for the induction of ischemia-induced arrhythmias. These include oxygen free radicals and increased levels of intracellular calcium [18].

Protection of ischemic preconditioning against arrhythmias is somewhat controversial and varies dependent on species [19]. It was reported that ischemic preconditioning decreased incidence of arrhythmias in in vivo rats [20-22] and dogs [11,23]. Also, it was suggested that the protective effect of ischemic preconditioning against arrhythmias is not likely to be involved in activation of K_{ATP} potassium channels during ischemia [20]. On the contrary, in in vivo dogs, possible mechanism of antiarrhythmic action by ischemic preconditioning is related to opening of mito K_{ATP} channels [22,23].

In the rabbit model, in which collateral flow is negligible [24], protection of ischemic preconditioning against arrhythmias was not observed. And as far as we have investigated, there have been no references that ischemic preconditioning has antiarrhythmic effect during ischemia and reperfusion in in vivo rabbit hearts.

As for anesthetic preconditioning, Kevin and Novalija suggested that isoflurane-induced preconditioning reduced the incidence of arrhythmia in in vitro rat hearts [6].

In this study, sevoflurane-induced preconditioning did not reduce ischemia-reperfusion arrhythmias. It may be due to the differences of species, length of ischemia and reperfusion, the choice of anesthetics, timing and concentration of anesthetics exposure and extent of collateral blood flow.

It has been reported that pretreatment with nicorandil and pinacidil, both K_{ATP} channel openers administered just prior to coronary occlusion resulted in protection against ischemia/reperfusion-induced arrhythmias in anesthetized rats due to activation of cardiomyocyte mitochondrial K_{ATP} channels. K_{ATP} channel openers may shorten the action potential duration, thereby reducing cellular calcium overload.
Antiarrhythmic protection must be the manifestation of a preservation of ionic homeostasis, whereas the essential requirements for protection against necrosis may be different. Indeed, from many of the studies, it is clear that it is possible to protect against necrosis without the prevention of arrhythmias and vice versa [26].

In the present investigation, though ischemic and sevoflurane-induced preconditioning reduced infarct size through opening of mitochondrial K\textsubscript{ATP} channels, it offered no protection against arrhythmias during ischemia and reperfusion.

From these observations, unlike nricorandil and pinacidil, ischemic preconditioning and this dose of sevoflurane might not open K\textsubscript{ATP} channel enough to exert antiarrhythmic action.

In summary, the present study indicates that ischemic preconditioning and sevoflurane-induced preconditioning exert infarct size limiting effects through opening of mitochondrial K\textsubscript{ATP} channels. However, ischemic preconditioning and sevoflurane-induced preconditioning do not have antiarrhythmic effects.

This suggests that the opening of mitochondrial K\textsubscript{ATP} channels by ischemic preconditioning and sevoflurane-induced preconditioning may not be enough to cause antiarrhythmic effects in in vivo rabbit hearts even though there is the common mechanism responsible for both infarct limiting and antiarrhythmic effects.

References


and preserving viability in ischemic/reperfused myocardium, which was initially proposed as the mechanism for protection of ischemic myocardium [25].


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