Effects of sevoflurane preconditioning and postconditioning on rat myocardial stunning in ischemic reperfusion injury*

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Abstract: Ischemic preconditioning and postconditioning distinctly attenuate ventricular arrhythmia after ischemia without affecting the severity of myocardial stunning. Therefore, we report the effects of sevoflurane preconditioning and postconditioning on stunned myocardium in isolated rat hearts. Isolated rat hearts were underwent 20 min of global ischemia and 40 min of reperfusion. After an equilibration period (20 min), the hearts in the preconditioning group were exposed to sevoflurane for 5 min and next washout for 5 min before ischemia. Hearts in the sevoflurane postconditioning group underwent equilibration and ischemia, followed immediately by sevoflurane exposure for the first 5 min of reperfusion. The control group received no treatment before and after ischemia. Left ventricular pressure, heart rate, coronary flow, electrocardiogram, and tissue histology were measured as variables of ventricular function and cellular injury, respectively. There was no significant difference in the duration of reperfusion ventricular arrhythmias between control and sevoflurane preconditioning group (P=0.195). The duration of reperfusion ventricular arrhythmias in the sevoflurane postconditioning group was significantly shorter than that in the other two groups (P<0.05). ±(dP/dt)max in the sevoflurane preconditioning group at 5, 10, 15, 20, and 30 min after reperfusion was significantly higher than that in the control group (P<0.05), and there were no significant differences at 40 min after reperfusion among the three groups (P>0.05). As expected, for a 20-min general ischemia, infarct size in heart slices determined by 2,3,5-triphenyltetrazolium chloride staining among the groups was not obvious. Sevoflurane postconditioning reduces reperfusion arrhythmias without affecting the severity of myocardial stunning. In contrast, sevoflurane preconditioning has no beneficial effects on reperfusion arrhythmias, but it is in favor of improving ventricular function and recovering myocardial stunning. Sevoflurane preconditioning and postconditioning may be useful for correcting the stunned myocardium.

Key words: Inhalation anesthetics, Sevoflurane, Postconditioning, Preconditioning, Ischemia-reperfusion injury, Myocardial stunning
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1 Introduction

Anesthetic preconditioning or postconditioning whereby the heart is exposed to a volatile anesthetic before or after prolonged ischemia, exerts a cardioprotective effect (Obal et al., 2005; Feng et al., 2008; Weber and Schlack, 2008), which has triggered an increasing interest in both basic science and clinical research and may ultimately cause an impact on anesthesia practice in patients of cardiac risk. Up to now, most studies have demonstrated similar efficacies on anesthetic postconditioning and ischemic postconditioning in protecting the myocardium (Wang et al.,
Postischemia, a reversible contractile dysfunction termed as myocardial stunning, is a significant phenomenon after cardiac surgery. Transient myocardial ischemia followed by reperfusion may lead to myocardial stunning. As for the stunned myocardium independent of myocardial infarction, it has been reported that ischemic preconditioning (Hagar et al., 1991) and ischemic postconditioning (Kloner et al., 2006; Sasaki et al., 2007; Dow et al., 2008; 2009) markedly attenuate ventricular arrhythmia after ischemia and prevent the recurrence of arrhythmia, and may be useful for correcting the stunned myocardium. Furthermore, the antiarrhythmic protection conferred by ischemic postconditioning is also present in old rats (Dow et al., 2008). The mechanisms have not been studied extensively. One study showed that this protective effect of ischemic preconditioning was not likely to be related to alterations in high-energy phosphate compounds (Hagar et al., 1991). In addition, the mechanism by which ischemic postconditioning reduced reperfusion-induced ventricular arrhythmias in the stunned myocardium was independent of known pathways including adenosine, mitochondrial K$_\text{ATP}$ channel, mitochondrial permeability transition pore, and PI3K pathways (Dow et al., 2009). However, the effects of anesthetic preconditioning or postconditioning on myocardial stunning remain unclear.

With these issues in mind, we investigated the effects of sevoflurane preconditioning and postconditioning on myocardial stunning in isolated rat hearts independent of myocardial infarction.

## 2 Materials and methods

Male Sprague-Dawley rats (200–250 g) were obtained from the Animal Center of Zhejiang University, Hangzhou, Zhejiang, China. Animals were handled in accordance with the principles of laboratory animal care and all experimental procedures were approved by the Research Commission for the Care and Use of Laboratory Animals of Zhejiang University.

### 2.1 Perfusion of isolated rat hearts

Rats were anaesthetized (pentobarbital sodium, 60 mg/kg, i.p.) and the hearts were excised rapidly, placed in ice-cold Krebs-Henseleit (K-H) buffer, mounted on a Langendorff apparatus, and perfused at 37 °C with K-H buffer (Stehr et al., 2007). Perfusion pressure was constant at 75 mmHg. The buffer containing (mmol/L) NaCl 118.0, KCl 4.7, CaCl$_2$ 1.25, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, NaHCO$_3$ 25.0, and glucose 11.0 was equilibrated with 95% O$_2$/5% CO$_2$ (pH 7.39±0.1). Hearts were subjected to global ischemia by stopping the K-H buffer perfusion, while reperfusion was achieved by restarting the perfusion.

Sevoflurane (Abbott Laboratories, Chuoku, Osaka, Japan) was bubbled into the perfusate using an agent specific vaporizer (Vapor 2000; Dräger Medizintechnik GmbH, Lübeck, Germany) placed in the O$_2$-CO$_2$ gas mixture line at a concentration of (1.2±0.02) mmol/L (8%, v/v) measured in the liquid phase by gas chromatography (Agilent Laboratories, Santa Clara, CA, USA). These concentrations, which are too high to maintain general anesthesia but may be used temporarily during mask induction (Epstein et al., 1998; Walpole and Logan, 1999), were chosen to induce sevoflurane preconditioning and postconditioning, as previously described (He et al., 2008; Yan et al., 2008; Zhang et al., 2009).

### 2.2 Experimental protocol

The rats were randomly divided into 3 groups (8 hearts each), 2 for treatment and 1 as control (Fig. 1). The timing of the preconditioning or postconditioning therapy was based on previous reports in necrosis models. Rat hearts in the treatment groups were exposed to sevoflurane. After a 20-min equilibration period, hearts in the sevoflurane preconditioning group (SPR) were exposed to sevoflurane for 5 min, followed by a 5-min washout period before a 20-min global ischemia and a 40-min reperfusion. Hearts in the sevoflurane postconditioning group (SPO) underwent equilibration, then 20 min of global ischemia, followed immediately by sevoflurane exposure in the first 5 min of the 40-min reperfusion period. The control group (CON) received no treatment before the 20-min global ischemia and during the 40 min-reperfusion period.