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Postconditioning of sevoflurane and propofol is associated with mitochondrial permeability transition pore*

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Abstract: Background: Sevoflurane and propofol are effective cardioprotective anaesthetic agents, though the cardioprotection of propofol has not been shown in humans. Their roles and underlying mechanisms in anesthetic postconditioning are unclear. Mitochondrial permeability transition pore (MPTP) opening is a major cause of ischemia-reperfusion injury. Here we investigated sevoflurane- and propofol-induced postconditioning and their relationship with MPTP. Methods: Isolated perfused rat hearts were exposed to 40 min of ischemia followed by 1 h of reperfusion. During the first 15 min of reperfusion, hearts were treated with either control buffer (CTRL group) or buffer containing 20 μmol/L atractyloside (ATR group), 3% (v/v) sevoflurane (SPC group), 50 μmol/L propofol (PPC group), or the combination of atractyloside with respective anesthetics (SPC+ATR and PPC+ATR groups). Infarct size was determined by dividing the total necrotic area of the left ventricle by the total left ventricular slice area (percent necrotic area). Results: Hearts treated with sevoflurane or propofol showed significantly better recovery of coronary flow, end-diastolic pressures, left ventricular developed pressure and derivatives compared with controls. Sevoflurane resulted in more protective alteration of hemodynamics at most time point of reperfusion than propofol. These improvements were paralleled with the reduction of lactate dehydrogenase release and the decrease of infarct size (SPC vs CTRL: (17.48±2.70)% vs (48.47±6.03)%, $P<0.05$; PPC vs CTRL: (35.60±2.10)% vs (48.47±6.03)%, $P<0.05$). SPC group had less infarct size than PPC group (SPC vs PPC: (17.48±2.70)% vs (35.60±2.10)%, $P<0.05$). Atractyloside coadministration attenuated or completely blocked the cardioprotective effect of postconditioning of sevoflurane and propofol. Conclusion: Postconditioning of sevoflurane and propofol has cardioprotective effect against ischemia-reperfusion injury of heart, which is associated with inhibition of MPTP opening. Compared to propofol, sevoflurane provides superior protection of functional recovery and infarct size.

Key words: Sevoflurane, Propofol, Postconditioning, Reperfusion injury, Mitochondrial permeability transition pore (MPTP)
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INTRODUCTION

Mitochondrial permeability transition is thought to be a major cause of reperfusion injury, and is probably responsible for damaging heart during ischemia reperfusion (Argaud *et al*., 2005a; Bopassa *et al*., 2006; Halestrap *et al*., 1998; 2004). Mitochondrial permeability transition pore (MPTP) opens during reperfusion (Griffiths and Halestrap, 1993; 1995), and the extent of functional recovery of a heart is inversely correlated with pore opening (Frokiaer *et al*., 1999; Griffiths and Halestrap, 1995; Halestrap *et al*., 1997). Most recently, inhibition of MPTP was shown to mediate the protective effects of repetitive, brief ischemic episodes conducted during early reperfusion after prolonged coronary artery occlusion (Argaud *et al*., 2005a; 2005b; Hausenloy *et al*., 2003), a phenomenon termed "ischemic postconditioning" (Vinten-Johansen *et al*., 2005; Zhao *et al*., 2003).

Anesthetic postconditioning, i.e., ischemic

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postconditioning, is elicited by anesthetic administration right at the onset of reperfusion, and is another promising therapeutic strategy of attaining myocardial protection against ischemia-reperfusion damage. Sevoflurane and propofol had been demonstrated to exert cardioprotection via many pathways during ischemia reperfusion (Javadov *et al*., 2000; Kokita *et al*., 1998; Mathur *et al*., 1999; Obal *et al*., 2003). However, whether their postconditioning is associated with MPTP is still unclear. Propofol is an anaesthetic agent frequently used during cardiac surgery. Propofol-induced postconditioning had been shown to protect the Langendorff-perfused heart against reperfusion injury when propofol was administered during the first 10 min of reperfusion (Kokita *et al*., 1998). Attenuation of lipid peroxidation was thought to be one mechanism to recover mechanical dysfunction and metabolic derangement during reperfusion. Although it is reported that propofol can dose-dependently inhibit the MPTP in isolated heart mitochondria (Sztark *et al*., 1995) and that propofol confers significant cardioprotection by less opening of MPTP when the propofol was administrated 10 min before the onset of ischemia and throughout the reperfusion phase (Javadov *et al*., 2000), little firm data support MPTP as an effector associated with propofol-induced proconditioning. Sevoflurane, a volatile anesthetic, was demonstrated to have cardioprotective effect in ischemic and reperfused rat hearts by postconditioning (Obal *et al*., 2005; 2003). Obal *et al*.(2005) has elucidated that the adenosine triphosphate-sensitive potassium (K_{ATP}) channel did involve the postconditioning of sevoflurane, which was in line with our previous results (Yan *et al*., 2008). Nevertheless, it might not be the only mediator underlying the complicating pathological lesion (Zhao and Vinten-Johansen, 2006). Glyburide, a K_{ATP} antagonist, pretreatment significantly attenuated the recovery of left ventricular developed pressure (LVDP), but did not abolish the cardioprotection observed with sevoflurane (Mathur *et al*., 1999). Taken together, the mechanism undergoing postconditioning of sevoflurane and propofol against reperfusion injury is not well established. Due to the important role of MPTP during reperfusion injury in the present study, we used atractyloside (ATR), an MPTP opener, to investigate the relationship between MPTP and postconditioning of sevoflurane and propofol.

MATERIALS AND METHODS

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 School of Medicine, Zhejiang University.

Langendorff heart perfusion

Male Spragu-Dawley rats (250 g) were heparinized (500 U i.p.) and killed by decapitation after being anesthetized (pentobarbital sodium, 60 mg/kg i.p.). The hearts were immediately excised and perfused in a non-circulation Langendorff apparatus with Krebs-Henseleit buffer (155 mmol/L Na^+, 5.6 mmol/L K^+, 138 mmol/L Cl^-, 2.1 mmol/L Ca^{2+}, 1.2 mmol/L PO_4^{3-}, 25 mmol/L HCO_3^-, 0.56 mmol/L Mg^{2+}, 11 mmol/L glucose) gassed with 95% O_2 and 5% CO_2 (pH 7.4, 37 °C). Perfusion pressure was set to 80 mmHg. LVDP and derivatives, end-diastolic pressure, epicardial electrocardiogram, and perfusion pressure were recorded on a personal computer (Uecker *et al*., 2003). Coronary flow (CF) was measured by timed collection of perfusate dripping from the right heart into a graduated cylinder.

Experimental protocol

Spontaneously beating hearts were equilibrated for 30 min. After 40 min of test ischemia, anesthetic postconditioning was induced by 3% (v/v) sevoflurane [1.5 minimum alveolar concentration (1.5 MAC)] administered for 15 min immediately at the onset of reperfusion. The buffer solution was equilibrated with sevoflurane using a Sevotec 3 vaporizer (Datex-Ohmeda, Tewksbury, MA) with an air bubbler. Sevoflurane concentrations were also measured in the buffer solution right before entering the aorta using a gas chromatograph (Perkin-Elmer, Norwalk, CT): 3% (v/v) sevoflurane (1.5 MAC in rats at 37 °C). Care was taken that all reservoirs were filled with buffer saturated with 1.5 MAC sevoflurane by adding sevoflurane to the perfusate 10 min before opening the stopcock for reperfusion. Propofol with the concentration of 50 μmol/L was achieved by the addition of "Diprivan" (Zeneca Pharma, UK) which contains 10 mg/ml of propofol in an intralipid emulsion. It was added during the first 15 min of reperfusion. In some experiments, 20 μmol/L ATR (Sigma Chemicals Co.,

USA) was coadministrated to sevoflurane or propofol. Hearts subjected to ischemia and reperfusion alone served as ischemic control. For each experimental group, six hearts were prepared, and functional parameters were recorded (Fig.1).

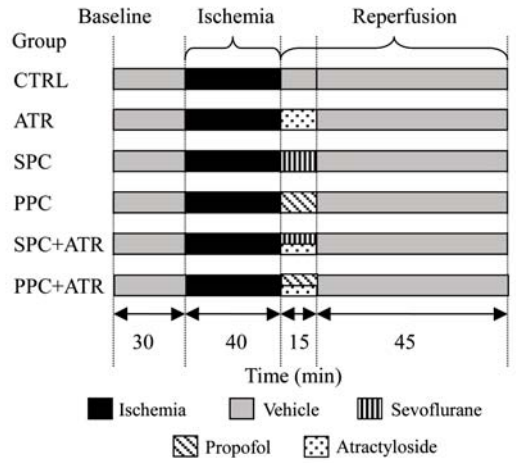

Fig.1 Scheme of treatment protocols

After 40 min of test ischemia, hearts were exposed to either control buffer or buffer containing atractyloside (20 μmol/L), sevoflurane (1.5 MAC), propofol (50 μmol/L) for 15 min immediately at the onset of reperfusion. The combination of sevoflurane or propofol with atractyloside was another two groups. In each group, six hearts were used. CTRL group: Unprotected hearts exposed to ischemia-reperfusion; ATR group: Atractyloside treated group; SPC group: Sevoflurane postconditioning; PPC group: Propofol postconditioning; SPC+ATR group: Sevoflurane postconditioning+atractyloside treatment; PPC+ATR group: Propofol postconditioning+atractyloside treatment

Lactate dehydrogenase measurement

In separate experiments, lactate dehydrogenase (LDH) was extracted from left ventricular tissue after 5 min of reperfusion, as previously described (Cao *et al*., 2004). LDH is released from dysfunctional myocardial and is washed out during reperfusion. Therefore, concentrations of LDH in the perfused buffer indicate severity of myocardial injury. LDH concentration was determined by spectrophotometry (Li *et al*., 1999).

Infarct size determination

Infarct size was determined by 2,3,5-triphenyltetrazolium chloride staining, as previously described (Zhong *et al*., 2004). Briefly, hearts were frozen at −20 °C for 2 h at the end of the experiment and subsequently sliced into five 2-mm cross-sections. The sections were incubated at 37 °C for 30 min in 1% (w/v) 2,3,5-triphenyltetrazolium chloride in 0.1 mol/L phosphate buffer (pH 7.4). Slices were fixed overnight in 10% (w/v) formaldehyde and digitally photographed. Planimetric analysis was performed using image system. Because the entire left ventricle was at risk (global ischemia), infarct size was determined by dividing the total necrotic area of the left ventricle by the total left ventricular slice area (percent necrotic area). Hearts subjected to ischemia and reperfusion alone served as ischemic control.

Statistics

All values are expressed as mean±*SD*. SPSS 11.5 was used for statistical analysis. For hemodynamics, time-dependent comparisons among groups were analyzed by repeated-measures ANOVA followed by post-hoc LSD analysis for multiple comparisons. Single time-point variables of hemodynamics, LDH and infarct size were analyzed by ANOVA followed by post-hoc LSD test for multiple comparisons. $P<0.05$ was considered significant.

RESULTS

Hemodynamic function

At the beginning of the experiments there were no differences in hemodynamics observed among groups (Table 1). After 40 min test ischemia followed by 60 min reperfusion, hemodynamic function of unprotected hearts (CTRL group) was significantly impaired as was demonstrated by lessening of LVDP, maximum left ventricular pressure increase and decrease rate ($\pm dp/dt$) and CF as well as increase of left ventricular end-diastolic pressure (LVEDP) compared with those in baseline. Anesthetic postconditioning with sevoflurane (1.5 MAC) or propofol (50 μmol/L) for 15 min immediately administrated at the onset of reperfusion significantly improved hemodynamic functional recovery of the damaged hearts. Sevoflurane postconditioning resulted in more protective alteration of LVDP, $\pm dp/dt$, LVEDP and CF at most time points of reperfusion. Further, we found that both sevoflurane and propofol postconditioning-induced hemodynamic effects were partially attenuated or totally abolished by coadministration of 20 μmol/L ATR. Specifically, more CF was still ob-

served in SPC+ATR group when compared with that in CTRL group, and the difference was statistically significant at the 15 min (SPC+ATR vs CTRL: (6.57±0.96) vs (5.43±0.81) ml/min, $P<0.05$) and 30 min (SPC+ATR vs CTRL: (5.01±0.29) vs (3.71±0.97) ml/min, $P<0.05$) of reperfusion.

Myocardial damage and infarct size

Myocardial damage was estimated by measuring the release of LDH from necrotic tissue. The concentration of LDH in coronary artery effluent was reduced to a comparable extent in SPC and PPC groups (Fig.2). ATR alone had no effect on LDH

Table 1 Haemodynamic variables in ischemic reperfused hearts

Group	LVDP (mmHg), LVEDP (mmHg), +dp/dt (mmHg/s), −dp/dt (mmHg/s), CF (ml/min), HR (beat/min)					
	Baseline	Reperfusion				
		5 min	10 min	15 min	30 min	60 min
CTRL	109.67±15.00, 5.47±0.19, 2546±266, 1946±213, 8.08±0.92, 278±20	43.54±21.79*, 52.39±10.64*, 1111±120*, 761±49*, 6.42±0.67*, 177±29*	44.51±10.70*, 47.53±5.63*, 1098±144*, 872±68*, 6.53±0.92*, 185±23*	49.47±4.05*, 47.16±10.02*, 1196±100*, 842±115*, 5.43±0.81*, 197±12*	47.78±8.57*, 48.24±6.97*, 1157±211*, 896±57*, 3.71±0.97*, 205±39*	44.11±5.58*, 44.50±1.34*, 1261±178*, 787±126*, 2.40±0.43*, 223±11*
ATR	105.82±18.71, 5.42±0.24, 2497±297, 1836±216, 8.33±0.93, 277±25	40.13±17.20*, 50.25±10.75*, 1047±117*, 761±38*, 6.29±0.55*, 181±36*	43.69±11.29*, 45.49±6.80*, 1046±159*, 845±86*, 6.21±0.95*, 193±31*	48.75±3.35*, 48.80±2.43*, 1129±78*, 871±15*, 5.64±0.74*, 192±17*	48.68±9.28*, 45.43±2.05*, 1135±276*, 909±66*, 3.74±0.97*, 202±43*	45.49±6.88*, 44.23±1.76*, 1185±223*, 740±125*, 2.16±0.26*, 219±3*
SPC	94.43±12.93, 5.38±0.25, 2562±179, 1815±247, 8.38±0.74, 283±18	56.13±21.16*, 31.19±10.66$^{*\#}$, 1481±116$^{*\#}$, 524±55$^{*\#}$, 10.67±1.22$^{*\#}$, 199±16*	57.85±13.67$^{*\#}$, 26.76±1.99$^{*\#}$, 1668±224$^{*\#}$, 858±90*, 11.58±1.05$^{*\#}$, 221±30$^{*\#}$	65.01±6.91$^{*\#}$, 26.60±4.69$^{*\#}$, 1825±120$^{*\#}$, 889±81*, 11.52±1.39$^{*\#}$, 238±13$^{*\#}$	73.74±12.23$^{\#}$, 24.51±3.12$^{*\#}$, 2056±215$^{*\#}$, 1125±49$^{*\#}$, 5.87±0.66$^{*\#}$, 260±27$^{\#}$	77.50±9.89$^{\#}$, 13.43±1.21$^{*\#}$, 2584±140$^{\#}$, 1380±45$^{*\#}$, 5.35±0.50$^{*\#}$, 233±9*
PPC	109.33±14.69, 5.35±0.19, 2594±262, 1735±226, 8.13±0.81, 297±19	56.10±12.44*, 41.78±6.56*, 1311±107$^{*\#\dagger}$, 657±82$^{*\#\dagger}$, 8.48±0.71$^{\#\dagger}$, 188±18*	35.82±7.89*†, 37.14±3.45$^{*\#\dagger}$, 1383±144$^{*\#\dagger}$, 865±59*, 8.60±1.05$^{\#\dagger}$, 202±14*	53.09±11.04*, 36.89±4.56$^{*\#\dagger}$, 1485±196$^{*\#\dagger}$, 865±84*, 7.00±0.41$^{*\#\dagger}$, 218±11$^{*\#\dagger}$	61.39±6.94$^{*\#\dagger}$, 35.77±3.24$^{*\#\dagger}$, 1606±161$^{*\#\dagger}$, 1011±42$^{*\#\dagger}$, 6.84±1.21$^{\#}$, 193±32*†	61.50±3.29$^{*\#\dagger}$, 28.28±2.20$^{*\#\dagger}$, 1923±134$^{*\#\dagger}$, 1083±69$^{*\#\dagger}$, 3.08±0.69*†, 228±6*
SPC+ATR	104.78±14.25, 5.34±0.19, 2506±244, 1711±215, 8.43±0.97, 279±24	32.69±25.07*, 50.53±6.38*, 965±102$^{*\#}$, 728±48*, 7.21±0.73, 148±11$^{*\#}$	47.59±7.86*, 48.31±4.24*, 957±118*, 764±84$^{*\#}$, 7.47±0.85, 178±9*	39.79±18.85*, 43.42±10.03*, 1184±94*, 779±113*, 6.57±0.96$^{*\#}$, 189±13*	42.12±6.72*, 44.32±5.78*, 1055±200*, 835±72*, 5.01±0.29$^{*\#}$, 194±38*	40.72±8.10*, 41.46±1.30$^{*\#}$, 1208±170*, 741±119*, 2.62±0.90*, 207±13$^{*\#}$
PPC+ATR	110.87±10.43, 5.64±0.28, 2602±317, 1724±96, 8.25±0.89, 278±18	48.55±15.66*, 49.31±10.06*, 1064±154*, 737±102*, 5.48±0.92*, 164±26*	38.11±13.64*, 44.78±5.42*, 1028±128*, 819±66*, 5.59±0.78*, 174±20*	39.22±16.92*, 44.13±9.54*, 1119±157*, 790±110*, 5.04±0.34*, 185±12*	45.52±8.19*, 45.09±6.90*, 1071±201*, 835±48*, 4.31±0.77*, 193±39*	41.86±9.32*, 40.55±4.28$^{*\#}$, 1170±160*, 728±112*, 1.83±0.44*, 207±10$^{*\#}$

Data presented as mean±*SD* (n=6); *Significantly ($P<0.05$) different from respective value of baseline (intragroup comparison); $^{\#}$Significantly ($P<0.05$) different from respective value of CTRL (intergroup comparison); †Significantly ($P<0.05$) different between SPC and PPC (intergroup comparison). LVDP: Left ventricular developed pressure; LVEDP: Left ventricular end-diastolic pressure; +dp/dt: Inotropy; −dp/dt: Lusitropy; CF: Coronary flow; HR: Heart rate; CTRL group: Unprotected hearts exposed to ischemia-reperfusion; ATR group: Atractyloside (20 μmol/L) treated group; SPC group: Sevoflurane postconditioning; PPC group: Propofol postconditioning; SPC+ATR group: Sevoflurane postconditioning+atractyloside treatment; PPC+ATR group: Propofol postconditioning+atractyloside treatment

release. However, it completely blocked the decrease of LDH by propofol postconditioning. Similarly to the effect on recovery in hemodynamic function, ATR only partially inhibited the effect of sevoflurane on LDH release (SPC+ATR vs CTRL: (0.33±0.073) vs (0.64±0.059) U/(min·g), $P<0.05$).

Further, we detected the infarct size of the damaged hearts in different treatment protocol. Sevoflurane and propofol administration reduced the infarct size by 63.9% and 26.6%, respectively, compared to the CTRL group (Fig.3). Sevoflurane postconditioning had less infarct size than propofol (SPC vs PPC: (17.48±2.70)% vs (35.60±2.10)%, $P<0.05$). Nevertheless, the protection induced by sevoflurane and propofol was fully reversed by ATR coadministration, respectively.

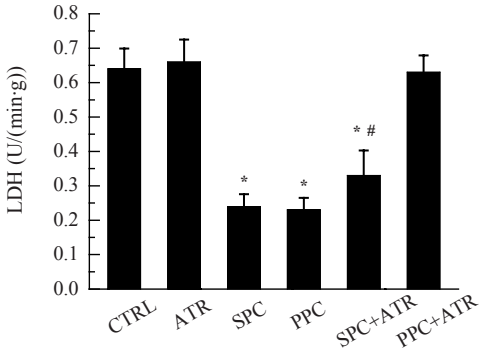

Fig.2 Lactate dehydrogenase (LDH) measurement in the various treatment groups

LDH content was determined after 5 min of reperfusion (n=6). Data are given as mean±*SD*. CTRL group: Unprotected hearts exposed to ischemia-reperfusion; ATR group: Atractyloside (20 μmol/L) treated group; SPC group: Sevoflurane postconditioning; PPC group: Propofol postconditioning; SPC+ATR group: Sevoflurane postconditioning+atractyloside treatment; PPC+ATR group: Propofol postconditioning+atractyloside treatment. $^{*}P<0.05$, compared to CTRL; $^{\#}P<0.05$, SPC+ATR vs SPC

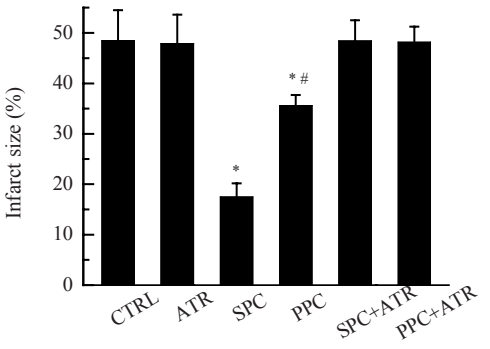

Fig.3 Infarct size was determined using 1% triphenyltetrazolium chloride staining (n=6)

Data are presented as mean±*SD*. CTRL group: Unprotected hearts exposed to ischemia-reperfusion; ATR group: Atractyloside (20 μmol/L) treated group; SPC group: Sevoflurane postconditioning; PPC group: Propofol postconditioning; SPC+ATR group: Sevoflurane postconditioning+atractyloside treatment; PPC+ATR group: Propofol postconditioning+atractyloside treatment. $^{*}P<0.05$, compared to CTRL; $^{\#}P<0.05$, PPC vs SPC

DISCUSSION

Reperfusion injury is a complex process involving numerous mechanisms exerted in the intracellular and extracellular environments (Piper *et al.*, 2003). Ischemic preconditioning and postconditioning represent interventions with multiple and interacting components marshaled against myocardial reperfusion injury by endogenous cardioprotective mechanisms. Postconditioning, theoretically, might be more clinically applicable than preconditioning in that therapy would not have to be administered prior to an ischemic episode, but could be administered at the time of reperfusion. In the present study, we administrated sevoflurane and propofol, two frequently used anesthetics in clinical setting, at the onset of reperfusion to induce a "postconditioned state", and presented evidence that postconditioning of sevoflurane and propofol significantly improved postischemic functional recovery of rat hearts. Such an improvement in postischemic functional recovery was paralleled to a significant attenuation in LDH release from coronary effluent and reduction in infarct size. These results confirmed and extended previous studies of postconditioning induced by distinct anesthetic agents.

Similar to the preconditioning, the concepts of triggers, mediators and end effectors have been used to describe the mechanisms involved in cardioprotective effects of postconditioning. Penna *et al*.(2006) reported in a recent study that reactive oxygen species may be acting as a trigger of protection in the very early phase of postconditioning. Classical ligand triggers have also been reported to be involved in postconditioning (Kin *et al*., 2005; Weihrauch *et al*., 2005). Furthermore, K_{ATP} channels and protein kinase C pathways may be evoked after the postconditioning trigger, and therefore may be acting as mediators (Obal *et al*., 2005; Penna *et al*., 2006). Our study showed that ATR, a specific MPTP opener, alone had no effect on diminishing reperfusion injury, whereas it totally blocked the infarct size-limiting effect of

sevoflurane and propofol postconditioning. This finding implies that MPTP is also involved in the sevoflurane- and propofol-induced postconditioning.

Under normal physiological conditions, the mitochondrial inner membrane is impermeable to all but a few selected metabolites and ions. However, under conditions of stress, a nonspecific pore known as MPTP can open in the mitochondrial inner membrane that allows free passage of any molecule of <1.5 kDa (Crompton, 1999). When the MPTP opens, the permeability barrier of the inner membrane becomes disrupted. It causes mitochondria to swell, and the outer membrane will break and lead to release of proteins in the intermembrane space such as cytochrome C and other factors that play a critical role in apoptotic cell death. In addition, opening of MPTP leads to hydrolyzing ATP, rather than synthesizing it. Under such conditions, intracellular ATP concentrations decline rapidly, leading to the disruption of ionic and metabolic homeostasis and the activation of degradative enzymes such as phospholipases, nucleases and proteases. The key factor responsible for MPTP opening is mitochondrial calcium overload, especially when this is accompanied by oxidative stress, adenine nucleotide depletion, elevated phosphate concentrations, and mitochondrial depolarization (Crompton, 1999; Halestrap *et al*., 1998). These conditions are exactly those that the heart experiences during postischemic reperfusion, and there is increasing evidence that MPTP opening is critical in the transition from reversible to irreversible reperfusion injury (Crompton, 1999; Halestrap *et al*., 1998). Previous investigations have disclosed the role of MPTP in some distinct models of postconditioning. It was reported that postconditioning consisting of four episodes (1 min of coronary occlusion and 1 min of reperfusion performed after the prolonged ischemia followed by 1 min of reflow) could inhibit opening of the MPTP and provide a powerful anti-ischemic protection in rabbits (Argaud *et al*., 2005a). Activation of PI3K inhibited the opening of MPTP and obtained beneficial effect in Langendorff rat heart reperfused with postconditioning, i.e., three episodes of 30 s of reperfusion followed by 30 s of ischemia (Bopassa *et al*., 2006). Isoflurane postconditioning induced by 15 min of 1.5 MAC isoflurane administered at the onset of reperfusion protected against reperfusion damage by preventing opening of MPTP through inhibition of GSK3β (Feng *et al*., 2005). Our study further extended the role of MPTP in sevoflurane- and propofol-induced postconditioning. As for sevoflurane, it was reported that mitochondrial K_{ATP} opened and superoxide decreased during ischemia and reperfusion was associated with sevoflurane-induced cardioprotection (Obal *et al*., 2005). Propofol acts as a free radical scavenger (Eriksson *et al*., 1992) and directly inhibits the opening of MPTP of isolated heart mitochondria (Sztark *et al*., 1995). These factors/roles will preserve mitochondrial function during ischemia and reperfusion and may facilitate less MPTP opening in sevoflurane- and propofol-induced postconditioning (Halestrap *et al*., 2004). However, the exact mechanism between MPTP opening and postconditioning of sevoflurane and propofol remains to be further investigated.

Also, we found that in assessment of hemodynamic function recovery, enhancement of CF by sevoflurane postconditioning could not be totally abrogated by ATR. Parallelly, ATR could not completely block sevoflurane postconditioning-induced reduction of LDH release during reperfusion. Yet it fully reversed the decrease of infarct size. Considering multiple sevoflurane-induced beneficial mediators on reperfusion (Kevin *et al*., 2003; Mathur *et al*., 1999; Obal *et al*., 2005) and the possible links among reactive oxygen species, K_{ATP} channel activation and MPTP opening (Halestrap *et al*., 2004), we speculate that sevoflurane postconditioning is mediated, at least in part, by MPTP. Other independent effectors cannot be excluded.

Further, in our experiment, sevoflurane (1.5 MAC) postconditioning had more protective effect than propofol (50 μmol/L) on hemodynamic functional recovery during reperfusion paralleled with more reduction of infarct size. No reasonable explanation could be given in the present study. In literature, the role of propofol against reperfusion injury was dose-dependent. Ebel *et al*.(1999) found no protective effect of propofol (at clinical relevant concentration) against myocardial reperfusion injury, but Ko *et al*.(1997) demonstrated that propofol at higher concentration (100 μmol/L) attenuated mechanical, biochemical and histologic changes caused by myocardial ischemia and reperfusion. Ansley *et al*.(1999) observed that high-dose propofol [a 2~2.5 mg/kg bolus followed by a continuous infusion of 200

μg/(kg·min)] enhanced erythrocyte antioxidant capacity during cardiopulmonary bypass in humans. Xia *et al*.(2006) related this increased antioxidant capacity in the high-dose propofol group of 120 μg/(kg·min) to improved myocardial function (high cardiac index) 12 h postoperatively. However, the cardiac index in the low-dose propofol group of 60 μg/(kg·min) was similar. On the other hand, it was reported that cardioprotection against reperfusion injury is maximal with only 2 min of sevoflurane (1 MAC) during the initial phase of reperfusion in rats compared with 5 and 10 min administration, respectively (Obal *et al*., 2003). Moreover, sevoflurane (with a concentration of 0.5%~2%) but not propofol (with a target plasma propofol concentration of 2~4 μg/ml) preserved left ventricular function after cardiopulmonary bypass with less evidence of myocardial damage in the first 36 h postoperatively (de Hert *et al*., 2002). Optimal dosage, regiment of postconditioning and underlying different pharmacological role (de Hert *et al*., 2004) should be taken into consideration to explain the differences in cardioprotection between the postconditioning of sevoflurane and propofol.

In summary, the current results suggest that postconditioning of sevoflurane and propofol improves functional recovery with reduction of myocardial damage and infarct size during reperfusion injury. This cardioprotective effect is associated with inhibition of MPTP opening. Sevoflurane of 1.5 MAC provides more beneficial effects on both functional hemodynamic parameters and infarct size than 50 μmol/L propofol. Our study should be interpreted with some degree of caution, particularly because it was performed using rat hearts, which may not be completely applicable to human tissue. Moreover, we did not study concentration-response relationships for each drug, but instead, relied on concentrations that have been established to produce the relevant effect for the respective agent. It cannot be excluded that different concentrations of these agents could produce other effects. Taken together, it is nonetheless attractive to suggest that sevoflurane and propofol may be exploited as cardioprotective anesthetics by postconditioning under clinical settings by the anesthesiologist. However, further studies are required to verify and optimize these beneficial effects as well as the underlying mechanism.

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