Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

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

An-lu DAI[1], Li-hua FAN[2], Feng-jiang ZHANG[1], Mei-juan YANG[1], Jing YU[1], Jun-kuan WANG[1], Tao FANG[3], Gang CHEN[1], Li-na YU[1], Min YAN[†‡1]

([1]*Department of Anesthesiology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China*)

([2]*Department of Anesthesiology, Lishui People's Hospital, Lishui 323600, China*)

([3]*Department of Anesthesiology, Jinhua People's Hospital, Jinhua 321300, China*)

[†]E-mail: yanminnina@hotmail.com

Received Dec. 20, 2009; Revision accepted Mar. 15, 2010; Crosschecked Mar. 16, 2010

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). $\pm(dP/dt)_{max}$ 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

doi:10.1631/jzus.B0900390 **Document code:** A **CLC number:** R614

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*.,

‡ Corresponding author

* Project supported by the National Natural Science Foundation of China (No. 30772090), the Natural Science Foundation of Zhejiang Province (No. Y204141), the Foundation from Science and Technology Department of Zhejiang Province (No. 2007R10034), and the Foundation from the Health Bureau of Zhejiang Province (No. 2007QN007), China

2006; Tsutsumi *et al*., 2007) and on anesthetic preconditioning (Riess *et al*., 2002; Weber and Schlack, 2008) just against prolonged ischemia containing obviously measured infarct size.

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_{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_2PO_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.

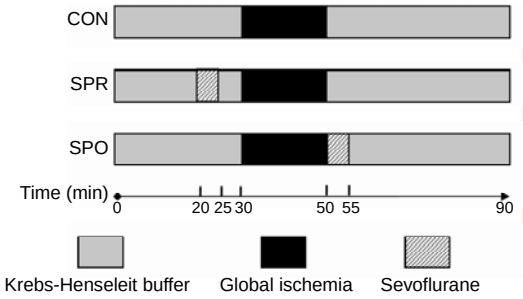

Fig. 1 Experimental protocols used in this study
CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

2.3 Assessment of cardiac function

A fluid-filled latex balloon was introduced into the left ventricle through the left atrial appendage and the balloon catheter was linked to a pressure transducer connected to a data-acquisition system (RM6240, Chengdu, China) to measure contractile function. The left ventricular end diastolic pressure (LVEDP) was adjusted to between 4 and 8 mmHg. The cardiac parameters of heart rate (HR), left ventricular-developed pressure (LVDP) (difference between left ventricular end systolic pressure and end diastolic pressure), and maximal rise/fall rate of left ventricular pressure $[\pm(dP/dt)_{max}]$ were monitored continuously. Electrocardiograms were recorded with carbon leads directly attached to the perfused heart surface. Analysis of arrhythmia incidence was carried out in accordance with the Lambeth Conventions (Walker *et al*., 1988). Total coronary flow (CF) was measured by timed collection of perfusate dripping from the right heart into a graduated cylinder.

2.4 Tissue histology

At the end of the 40-min reperfusion, the left ventricle was sliced transversely from apex to base into 7 slices. Four hearts in each group were used to determine infarct size by 1% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC) staining, as previously described (Riess *et al*., 2004; Deyhimy *et al*., 2007). Slices of the remaining hearts in each group were fixed with 10% (v/v) formalin, embedded in paraffin, and processed for histologic analysis. The slices for histologic analysis were stained with hematoxylin and eosin.

2.5 Statistical analysis

SPSS 10.0 was used for statistical analysis. Data were presented as mean±standard deviation (SD). Differences in functional data at each time point were evaluated using one-way analysis of variance (ANOVA) followed by post hoc analysis with Student-Newman-Keuls for multiple comparisons. The same analysis was used for detection of differences in duration of reperfusion arrhythmias. If variances are not homogeneous, nonparametric tests (Kruskal-Wallis *H* test) were used to compare the three groups by post hoc analysis with Mann-Whitney *U* test for multiple comparisons. A value of $P<0.05$ was considered significant.

3 Results

Twenty-eight rats were instrumented. Three rats were excluded because of severe hypotension during the baseline time (1 CON, 1 SPR, and 1 SPO) and one rat was excluded because of persistent ventricular fibrillation during reperfusion. There were eight successful rats in each group.

3.1 Duration of reperfusion arrhythmias

The incidence of reperfusion ventricular arrhythmia was 100% in all three groups. Results of duration of reperfusion arrhythmias are shown in Fig. 2. The variances of duration of reperfusion arrhythmias were not homogeneous, so nonparametric tests were used to compare two or three groups. There was no significant difference in the duration of reperfusion ventricular arrhythmias between the CON group and SPR group ($P=0.195$). The duration of reperfusion ventricular arrhythmias in the SPO group was markedly less than that in other two groups (SPO

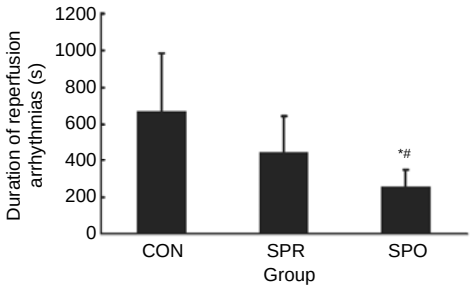

Fig. 2 Duration of reperfusion arrhythmias
Data are mean±SD, $n=8$ in each groups; $^{*}P<0.05$ vs. CON; $^{\#}P<0.05$ vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

vs. CON, P=0.010; SPO vs. SPR, P=0.028). In addition, no ventricular arrhythmia was observed after administration of sevoflurane in the SPR group.

3.2 Hemodynamic function

Systemic hemodynamics during baseline was not significantly different among groups (Figs. 3–7). ±dP/dt and LVDP after a 5-min administration of sevoflurane in the SPR were significantly lower than those in the CON and SPO groups, which were recovered 5 min later, i.e., at the moment before the global ischemia (Figs. 3–5). After 20 min of global ischemia, sevoflurane postconditioning, in which hearts were exposed to sevoflurane for 5 min at the onset of reperfusion, significantly inhibited functional recovery when compared with the CON and SPR groups (P<0.05, Figs. 3–5 and 7), and increased the CF at 5 min after reperfusion (Fig. 6). As shown in

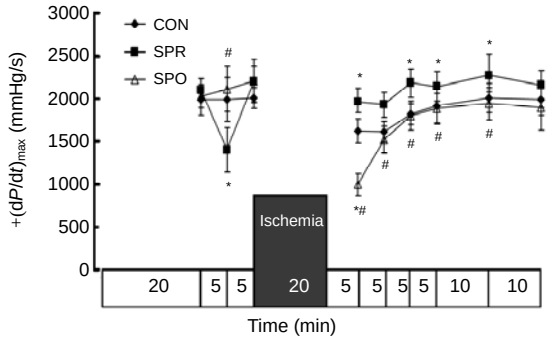

Fig. 3 Maximal rise/fall rate of left ventricular pressure [+(dP/dt)$_{max}$]
Data are mean±SD, n=8 in each groups; * P<0.05 vs. CON; # P<0.05 vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

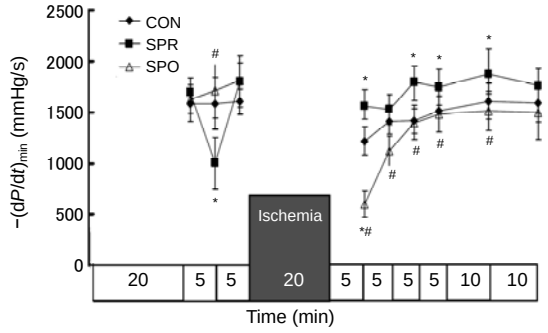

Fig. 4 Minimal rise/fall rate of left ventricular pressure [−(dP/dt)$_{min}$]
Data are mean±SD, n=8 in each groups; * P<0.05 vs. CON; # P<0.05 vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

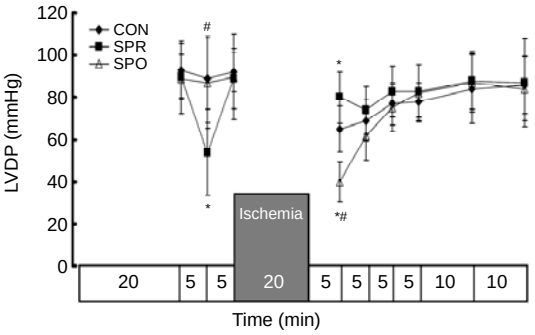

Fig. 5 Left ventricular developed pressure (LVDP)
Data are mean±SD, n=8 in each groups; * P<0.05 vs. CON; # P<0.05 vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

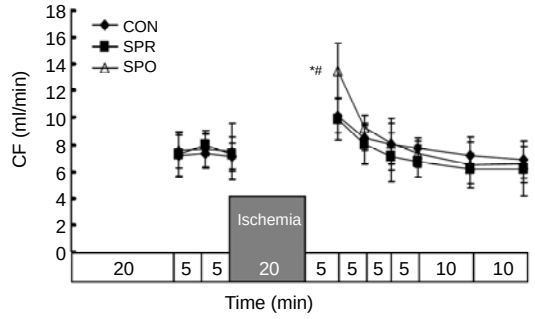

Fig. 6 Coronary flow (CF)
Data are mean±SD, n=8 in each groups; * P<0.05 vs. CON; # P<0.05 vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

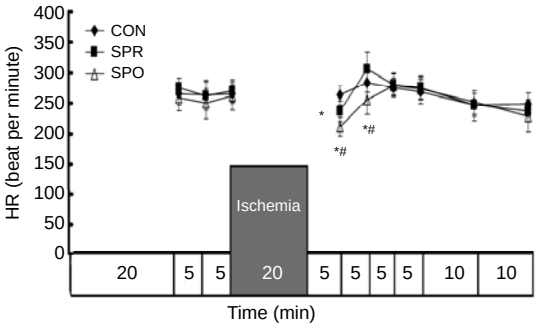

Fig. 7 Heart rate (HR)
Data are mean±SD, n=8 in each groups; * P<0.05 vs. CON; # P<0.05 vs. SPR. CON: control group; SPR: sevoflurane preconditioning group; SPO: sevoflurane postconditioning group

Figs. 3 and 4, ±dP/dt in the SPR group at 5, 10, 15, 20, and 30 min after reperfusion was significantly higher than that in the CON group (P<0.05). There were no significant differences in ±dP/dt at 40 min after reperfusion between SPR and CON groups

[(2164±165) mmHg/s vs. (1993±183) mmHg/s, $P>0.05$]. ±dP/dt, LVDP, CF, and HR were similar among the three groups at the end of the experiment ($P>0.05$, Figs. 3–7).

3.3 Infarct size and histologic analysis

As expected, for a 20-min general ischemia, infarct size in heart slices by TTC staining among the groups was not obvious. Histologic analysis revealed intact architecture of the cardiomyocytes. Specifically, contraction band necrosis or sarcolemmal blebs were not observed. Occasionally, cells showed mild edema and waviness.

4 Discussion

In this study, we investigated the effects of sevoflurane preconditioning and postconditioning on myocardial stunning using isolated rat hearts as the ischemia reperfusion model. We found that 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.

Myocardial stunning, whose diagnosis is based on experimental findings, is defined as a reversible contractile dysfunction during reperfusion after brief ischemia, and it happens in absence of necrosis (Braunwald and Kloner, 1982). In isolated perfused heart preparations, myocardial stunning can be generated after 20–25 min of global no-flow ischemia followed by reperfusion (Palmer *et al*., 2004; Chow *et al*., 2007; Tiwari *et al*., 2008). If the period of ischemia extends to 30 min, irreversible functional impairment occurs as cells undergo necrosis, i.e., infarction (Hansen, 1995; Kloner *et al*., 1998; Bolli and Marban, 1999; Park and Lucchesi, 1999; Palmer *et al*., 2004; Grund *et al*., 2006; Chow *et al*., 2007). All hearts in the experiments underwent 20 min of ischemia and 40 min of reperfusion, and absence of infarction was confirmed by TTC staining. Therefore, the experimental model used can also be considered as an experimental model of the stunned myocardium. There are, to our knowledge, no published data examining the effects of sevoflurane preconditioning and postconditioning on the stunned myocardium.

Arrhythmias developed by reperfusion after ischemia are also a type of ischemia reperfusion injury. The mechanisms of reperfusion arrhythmias are complex and are incompletely understood. Reperfusion arrhythmias occur due to heterogeneous recovery of conduction and the refractory period caused by incomplete reperfusion, reentry, abnormal automaticities, and activities triggered by Ca^{2+} overload (Dennis *et al*., 1990). Another common reperfusion arrhythmia mechanism is formation of free radicals and accentuation of preexisting heterogeneity of refractoriness occurring during reperfusion (Wit and Janse, 2001).

This study demonstrates that sevoflurane postconditioning markedly reduces duration of reperfusion arrhythmias. The degree of reduction of arrhythmia by sevoflurane postconditioning has been little investigated, but in the present study it was found quite striking. As for ischemic postconditioning, some studies (Kloner *et al*., 2006; Sasaki *et al*., 2007; Dow *et al*., 2008; 2009) reported that ischemia postconditioning markedly attenuates ventricular arrhythmias after ischemia-reperfusion in the necrosis-free model. Ischemic postconditioning's benefit on reperfusion ventricular arrhythmias was also maintained in the senescent heart (Dow *et al*., 2008). In addition, the mechanism by which ischemic postconditioning-reduced reperfusion induced ventricular arrhythmias was independent of known pathways including adenosine, mitochondrial K_{ATP} channel, mitochondrial permeability transition pore, and PI3K pathways (Dow *et al*., 2009). The proposed mechanism for anesthetic postconditioning's cardioprotection includes many of the same pathways proposed for postconditioning's benefits, including adenosine (Weihrauch *et al*., 2005), opening of the mitochondrial K_{ATP} channel (Obal *et al*., 2005), nitric oxide (Krolikowski *et al*., 2006), closing of the mitochondrial permeability transition pore (He *et al*., 2008), and intracellular survival pathways such as ERK1/2 and PI3K/Akt (Chiari *et al*., 2005; Feng *et al*., 2006; Krolikowski *et al*., 2006). In addition, administration of sevoflurane may make the myocardium more electrically homogenous and, therefore, affect such a mechanism of arrhythmia as reentrant circuits. However, exact mechanisms underlying the described effect remain to be investigated, especially in the

stunned myocardium. The mechanism of anesthetic postconditioning's ability to reduce arrhythmias was not the focus of the present study; further studies are needed in the future.

On the other hand, sevoflurane preconditioning performed until 5 min before the 20-min ischemia did not affect the duration of reperfusion arrhythmias in this study. As for ischemia preconditioning, Hagar *et al*. (1991) reported that ischemic preconditioning of hearts could reduce the incidence of reperfusion arrhythmias, but another study (Sasaki *et al*., 2007) suggested that ischemic preconditioning had no effects on reperfusion arrhythmias. The effect of preconditioning on reperfusion arrhythmias has not been studied extensively. No matter what the effect of sevoflurane preconditioning on arrhythmias is like, sevoflurane preconditioning improved ventricular function in the early reperfusion 20 min after ischemia, which may be also useful for correcting the stunned myocardium. Although the pathogenesis of myocardial stunning has not been definitively established, the two major hypotheses can be that it is caused by the generation of oxygen-derived free radicals (oxyradical hypothesis) or that by a transient calcium overload (calcium hypothesis) on reperfusion (Bolli and Marban, 1999). In the necrosis model, sevoflurane preconditioning could reduce formation of oxygen-derived free radicals during reperfusion (Novalija *et al*., 2002; 2003; Kevin *et al*., 2003) and enhance Ca^{2+} handling and mechanical and metabolic functions elicited by Na^{+}-Ca^{2+} exchange inhibition (An *et al*., 2006; Bouwman *et al*., 2006). However, there are no published data examining these events in the stunned myocardium.

Limitation of the present study is that only one dose and single time points of administration of sevoflurane preconditioning or postconditioning were used. In addition, we have not tested the potential mechanisms, which, indeed, could be an important approach to further explore. The implementation of the isolated rat heart system of ischemia-reperfusion injury has been considered necessary for the intrada for future in vivo studies. However, the ex vivo system of ischemia-reperfusion injury has several significant limitations such as its dependence on a physiological (non-blood based) buffer for perfusion purposes.

In summary, we were able to characterize an ischemia reperfusion-induced myocardial stunning in a rat model. 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. Therefore, this study helps broaden the understanding of effects of sevoflurane preconditioning and postconditioning on the stunned myocardium. Sevoflurane preconditioning and postconditioning may be useful for correcting the stunned myocardium.

References

An, J., Rhodes, S.S., Jiang, M.T., Bosnjak, Z.J., Tian, M., Stowe, D.F., 2006. Anesthetic preconditioning enhances Ca^{2+} handling and mechanical and metabolic function elicited by Na^{+}-Ca^{2+} exchange inhibition in isolated hearts. *Anesthesiology*, **105**(3):541-549. [doi:10.1097/00000542-200609000-00018]

Bolli, R., Marban, E., 1999. Molecular and cellular mechanisms of myocardial stunning. *Physiol. Rev.*, **79**(2):609-634.

Bouwman, R.A., Salic, K., Padding, F.G., Eringa, E.C., van Beek-Harmsen, B.J., Matsuda, T., Baba, A., Musters, R.J., Paulus, W.J., de Lange, J.J., Boer, C., 2006. Cardioprotection via activation of protein kinase C-delta depends on modulation of the reverse mode of the Na^{+}/Ca^{2+} exchanger. *Circulation*, **114**(1 Suppl.):I226-I232. [doi:10.1161/CIRCULATIONAHA.105.000570]

Braunwald, E., Kloner, R.A., 1982. The stunned myocardium: prolonged, postischemic ventricular dysfunction. *Circulation*, **66**(6):1146-1149.

Chiari, P.C., Bienengraeber, M.W., Pagel, P.S., Krolikowski, J.G., Kersten, J.R., Warltier, D.C., 2005. Isoflurane protects against myocardial infarction during early reperfusion by activation of phosphatidylinositol-3-kinase signal transduction: evidence for anesthetic-induced postconditioning in rabbits. *Anesthesiology*, **102**(1):102-109. [doi:10.1097/00000542-200501000-00018]

Chow, A.K., Cena, J., Schulz, R., 2007. Acute actions and novel targets of matrix metalloproteinases in the heart and vasculature. *Br. J. Pharmacol.*, **152**(2):189-205. [doi:10.1038/sj.bjp.0707344]

Dennis, S.C., Coetzee, W.A., Cragoe, E.J., Opie, L.H., 1990. Effects of proton buffering and of amiloride derivatives on reperfusion arrhythmias in isolated rat hearts. Possible evidence for an arrhythmogenic role of Na^{+}-H^{+} exchange. *Circ. Res.*, **66**(4):1156-1159.

Deyhimy, D.I., Fleming, N.W., Brodkin, I.G., Liu, H., 2007. Anesthetic preconditioning combined with postconditioning offers no additional benefit over preconditioning or postconditioning alone. *Anesth. Analg.*, **105**(2):316-324. [doi:10.1213/01.ane.0000267524.71445.e7]

Dow, J., Bhandari, A., Kloner, R.A., 2008. Ischemic postconditioning's benefit on reperfusion ventricular arrhythmias is maintained in the senescent heart. *J. Cardiovasc. Pharmacol. Ther.*, **13**(2):141-148. [doi:10.1177/1074248408317705]

Dow, J., Bhandari, A., Kloner, R.A., 2009. The mechanism by which ischemic postconditioning reduces reperfusion arrhythmias in rats remains elusive. *J. Cardiovasc. Pharmacol. Ther.*, **14**(2):99-103. [doi:10.1177/1074248408329606]

Epstein, R.H., Stein, A.L., Marr, A.T., Lessin, J.B., 1998. High concentration versus incremental induction of anesthesia with sevoflurane in children: a comparison of induction times, vital signs, and complications. *J. Clin. Anesth.*, **10**(1):41-45. [doi:10.1016/S0952-8180(97)00218-3]

Feng, J., Fischer, G., Lucchinetti, E., Zhu, M., Bestmann, L., Jegger, D., Arras, M., Pasch, T., Perriard, J.C., Schaub, M.C., Zaugg, M., 2006. Infarct-remodeled myocardium is receptive to protection by isoflurane postconditioning: role of protein kinase B/Akt signaling. *Anesthesiology*, **104**(5):1004-1014. [doi:10.1097/00000542-200605000-00017]

Feng, J., Zhu, M., Schaub, M.C., Gehrig, P., Roschitzki, B., Lucchinetti, E., Zaugg, M., 2008. Phosphoproteome analysis of isoflurane-protected heart mitochondria: phosphorylation of adenine nucleotide translocator-1 on Tyr194 regulates mitochondrial function. *Cardiovasc. Res.*, **80**(1):20-29. [doi:10.1093/cvr/cvn161]

Grund, F., Treiman, C., Ilebekk, A., 2006. How to discriminate between hibernating and stunned myocardium. *J. Cardiovasc. Surg. (Torino)*, **47**(3):323-328.

Hagar, J.M., Hale, S.L., Kloner, R.A., 1991. Effect of preconditioning ischemia on reperfusion arrhythmias after coronary artery occlusion and reperfusion in the rat. *Circ. Res.*, **68**(1):61-68.

Hansen, P.R., 1995. Myocardial reperfusion injury: experimental evidence and clinical relevance. *Eur. Heart. J.*, **16**(6):734-740.

He, W., Zhang, F.J., Wang, S.P., Chen, G., Chen, C.C., Yan, M., 2008. Postconditioning of sevoflurane and propofol is associated with mitochondrial permeability transition pore. *J. Zhejiang. Univ.-Sci. B.*, **9**(2):100-108. [doi:10.1631/jzus.B0710586]

Kevin, L.G., Novalija, E., Riess, M.L., Camara, A.K., Rhodes, S.S., Stowe, D.F., 2003. Sevoflurane exposure generates superoxide but leads to decreased superoxide during ischemia and reperfusion in isolated hearts. *Anesth. Analg.*, **96**(4):949-955. [doi:10.1213/01.ANE.0000052515.25465.35]

Kloner, R.A., Bolli, R., Marban, E., Reinlib, L., Braunwald, E., 1998. Medical and cellular implications of stunning, hibernation, and preconditioning: an NHLBI workshop. *Circulation*, **97**(18):1848-1867.

Kloner, R.A., Dow, J., Bhandari, A., 2006. Postconditioning markedly attenuates ventricular arrhythmias after ischemia-reperfusion. *J. Cardiovasc. Pharmacol. Ther.*, **11**(1):55-63. [doi:10.1177/107424840601100105]

Krolikowski, J.G., Weihrauch, D., Bienengraeber, M., Kersten, J.R., Warltier, D.C., Pagel, P.S., 2006. Role of Erk1/2, p70s6K, and eNOS in isoflurane-induced cardioprotection during early reperfusion in vivo [Le rôle des Erk1/2, p70s6K et eNOS dans la cardioprotection induite par l'isoflurane pendant la reperfusion in vivo précoce]. *Can. J. Anesth.*, **53**(2):174-182. [doi:10.1007/BF03021824]

Novalija, E., Varadarajan, S.G., Camara, A.K., An, J., Chen, Q., Riess, M.L., Hogg, N., Stowe, D.F., 2002. Anesthetic preconditioning: triggering role of reactive oxygen and nitrogen species in isolated hearts. *Am. J. Physiol. Heart Circ. Physiol.*, **283**(1):H44-H52.

Novalija, E., Kevin, L.G., Eells, J.T., Henry, M.M., Stowe, D.F., 2003. Anesthetic preconditioning improves adenosine triphosphate synthesis and reduces reactive oxygen species formation in mitochondria after ischemia by a redox dependent mechanism. *Anesthesiology*, **98**(5):1155-1163. [doi:10.1097/00000542-200305000-00018]

Obal, D., Dettwiler, S., Favoccia, C., Scharbatke, H., Preckel, B., Schlack, W., 2005. The influence of mitochondrial KATP-channels in the cardioprotection of preconditioning and postconditioning by sevoflurane in the rat in vivo. *Anesth. Analg.*, **101**(5):1252-1260. [doi:10.1213/01.ANE.0000181336.96511.32]

Palmer, B.S., Hadziahmetovic, M., Veci, T., Angelos, M.G., 2004. Global ischemic duration and reperfusion function in the isolated perfused rat heart. *Resuscitation*, **62**(1):97-106. [doi:10.1016/j.resuscitation.2003.12.027]

Park, J.L., Lucchesi, B.R., 1999. Mechanisms of myocardial reperfusion injury. *Ann. Thorac. Surg.*, **68**(5):1905-1912. [doi:10.1016/S0003-4975(99)01073-5]

Riess, M.L., Camara, A.K., Chen, Q., Novalija, E., Rhodes, S.S., Stowe, D.F., 2002. Altered NADH and improved function by anesthetic and ischemic preconditioning in guinea pig intact hearts. *Am. J. Physiol. Heart Circ. Physiol.*, **283**(1):H53-H60.

Riess, M.L., Kevin, L.G., Camara, A.K., Heisner, J.S., Stowe, D.F., 2004. Dual exposure to sevoflurane improves anesthetic preconditioning in intact hearts. *Anesthesiology*, **100**(3):569-574. [doi:10.1097/00000542-200403000-00016]

Sasaki, H., Shimizu, M., Ogawa, K., Okazaki, F., Taniguchi, M., Taniguchi, I., Mochizuki, S., 2007. Brief ischemia-reperfusion performed after prolonged ischemia (ischemic postconditioning) can terminate reperfusion arrhythmias with no reduction of cardiac function in rats. *Int. Heart J.*, **48**(2):205-213. [doi:10.1536/ihj.48.205]

Stehr, S.N., Pexa, A., Hannack, S., Heintz, A., Heller, A.R., Deussen, A., Koch, T., Hubler, M., 2007. Insulin effects on myocardial function and bioenergetics in L-bupivacaine toxicity in the isolated rat heart. *Eur. J. Anaesthesiol.*, **24**(4):340-346. [doi:10.1017/S0265021506002109]

Tiwari, M., Hemalatha, T., Ganesan, K., Nayeem, M., Manohar, B.M., Balachandran, C., Vairamuthu, S., Subramaniam, S., Puvanakrishnan, R., 2008. Myocardial ischemia and reperfusion injury in rats: lysosomal hydrolases and matrix metalloproteinases mediated cellular damage. *Mol.*

Cell. Biochem., **312**(1-2):81-91. [doi:10.1007/s11010-008-9723-7]

Tsutsumi, Y.M., Yokoyama, T., Horikawa, Y., Roth, D.M., Patel, H.H., 2007. Reactive oxygen species trigger ischemic and pharmacological postconditioning: in vivo and in vitro characterization. *Life Sci.*, **81**(15):1223-1227. [doi:10.1016/j.lfs.2007.08.031]

Walker, M.J., Curtis, M.J., Hearse, D.J., Campbell, R.W., Janse, M.J., Yellon, D.M., Cobbe, S.M., Coker, S.J., Harness, J.B., Harron, D.W., 1988. The Lambeth Conventions: guidelines for the study of arrhythmias in ischaemia infarction, and reperfusion. *Cardiovasc. Res.*, **22**(7):447-455. [doi:10.1093/cvr/22.7.447]

Walpole, R., Logan, M., 1999. Effect of sevoflurane concentration on inhalation induction of anaesthesia in the elderly. *Br. J. Anaesth.*, **82**(1):20-24.

Wang, C., Neff, D.A., Krolikowski, J.G., Weihrauch, D., Bienengraeber, M., Warltier, D.C., Kersten, J.R., Pagel, P.S., 2006. The influence of B-cell lymphoma 2 protein, an antiapoptotic regulator of mitochondrial permeability transition, on isoflurane-induced and ischemic postconditioning in rabbits. *Anesth. Analg.*, **102**(5):1355-1360. [doi:10.1213/01.ane.0000202463.28618.64]

Weber, N.C., Schlack, W., 2008. Inhalational Anaesthetics and Cardioprotection. *In*: Handbook of Experimental Pharmacology. Modern Anesthetics. Vol. 182, Springer Berlin Heidelberg, p.187-207. [doi:10.1007/978-3-540-74806-9_9]

Weihrauch, D., Krolikowski, J.G., Bienengraeber, M., Kersten, J.R., Warltier, D.C., Pagel, P.S., 2005. Morphine enhances isoflurane-induced postconditioning against myocardial infarction: the role of phosphatidylinositol-3-kinase and opioid receptors in rabbits. *Anesth. Analg.*, **101**(4):942-949. [doi:10.1213/01.ane.0000171931.08371.a2]

Wit, A.L., Janse, M.J., 2001. Reperfusion arrhythmias and sudden cardiac death: a century of progress toward an understanding of the mechanisms. *Circ. Res.*, **89**(9):741-743.

Yan, M., Chen, C., Zhang, F., Chen, G., 2008. Lidocaine abolishes the myocardial protective effect of sevoflurane post-conditioning. *Acta Anaesthesiol. Scand.*, **52**(1):111-116.

Zhang, F.J., Chen, G., Chen, C., Yan, M., 2009. Sevoflurane postconditioning converts persistent ventricular fibrillation into regular rhythm. *Eur. J. Anaesthesiol.*, **26**(9):766-771. [doi:10.1097/EJA.0b013e32832a58fa]