Activation of Akt and cardioprotection against reperfusion injury are maximal with only five minutes of sevoflurane postconditioning in isolated rat hearts

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Abstract: It had been proved that administration of sevoflurane for the first two minutes of reperfusion effectively protects the heart against reperfusion injury in rats in vivo. Our aim was to investigate the duration of effective sevoflurane administration and its underlying mechanism in isolated rat hearts exposed to global ischemia/reperfusion (I/R) injury. Adult male Sprague-Dawley rats were randomly divided into six groups (n=12): a sham-operation group, an I/R group, and four sevoflurane postconditioning groups (S2, S5, S10, and S15). In the S2, S5, S10, and S15 groups, the duration times of sevoflurane administration were 2, 5, 10, and 15 min after the onset of reperfusion, respectively. The isolated rat hearts were mounted on the Langendorff system, and after a period of equilibrium were subjected to 40 min global ischemia and 120 min reperfusion. Left ventricular (LV) hemodynamic parameters were monitored throughout each experiment and the data at 30 min of equilibrium and 30, 60, 90, and 120 min of reperfusion were analyzed. Myocardial infarct size at the end of reperfusion (n=7 in each group) and the expression of myocardial phosphorylated Akt (p-Akt) after 15-min reperfusion were determined in a duplicate set of six groups of rat hearts (n=5 in each group). Compared with the I/R group, the S5, S10, and S15 groups had significantly improved left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP), and the maximal rate of rise or fall of the LV pressure (±dP/dtmax), and decreased myocardial infarct size (P<0.05), but not the S2 group. After 15 min of reperfusion, the expression of p-Akt was markedly up-regulated in the S5, S10, and S15 groups compared with that in the I/R group (P<0.05), but not in the S2 group. Sevoflurane postconditioning for 5 min was sufficient to activate Akt and exert maximal cardioprotection against I/R injury in isolated rat hearts.

Key words: Sevoflurane postconditioning, Ischemia/reperfusion (I/R) injury, Cardioprotection, Duration of administration, Akt


1 Introduction

Administering inhaled anesthetics, such as isoflurane and sevoflurane, at the onset of reperfusion, so called anesthetic postconditioning (APO), has been well demonstrated to provide cardioprotection against ischemia/reperfusion (I/R) injury in extensive animal experiments (Chen et al., 2008; Redel et al., 2009;
Pravdic et al., 2010). Because ischemia is usually unpredictable and happens suddenly, APO, which can be applied after ischemia, is attracting considerable clinical attention. However, the duration of effective APO administration both in patients and animals remains controversial, and the effect of APO against the myocardial I/R injury in patients is not as potent as in animals, partly due to the distinct administration time of APO adopted in different studies (Smul et al., 2009).

Sevoflurane is widely used in cardiac surgery, since induction and recovery with sevoflurane are faster and smoother than those with other inhaled anesthetics (Wallin et al., 1975; Sakai et al., 2005). A meta-analysis showed that sevoflurane reduces the rate of myocardial infarct size and mortality in patients undergoing cardiac surgery, though the underlying mechanism remains unclear (Landoni et al., 2007). Several recent studies have confirmed that sevoflurane postconditioning spares myocardial infarct size and improves contractile functions in I/R animals (Inamura et al., 2010; Yao et al., 2010b; Yu et al., 2010; Zheng et al., 2011). It is widely accepted that activating the phosphatidylinositol-3-kinase (PI3K)/Akt pathway is pivotal to cardioprotection by sevoflurane postconditioning against I/R injury (Yao et al., 2010a; Yu et al., 2010). However, there was no agreement among the different studies about the optimum duration of effective sevoflurane administration, even in similar myocardial I/R models. The protection of rat hearts by sevoflurane postconditioning was achieved within the first few minutes of reperfusion (Obal et al., 2003; Inamura et al., 2010). Longer administration (more than two minutes) of sevoflurane had no extra cardioprotective effects (Obal et al., 2003), although it was reported to work after 15 min in some cases (He et al., 2008; Yao et al., 2010b). It seems that sevoflurane postconditioning needs time to activate downstream effectors, even though the exact duration of postconditioning and, especially, the underlying mechanisms remain unclear. Therefore, the purpose of this study was to investigate the exact duration of effective sevoflurane postconditioning in isolated rat hearts subjected to global I/R injury and to determine whether this time effect is related to the activation of Akt.

2 Materials and methods

2.1 Animals

Male Sprague-Dawley rats (230–250 g) were obtained from the Experimental Animal Center of Zhejiang Academy of Medical Sciences, China. All procedures were performed according to protocols approved by the Institutional Committee for Use and Care of Laboratory Animals published by the US National Institutes of Health (NIH Publication Nos. 85–23, revised in 1996). The experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Zhejiang University, China.

2.2 Reagents

Sevoflurane was purchased from the Maruishi Pharmaceutical Company (Osaka, Japan) and 2,3,5-triphenyltetrazolium chloride (TTC) from the Sigma-Aldrich Inc. (USA). Rabbit monoclonal Akt and phospho-Akt (p-Akt, Ser473) antibodies were purchased from the Cell Signaling Technology (USA). Unless indicated otherwise, all other chemicals were of analytical purity.

2.3 Isolated perfused rat heart preparation

Rats of 230–250 g were anesthetized [40 g/L chloral hydrate, 8 ml/kg intraperitoneal (i.p.)] and heparinized (500 U/kg, i.p.). After median sternotomy, the heart was rapidly isolated and perfused on the Langendorff apparatus at (37±0.1) °C with a constant pressure (80 mmHg) using Krebs-Henseleit (K-H) buffer composed of (mmol/L): NaCl 118, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, CaCl2 1.25, and glucose 10, and equilibrated with a mixture of 95% O2 and 5% CO2 (pH 7.4). A latex, fluid-filled balloon was introduced into the left ventricle via the left atrium, and the balloon catheter was linked to a pressure transducer connected to the physiological signal acquisition system (PowerLab, ADInstruments Shanghai Trading Co., Ltd., China) to monitor the contractile function. At the beginning of perfusion, the left ventricular end-diastolic pressure (LVEDP) was adjusted to 4–6 mmHg. All hearts were allowed to equilibrate for 30 min. After that, the flow in the I/R group was turned off for 40 min to elicit global ischemia, and then the hearts were reperfused for 120 min. Left ventricular developed pressure (LVDP),