

# Sevoflurane induces cardioprotection through reactive oxygen species-mediated upregulation of autophagy in isolated guinea pig hearts

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Received: 10 September 2013 / Accepted: 11 November 2013 / Published online: 12 December 2013  
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## Abstract

**Purpose** Sevoflurane increases reactive oxygen species (ROS), which mediate cardioprotection against myocardial ischemia–reperfusion injury. Emerging evidence suggests that autophagy is involved in cardioprotection. We examined whether reactive oxygen species mediate sevoflurane preconditioning through autophagy.

**Methods** Isolated guinea pigs hearts were subjected to 30 min ischemia followed by 120 min reperfusion (control). Anesthetic preconditioning was elicited with 2 % sevoflurane for 10 min before ischemia (SEVO). The ROS-scavenger, *N*-(2-mercaptopyrionyl) glycine (MPG, 1 mmol/l), was administered starting 30 min before ischemia to sevoflurane-treated (SEVO + MPG) or non-sevoflurane-treated (MPG) hearts. Infarct size was determined by triphenyltetrazolium chloride stain. Tissue

samples were obtained after reperfusion to determine autophagy-related protein (microtubule-associated protein light chain I and II: LC3-I, -II) and 5' AMP-activated protein kinase (AMPK) expression using Western blot analysis. Electron microscopy was used to detect autophagosomes.

**Results** Infarct size was significantly reduced and there were more abundant autophagosomes in SEVO compared with control. Western blot analysis revealed that the ratio of LC3-II/I and phosphorylation of AMPK were significantly increased in SEVO. These effects were abolished by MPG.

**Conclusions** Sevoflurane induces cardioprotection through ROS-mediated upregulation of autophagy.

**Keywords** Sevoflurane · Preconditioning · Autophagy · Ischemia–reperfusion · Reactive oxygen species

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## Introduction

Sevoflurane is a popular anesthetic with few clinical side effects and is used worldwide. During the past 15 years, experimental [1–5] and clinical studies [6] have demonstrated that volatile anesthetics, including sevoflurane, exert cardioprotective effects against myocardial ischemia–reperfusion injury such as ischemic preconditioning where brief episodes of ischemia and reperfusion before prolonged ischemia dramatically limit infarct size [7]. Many potential mechanisms for this cardioprotection, known as anesthetic preconditioning (APC), have been proposed [8]. Novalija et al. [9] demonstrated that APC by sevoflurane is initiated by reactive oxygen species (ROS) produced during sevoflurane exposure and reduces the burst formation

of ROS after ischemia–reperfusion in isolated guinea pig hearts. Recently, cardioprotection by sevoflurane has been shown to be mediated by ROS-induced 5' AMP-activated protein kinase (AMPK) activation, which is a well-known regulator of cellular energy status [10].

AMPK is a key enzyme for the initiation of autophagy, which is a tightly regulated intracellular catabolic process for the disposal of damaged and dysfunctional organelles and protein aggregates to maintain homeostasis [11]. Growing evidence suggests that autophagy is activated during various pathological conditions, including myocardial ischemia–reperfusion injury [12]. It has been reported that excessive activation of autophagy can be detrimental and may even lead to the cell death called autophagic cell death [13]. In contrast, Sala-Mercado et al. [14] demonstrated that pre-ischemic induction of autophagy limits myocardial infarct size in *in vivo* swine hearts. Qiao et al. [15] demonstrated that cardioprotection of delayed preconditioning by sevoflurane is mediated by upregulation of autophagy. However, it has not been determined whether activation of autophagy is beneficial or detrimental in myocardial ischemia–reperfusion injury. Although ROS mediate sevoflurane preconditioning, it is not known whether autophagy is involved in this ROS-induced cardioprotection. Thus, we hypothesized that ROS produced during sevoflurane exposure mediates sevoflurane cardiac preconditioning through activation of autophagy. Elucidating the role of autophagy in sevoflurane preconditioning could prove clinically useful in patients at cardiovascular risk during perioperative periods.

## Materials and methods

This study was conducted in accordance with the Guidelines for Animal Research at Osaka Dental University, and with the approval of the Animal Experiment Committee of Osaka Dental University, Osaka, Japan. These guidelines conform to those laid out in the Guide for the Care and Use of Laboratory Animals, available from the National Academy of Science. Male Hartley guinea pigs were fed Lab Diet (RC4; Oriental Yeast, Tokyo, Japan) and given water *ad libitum*. A ROS scavenger, *N*-(2-mercaptopyrroline) glycine (MPG), was purchased from Sigma Aldrich (Ann Arbor, MI, USA).

### Isolated heart perfusion and measurement of function

Male guinea pigs weighing 550–700 g (12–13 weeks old) were given heparin (1,000 units intraperitoneally), then anesthetized with pentobarbital (60 mg/kg, intraperitoneally). Hearts were excised and immediately arrested in cold iso-osmotic saline containing 20 mM KCl. The aorta was

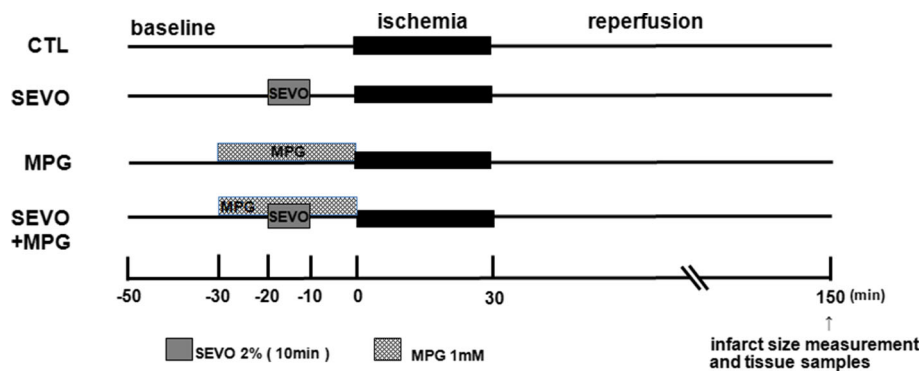
cannulated and the isolated hearts were perfused at 70 mmHg on a nonrecirculating isovolumic perfused heart apparatus, using a Krebs-Henseleit perfusate and paced at 240 beats/min as previously described [3]. Left ventricular developed pressure (LVDP, mmHg) was measured using a 2.5 French, high-fidelity micromanometer (Nihon-Kohden, Tokyo, Japan) passed into a compliant latex balloon, inserted into the left ventricle, and recorded on a PowerLab 2/20 Data Recording System (ADInstruments, Hayward, Australia). The balloon was connected to a Y-adaptor with one end used to advance the micromanometer and the other used to fill the left ventricular balloon with bubble-free water to an end-diastolic pressure (LVEDP) of 10 mmHg. Coronary flow (CF, ml/min) was measured by collecting effluent. Global ischemia was achieved by clamping the aortic inflow line. During ischemia, hearts were maintained at 37 °C by enclosure in a water-jacketed air chamber. Warmed perfusate kept in the lower part of the chamber saturated the air with humidity and prevented cooling by evaporation. Heart temperature was continuously monitored by a digital thermometer (PTW-100A; Unique Medical, Tokyo, Japan). Sevoflurane was insufflated by passing the 95 %O<sub>2</sub>/5 %CO<sub>2</sub> gas mixture through a calibrated vaporizer (ACOMA, Tokyo, Japan).

### Measurement of sevoflurane concentration

Samples of coronary perfusate were collected anaerobically from the aortic cannula for measurement of sevoflurane concentration by an organic vapor sensor (OSP, Saitama, Japan). The organic vapor sensor is designed to detect volatile organic compounds (VOC) in the air. Before measurement of sevoflurane concentration, calibration of the organic vapor sensor was performed to make a standard VOC (ppm)–sevoflurane (mmol/l) curve. The concentration of sevoflurane was determined using the well-described headspace method [16]. The 150 ml vial containing perfusate obtained from the aortic cannula with closed air space in the upper portion (headspace) was shaken for 1 min. After reaching equilibration in the vial, the organic vapor sensor was connected to the vial to measure VOC (ppm) in the closed air space. The obtained data (ppm) were converted to the concentration of sevoflurane in the perfusate (mmol/l) using a standard VOC–sevoflurane curve. This concentration correlates well with that measured by the headspace method with gas chromatography that is usually used to measure sevoflurane concentration.

### Experimental protocol

Animals were assigned to one of four groups ( $n = 8$  each; Fig. 1). After a 20 min equilibration, baseline LVDP, LVEDP, and CF were recorded. Hearts were subjected to



**Fig. 1** Schematic illustration of the experimental protocol using four groups. After a 20 min baseline period, isolated perfused hearts were subjected to 30-min global ischemia and 120-min reperfusion. Anesthetic preconditioning was elicited by administration of 10 min of SEVO (2 %) with 10 min washout before 30 min ischemia. MPG

was administered starting 30 min before ischemia in hearts from SEVO-treated or non-SEVO-treated animals. Tissue samples were obtained after reperfusion. *CTL* control, *MPG* *N*-(2-mercaptopropionyl) glycine, *SEVO* sevoflurane

30 min (control; CTL) of ischemia followed by 120 min of reperfusion. Anesthetic preconditioning was elicited by administration of sevoflurane (2 %) for 10 min followed by 10 min washout before 30 min (SEVO). A ROS scavenger, MPG (1 mmol/l), was administered starting 30 min before ischemia in sevoflurane-treated (SEVO + MPG) or non-sevoflurane-treated (MPG) hearts. MPG was dissolved in distilled water and was added to the Krebs-Henseleit perfusate to a final concentration of 1 mmol/l.

#### Determination of myocardial infarct size

At the end of experiments, hearts were quickly frozen at  $-80^{\circ}\text{C}$  for 15 min, and then sliced into 2 mm thick transverse sections from apex to base (6 slices/heart). After removing the right ventricle and defrosting, each slice was weighed and incubated at  $37^{\circ}\text{C}$  with 1 % triphenyltetrazolium chloride (Sigma Chemicals) in phosphate buffer (pH 7.4) for 10 min and then fixed in 10 % formalin for at least 5 h to distinguish red-stained viable tissue from pale unstained necrotic tissue [17]. Each slice was photographed, and the necrotic area was determined using Adobe Photoshop CS (Adobe, San Jose, CA, USA) and multiplied by the weight of the slice, then expressed as a fraction of the left ventricle.

#### Western blot analysis

Separate experiments were performed ( $n = 4$  for each group) to examine expression of autophagy-related protein (microtubule-associated protein light chain I and II, LC3-I, II) and AMPK. Myocardial tissue samples were collected after 120 min reperfusion and homogenized in ice-cold homogenizing buffer containing 250 mM sucrose, 20 mM HEPES (pH 7.5), 10 mM KCl, 2 mM EGTA, 2 mM  $\text{MgCl}_2$ , 25 mM NaF, 50 mM  $\beta$ -glycerophosphate, 1 mM

$\text{Na}_3\text{VO}_4$ , 1 mM PMSF, and protease inhibitor leupeptin (10  $\mu\text{g/ml}$ ). The homogenate was centrifuged at 1,000 g for 5 min at  $4^{\circ}\text{C}$  to clean up. The supernatant was recentrifuged at 10,000 g for 15 min at  $4^{\circ}\text{C}$  to obtain the cytosolic fraction. The protein concentration was estimated with a Bradford assay. Equivalent amounts (20  $\mu\text{g}$ ) of protein samples were loaded and separated on 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gradient gel, then electrically transferred overnight to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). After blocking with 5 % skim milk in Tris-buffered saline containing 0.1 % Tween-20 (TBS-T), the membranes were incubated for 2 h at  $4^{\circ}\text{C}$  in TBS-T containing 5 % skim milk and overnight 1:1,000 dilution of rabbit primary antibody for LC3 (Medical & Biological Laboratories, Boston, MA, USA), phospho-AMPK- $\alpha$  (Thr172), and total AMPK (Cell Signaling Technology, Boston, MA, USA). Membranes were incubated with a 1:1,000 dilution of horseradish peroxidase-labeled anti-rabbit immunoglobulin G (NA 934 V; GE Healthcare, Buckinghamshire, UK). The same blot was stripped and reblotted with antibodies to  $\alpha$ -tubulin (Santa Cruz Biotechnology) to confirm equal protein loading. Bound antibody signals were detected with enhanced chemiluminescence (Pierce Biotechnology, Rockford, IL, USA) and visualized using the VersaDoc 5000 Imaging System (Bio-Rad). Quantitative analysis of the band densities was performed by Quantity One software (Bio-Rad), and the results are presented as the ratio of phospho-AMPK to total AMPK. The average light intensity was multiplied by 100 to facilitate presentation of an x-fold increase.

#### Electron microscopy

To confirm the induction of autophagy, cardiac specimens were obtained at 120 min of reperfusion ( $n = 4$  for each

group). They were fixed with phosphate-buffered 2.5 % glutaraldehyde (pH 7.4) and postfixed with 1 % osmium tetroxide, after which they were conventionally prepared for transmission electron microscopy (H-800; Hitachi, Tokyo, Japan). We performed a morphometric analysis under an electron microscope using the method described previously [18] with some modification. In brief, a uniform sampling of electron micrographs was used for the morphometric assay of each group. Ten random fields micrographed at 6,000 $\times$  from each of three blocks were printed at a final magnification of 15,000 $\times$  and analyzed on composite grids as described previously to calculate the density of autophagic vacuoles (number/100  $\mu\text{m}^2$ ), i.e., both autophagosomes and autolysosomes, within a cardiomyocyte.

#### Statistical analysis

All data are expressed as mean  $\pm$  SD. Statistical power analysis revealed that a sample size of  $n = 8$  would provide sufficient power (0.8) to detect a difference between mean infarct size indices of 15 % (SD = 9,  $\alpha = 0.05$ ). A group size of  $n = 4$  was used for Western blot to provide a power of 0.8 to detect a difference between means of 20 % (SD = 10,  $\alpha = 0.05$ ). Hemodynamic data were tested for normal distribution and subsequently analyzed by a two-

factor repeated-measures analysis of variance (ANOVA) for time and treatment. If an overall difference between the variables was observed, comparisons were performed as one-way ANOVA followed by Tukey's post hoc test for intergroup differences and by Dunnett's for intragroup differences with baseline values as the reference time point. Analyses of infarct size and Western blot were performed using one-way ANOVA followed by Student's  $t$  test with Bonferroni's correction for multiple comparisons to avoid type I error. For changes within and between groups, a two-tailed  $p$  value  $<0.05$  was considered significant in advance. (SPSS17 for Windows; SPSS Japan, Tokyo, Japan).

#### Results

Of a total of 66 hearts, 1 heart in the SEVO + MPG group was not used secondary to intractable ventricular fibrillation after reperfusion and 1 heart was not used because of aortic rupture. Additional hearts were studied until each group had  $n = 8$  successful experiments for the infarct size study. There was no significant difference in body weight among groups. The concentration of sevoflurane in the coronary perfusate after 10 min of exposure was  $0.25 \pm 0.02$  mM. Sevoflurane was not detected in the effluent during the baseline, ischemic, and reperfusion periods.

**Table 1** Hemodynamic variables

	Baseline	After treatment (SEVO/MPG)	Before ischemia	Reperfusion (min)		
				30	60	120
<b>LVDP (mmHg)</b>						
CTL	113 $\pm$ 15	117 $\pm$ 13	116 $\pm$ 13	39 $\pm$ 7	34 $\pm$ 9	26 $\pm$ 6
SEVO	101 $\pm$ 13	100 $\pm$ 12	103 $\pm$ 12	63 $\pm$ 7*	60 $\pm$ 8*	52 $\pm$ 8*
MPG	113 $\pm$ 22	107 $\pm$ 23	104 $\pm$ 24	46 $\pm$ 19*	48 $\pm$ 15*	48 $\pm$ 9*
SEVO + MPG	102 $\pm$ 17	108 $\pm$ 21	110 $\pm$ 21	51 $\pm$ 18*	50 $\pm$ 17*	46 $\pm$ 16*
<b>LVEDP (mmHg)</b>						
CTL	10 $\pm$ 0	11 $\pm$ 2	11 $\pm$ 2	50 $\pm$ 10	55 $\pm$ 15	57 $\pm$ 15
SEVO	10 $\pm$ 0	10 $\pm$ 3	10 $\pm$ 2	24 $\pm$ 7*	23 $\pm$ 4*	23 $\pm$ 5*
MPG	10 $\pm$ 0	11 $\pm$ 7	11 $\pm$ 7	48 $\pm$ 26	48 $\pm$ 25	40 $\pm$ 26
SEVO + MPG	10 $\pm$ 0	6 $\pm$ 6	6 $\pm$ 6	39 $\pm$ 16	36 $\pm$ 16	35 $\pm$ 18*
<b>CF (ml/min)</b>						
CTL	27 $\pm$ 6	28 $\pm$ 6	28 $\pm$ 6	20 $\pm$ 4	19 $\pm$ 4	18 $\pm$ 4
SEVO	32 $\pm$ 8	28 $\pm$ 5	27 $\pm$ 5	22 $\pm$ 4	21 $\pm$ 5	21 $\pm$ 5
MPG	30 $\pm$ 9	30 $\pm$ 9	29 $\pm$ 8	22 $\pm$ 6	20 $\pm$ 6	19 $\pm$ 7
SEVO + MPG	26 $\pm$ 5	26 $\pm$ 5	27 $\pm$ 4	21 $\pm$ 7	18 $\pm$ 6	16 $\pm$ 6

Data are presented as mean  $\pm$  SD

LVDP left ventricular developed pressure, LVEDP left ventricular end-diastolic pressure, CF coronary flow, CTL control, MPG N-2-mercapto-propionyl glycine, SEVO sevoflurane

\*  $p < 0.05$  versus CTL;  $n = 8$  for each group

## Hemodynamics

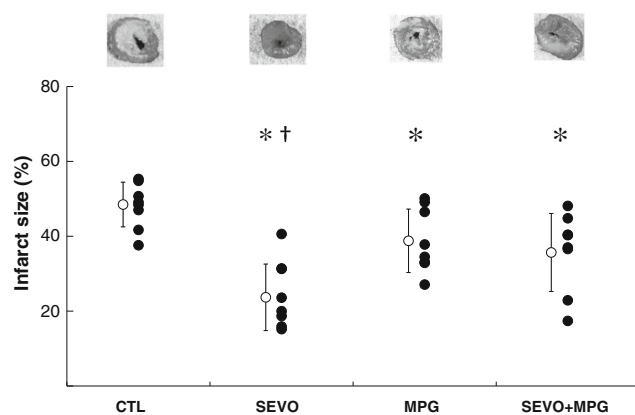
Hemodynamic data are shown in Table 1. Baseline LVDP and CF were similar among the four groups. Treatment of sevoflurane and/or MPG did not significantly change LVDP, LVEDP, and CF before ischemia. Recovery of LVDP was significantly greater in SEVO, MPG, and SEVO + MPG compared with CTL throughout the reperfusion period. After 120 min reperfusion, SEVO had significantly lower LVEDP compared to CTL. There was no significant difference in CF among all the groups throughout the experiment, suggesting that changes in CF could not account for the improved contractile recovery of the SEVO, MPG, and SEVO + MPG groups.

## Myocardial infarct size

Myocardial infarct size data are shown in Fig. 2. Myocardial infarct size in SEVO was significantly reduced by approximately 50 % compared with CTL hearts (SEVO  $25 \pm 8$  % vs. CTL  $48 \pm 6$  %,  $p < 0.05$ ). This cardioprotection achieved by sevoflurane was abolished by MPG (SEVO  $25 \pm 8$  % vs. SEVO + MPG  $36 \pm 10$  %,  $p < 0.05$ ). Treatment with MPG alone decreased the infarct size compared with CTL (MPG  $39 \pm 8$  % vs. CTL  $48 \pm 6$  %,  $p < 0.05$ ).

## Electron microscopy

Figure 3 shows representative electron micrographs of each study group. Although autophagic vacuoles were abundantly seen in cardiomyocytes from the untreated control heart



**Fig. 2** Infarct size as a percentage of left ventricle in four groups. After 120 min reperfusion, infarct size was significantly reduced in SEVO compared to CTL. This cardioprotection of SEVO was abolished by MPG. MPG alone decreased infarct size compared to CTL. Data are presented as mean  $\pm$  SD. \* $p < 0.05$  versus CTL; † $p < 0.05$  versus MPG and SEVO + MPG. CTL control; MPG *N*-(2-mercaptopropionyl) glycine, SEVO sevoflurane.  $n = 8$  for each group

(Fig. 3a, CTL), typical autophagosomes containing intracellular organelles, such as mitochondria and membrane-like structures, were increased even more after sevoflurane administration (Fig. 3b, SEVO). Autophagosomes were apparently diminished by addition of MPG (Fig. 3c, d, MPG and SEVO + MPG). Morphometric analysis of autophagosome density confirmed these findings (Fig. 3e).

## Western blot analysis

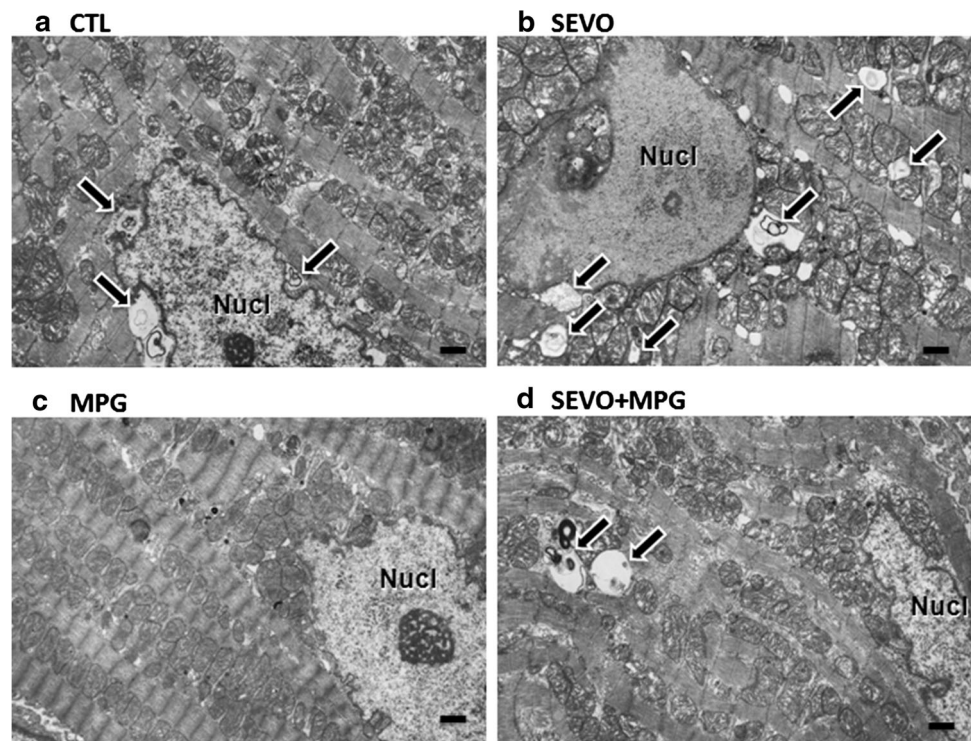
LC3-I, II, total AMPK, and the phosphorylation state of AMPK after treatment with sevoflurane and MPG are illustrated by a representative Western blot in Fig. 4a. In hearts pretreated with sevoflurane, myocardial expression of LC3-II was increased compared with control hearts. This increased expression of LC3-II was abolished by administration of MPG (Fig. 4b). The ratio of phospho-AMPK to total AMPK was significantly increased in SEVO compared with CTL (Fig. 4c). This increase was not caused by unequal loading of the Western blot, as shown by the detection of  $\alpha$ -tubulin. The combination of sevoflurane and MPG abolished this increased expression in SEVO.

## Discussion

This study demonstrated that ROS produced during sevoflurane exposure have a role in sevoflurane-induced cardioprotection through autophagy. Growing evidence suggests that ROS and autophagy are involved in cardioprotection by ischemic and volatile anesthetics preconditioning. The present study for the first time showed a link between these two mediators in sevoflurane-induced cardioprotection.

It was well documented that a burst ROS formation during the very early reperfusion period after myocardial ischemia–reperfusion causes serious damage to cardiomyocytes [19]. Scavenging ROS have been shown to attenuate myocardial reperfusion injury [20]. This finding is consistent with the present study demonstrating that treatment with MPG alone before ischemia reduced infarct size compared with control. MPG is a highly diffusible low molecular weight compound that scavenges superoxide and hydroxyl radicals produced both intra- and extracellularly [21, 22]. In contrast, a low level of ROS was reported to function as a protective second messenger during ischemic preconditioning [23]. It has been shown that APC by sevoflurane is triggered by production of ROS during the preconditioning period [24]. In turn, this generation of ROS reduces the burst production of ROS during the very early reperfusion period, which results in reduction of myocardial infarct size. In the present study, administration of MPG to sevoflurane-treated hearts abolished infarct size,

**Fig. 3** Electron micrographs of cardiomyocytes. Autophagic vacuoles were seen in cardiomyocytes from the untreated control (a). Typical autophagosomes containing intracellular organelles, such as mitochondria and membrane-like structures (arrows), were increased more after sevoflurane administration (b, SEVO). Autophagosomes were apparently diminished by addition of MPG (c, MPG; d, SEVO + MPG). e Graph shows density of autophagic vacuoles (number/100  $\mu\text{m}^2$ ) within a cardiomyocyte in each group. † $p < 0.05$  versus other groups. Nucl nucleus, CTL control, MPG N-(2-mercaptopropionyl) glycine, SEVO sevoflurane. Bars 1  $\mu\text{m}$

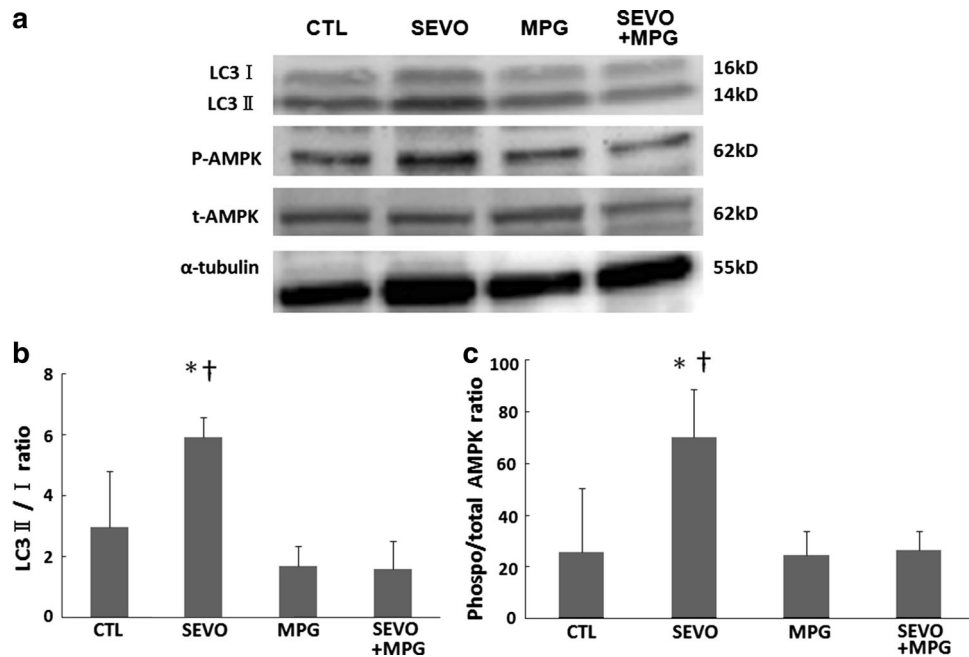


limiting effect of sevoflurane preconditioning. This result suggests that production of ROS during sevoflurane exposure plays an important role in sevoflurane preconditioning.

There were no significant differences in LVDP and LVEDP among the groups at 120 min of reperfusion (e.g., SEVO vs. MPG or SEVO + MPG), despite the difference in infarct size. A possible reason for this discrepancy may be stunning caused by global ischemia in a much greater area than the infarcted area of LV. Cohen et al. [25] demonstrated that the enhanced functional recovery resulting from infarct size reduction by preconditioning becomes apparent 1–3 days later because of stunning.

Autophagy is a bulk degradation process in which cytosolic proteins and organelles are degraded through lysosomes: this is an evolutionally conserved process

crucial for normal tissue homeostasis. Accumulating evidence suggests that autophagy is stimulated by ischemia and actually contributes to cardiomyocyte survival [26, 27]. A recent study has shown that autophagy induced by ischemic preconditioning is essential for cardioprotection [28]. McCormick et al. [29] demonstrated that enhancing autophagy confers protection against ischemia–reperfusion injury in cardiomyocytes. Using in vivo swine models, Sala-Mercado et al. [14] showed that induction of autophagy by chloramphenicol succinate before ischemia limited infarct size. However, it is not known whether this upregulation of autophagy is mediated by ROS produced during sevoflurane exposure. Recently, Dai and Rabinovitch [30] demonstrated that mitochondrial oxidative stress mediates induction of



**Fig. 4** **a** Representative Western blot analysis of LC3-I, LC3-II, phosphorylated AMPK, and total AMPK from left ventricular samples obtained after 120 min of reperfusion. **b** Ratio of LC3-II to LC3-I (LC3-II/I) was increased in SEVO compared to CTL. Administration of MPG abolished this increased LC3-II/I in the SEVO group. \* $p < 0.05$  versus CTL; † $p < 0.05$  versus MPG and SEVO + MPG. **c** Densitometric evaluation of four experiments as the x-fold increase in average light density versus CTL. The results are

presented as the ratio of the phosphorylated AMPK to total AMPK. The average light intensity was multiplied by 100 to facilitate the presentation of an x-fold increase. Data are presented as mean  $\pm$  standard deviation. \* $p < 0.05$  versus CTL; † $p < 0.05$  versus MPG and SEVO + MPG. AMPK 5' AMP-activated protein kinase, CTL control, LC3-I, II microtubule-associated protein light chain I and II, MPG N-(2-mercapto-propionyl) glycine, SEVO sevoflurane

autophagy in angiotensin II-treated mouse hearts. Hariharan et al. [31] have shown that oxidative stress stimulates autophagic flux during myocardial ischemia–reperfusion. In the present study, electron microscopy demonstrated that there were more abundant autophagosomes in sevoflurane-treated hearts than in the controls. This upregulation of autophagy was abolished by MPG. Furthermore, Western blot analysis showed that the ratio of LC3 II/I (a marker of autophagy) and phosphorylation of AMPK were increased in sevoflurane preconditioning, which was abolished by MPG. Both LC3 and AMPK are key enzymes for formation of autophagosomes. Cardiac AMPK activation has been shown to be linked to inhibition of GSK3 $\beta$  [32], which is a critical mediator of cardioprotection by APC [5], suggesting that ROS produced during sevoflurane exposure induced upregulation of autophagy in sevoflurane preconditioning.

The following study limitations should be acknowledged. The isolated Langendorff-perfused heart may not completely reflect conditions in vivo. However, the ex vivo conditions of the present study were designed to exclude possible confounding systemic hemodynamic and humoral effects of sevoflurane. Although we did not directly measure ROS during sevoflurane exposure, the dose (1 mM) of MPG used in the present study has been shown to abolish the

infarct size-limiting effect of sevoflurane preconditioning [10].

In conclusion, sevoflurane exerts a cardioprotection against ischemia–reperfusion injury by ROS-mediated upregulation of autophagy. A more thorough understanding of the multiple signaling steps and the ultimate cytoprotective mechanisms underlying APC may lead to improvements in the management of patients at risk during perioperative periods.

**Acknowledgments** We thank Akiko Tsujimoto (Gifu University Graduate School of Medicine) for technical assistance. This study was conducted at the Laboratory Animal Facilities and Dental Bioscience I, Institute of Dental Research, Osaka Dental University, and was supported by Grant-in-Aid for Scientific Research (C) 23593008 from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Masami Miyamae) (Tokyo, Japan).

**Conflict of interest** The authors have no conflicts of interest to report.

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