Sevoflurane and nitrous oxide exert cardioprotective effects against hypoxia-reoxygenation injury in the isolated rat heart

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Abstract It is unclear whether nitrous oxide (N₂O) has a protective effect on cardiac function in vitro. In addition, little is known about the cardioprotective effect of anesthesia administered during hypoxia or ischemia. We therefore studied the cardioprotective effects of N₂O and sevoflurane administered before or during hypoxia in isolated rat hearts. Rat hearts were excised and perfused using the Langendorff technique. For hypoxia-reoxygenation, hearts were made hypoxic (95% N₂, 5% CO₂) for 45 min and then reoxygenated (95% O₂, 5% CO₂) for 40 min (control: CT group). Preconditioning was achieved through three cycles of application of 4% sevoflurane (sevo-pre group) or 50% N₂O (N₂O-pre group) for 5 min with 5-min washouts in between. Hypoxic conditions were achieved by administering the 4% sevoflurane (sevo-hypo group) or 50% N₂O (N₂O-hypo group) during the 45-min hypoxic period. L-type calcium channel currents (I_{Ca,L}) were recorded on rabbit myocytes. (1) Both 4% sevoflurane and 50% N₂O significantly reduced left ventricular developed pressure (LVDP). Sevoflurane also increased left ventricular end-diastolic pressure, though N₂O did not. (2) The recoveries of LVDP and pressure-rate product (PRP) after hypoxia-reoxygenation were better in the sevo-pre group than in the CT or N₂O-pre group. (3) Application of either sevoflurane or N₂O during hypoxia improved recovery of LVDP and PRP, and GOT release was significantly lower than in the CT group. (4) Sevoflurane and N₂O reduced I_{Ca,L} to similar extents. Although sevoflurane administered before or during hypoxia exerts a cardioprotective effect, while N₂O shows a cardioprotective effect only when administered during hypoxia.

Keywords Cardiac function · Cardioprotection · Hypoxia

Introduction

It is now well established that preconditioning with volatile anesthetics protects the heart against ischemia and reperfusion injury [1–7]. Comparatively little is known about the effects of anesthetic preconditioning (APC) with nitrous oxide (N₂O), though it has been shown that brief, repetitive administrations of 60% N₂O before prolonged coronary occlusion and reperfusion does not protect rat myocardium in vivo [8]. Little is also known about cardioprotective effect of anesthetic, including sevoflurane and N₂O, administered during hypoxia or ischemia. Our aim, therefore, was to determine whether preconditioning with sevoflurane or N₂O, or their administration during hypoxia, might exert a cardioprotective effect against hypoxia-reperfusion injury in the isolated rat heart.

Materials and methods

This investigation conformed to the Guide for the Care and Use of Laboratory Animals (US National Institute of Health publication, DHEW publication No. (NIH) 85-23, 1996) and was approved by the Juntendo University School of Medicine (Tokyo, Japan) animal experimentation committee.
Langendorff perfusion

Male Sprague-Dawley (SD) rats weighing 280–340 g were used. After anesthetizing the rats with diethyl ether, they were decapitated and their hearts were quickly excised for Langendorff perfusion. Each heart was perfused with modified Krebs–Henseleit solution (containing in mM: NaCl 116.0, NaHCO3 25.0, MgSO4 1.2, KCl 4.7, KH2PO4 1.2, CaCl2 2.0, glucose 5.5; pH 7.4) in a retrograde direction at a constant flow rate of 13 ml min\(^{-1}\) without recirculation. The perfusate was warmed to 38°C and oxygenated with a 95% O\(_2\)-5% CO\(_2\) gas mixture to maintain the partial pressure of O\(_2\) above 400 mmHg. During the period of hypoxia, glucose in the perfusate was replaced to adjust osmotic pressure with equimolar sucrose, and the solution was saturated with a 95% N\(_2\)-5% CO\(_2\) gas mixture to maintain the partial pressure of O\(_2\) at approximately 20 mmHg (hypoxic solution). In addition, a latex balloon was inserted through the mitral annulus into the left ventricular cavity, and distilled water (0.1–0.2 ml) was injected into the balloon until it was inflated to just above the level required to produce a visible (1–2 mmHg) elevation of the left ventricular end-diastolic pressure (LVEDP) and left ventricular developed pressure (LVDP), LVEDP, heart rate (HR) and coronary perfusion pressure were monitored throughout the experiments. The extent of irreversible myocardial damage was assessed by measuring the amount of glutamic oxaloacetic transaminase (GOT) released into the coronary effluent. GOT activities in 10-μl aliquots of coronary effluent were estimated using a dry chemical method with a commercially available kit (Fuji Film Ltd. Co., Tokyo, Japan). The released GOT activity was normalized to IU g\(^{-1}\) (dry tissue) min\(^{-1}\).

Experimental protocols

Effects of 4% sevoflurane or 50% N\(_2\)O on the function of rat hearts (Fig. 1a)

After stabilization for 20 min, hearts were perfused for 45 min with Krebs solution equilibrated with 95% O\(_2\) plus 5% CO\(_2\) in the time-matched control (TC; n = 6) group. In the N\(_2\)O (n = 5), sevoflurane (sevo; n = 6) and N\(_2\) (n = 6) groups, hearts were, respectively, perfused for 30 min with solutions equilibrated with N\(_2\)O (50% N\(_2\)O, 45% O\(_2\), 5% CO\(_2\)), sevoflurane (4% sevo, 91% O\(_2\), 5% CO\(_2\)), or N\(_2\) (50% N\(_2\), 45% O\(_2\), 5% CO\(_2\)), and then with normal Krebs solution for 15 min. Sevoflurane was vaporized using a vaporizer (Sevotec 3, Ohmeda, West Yorkshire, UK) and then mixed into 95% O\(_2\), 5% CO\(_2\), subsequently bubbled through the bathing solution. The volume percentage of sevoflurane in the gas phase above the Krebs–Henseleit solution was continuously monitored using an anesthetic gas analyzer (Capnomac Ultima, Datax, Helsinki, Finland).

Effects of preconditioning with 4% sevoflurane or 50% N\(_2\)O (Fig. 1b)

After stabilization for 20 min, hearts in the control group (CT; n = 7) were subjected to hypoxia for 45 min and reoxygenated for 40 min. In the sevoflurane-preconditioning (sevo-pre; n = 7) and N\(_2\)O-preconditioning (N\(_2\)O-pre; n = 6) groups, three cycles of application of sevoflurane (4% sevo, 91% O\(_2\), 5% CO\(_2\)) or N\(_2\)O (50% N\(_2\)O, 45% N\(_2\), 5% CO\(_2\)) for 5 min with 5-min washouts in between the anesthetics application were followed by hypoxia-reoxygenation.

Effects of 4% sevoflurane or 50% N\(_2\)O administered during hypoxia (Fig. 1c)

After stabilization for 20 min, hearts in the sevoflurane-hypoxia (sevo-hypo; n = 6) and N\(_2\)O-hypoxia (N\(_2\)O-hypo; n = 7) groups were perfused for 45 min with Krebs solution (sucrose), respectively, equilibrated with sevoflurane (4% sevo, 91% O\(_2\), 5% CO\(_2\)) or N\(_2\)O (50% N\(_2\)O, 45% N\(_2\), 5% CO\(_2\)) for 5 min with 5-min washouts in between the anesthetics application were followed by hypoxia-reoxygenation.

![Fig. 1](https://example.com/fig1.png)
L-type Ca\(^{2+}\) (I_{Ca,L}) current recording

For electrophysiological experiments, ventricular myocytes were enzymatically isolated from the hearts of Japanese white rabbits (1.6 kg). After anesthetizing the rabbits by injection of pentobarbital sodium (50 mg kg\(^{-1}\)) into the auricular vein, the hearts were excised, prepared for Langendorff perfusion, and then sequentially perfused at 37\(^\circ\)C with normal Tyrode solution (in mM: NaCl 135, KCl 5.4, CaCl\(_2\) 1.8, HEPES 10, glucose 10, pH 7.4) for 5 min, Ca\(^{2+}\)-free Tyrode for 5 min, collagenase (0.6 mg ml\(^{-1}\); Type I, Sigma, Chemical Company; St Louis, MO, USA) solution for 30 min and Kraft–Briëhe (KB; in mM: taurine 10, oxalic acid 10, glutamic acid 70, KCl 25, KH\(_2\)PO\(_4\) 10, EGTA–Tris 0.5, HEPES 5, glucose 10, pH 7.4) for 5 min. The digested ventricles were cut into small pieces with scissors and gently shaken in a warmed water bath for 1 min. The tissue fragments were then passed through a filter (mesh: 100 \(\mu\)m) into a beaker containing KB solution. Isolated cells were stored in normal Tyrode solution at room temperature (22–25\(^\circ\)C) prior to experimentation.

Whole cell patch-clamp recordings were made in normal Tyrode solution at room temperature. Cells were gently dispersed onto a cover glass fixed at the bottom of an experimental chamber. Once a giga-seal was made, the whole cell configuration was established by applying negative pressure to rupture the patch membrane. The myocytes were then allowed to equilibrate with the pipette solution for about 10 min. To record I_{Ca,L}, normal Tyrode solution was replaced with Cs\(^{+}\) solution (in mM: NaCl 135, CsCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 1, HEPES 10, glucose 10, pH 7.4). After obtaining baseline recordings, solution bubbled with 50% N\(_2\)O plus 50% O\(_2\) for 20 min. This was followed a continued gradual decline until, after 45 min of hypoxia, and PRP reached 4.8 \pm 1.7% of baseline in the CT group, 8.2 \pm 0.3% in the sevo-pre group, and 5.6 \pm 3.7% in the N\(_2\)O-pre group. There was no significant difference among these three groups (Fig. 3b). After 40 min of reoxygenation, LVDP recovered to 60.1 \pm 3.8% in the CT group, 77.0 \pm 4.3% in the sevo-pre group, and 66.7 \pm 6.4% in the N\(_2\)O-pre group. PRP recovered to 54.4 \pm 7.7% in the CT group, 68.2 \pm 4.5% in the sevo-pre group, and 58.3 \pm 6.3% in the N\(_2\)O-pre group. The recoveries of LVDP and PRP in the sevo-pre group, but not N\(_2\)O-pre group, were significantly (\(P < 0.001\)) greater than those in the CT group (Fig. 3a, b). There were no significant differences in HR among the three groups (data not shown).

GOT release remained near baseline during the 45 min hypoxic period, but it increased rapidly during the first 5 min of reoxygenation and then declined gradually in all
groups. After the first 2 min of reoxygenation, the amount of GOT released was significantly lower in the sevo-pre group (0.8 ± 0.1, P < 0.01) than in the CT group (1.8 ± 0.3). By contrast, preconditioning with N\(_2\)O (1.8 ± 0.7 IU g\(^{-1}\) min\(^{-1}\)) did not reduce the amount of GOT released (Fig. 3c).

Effects of 4% sevoflurane or 50% N\(_2\)O administered during hypoxia

When 4% sevoflurane or 50% N\(_2\)O was administered during the hypoxic period, the recoveries of LVDP and PRP upon reoxygenation in the N\(_2\)O-hypo and sevo-hypo...
groups were facilitated in comparison to those in the CT group. After 20 min of reoxygenation, LVDP had recovered to 32.3 ± 9.3% in the CT group, 56.7 ± 7.9% in the sevo-hypo group (P < 0.05 vs. CT), and 50.6 ± 8.6% in the N₂O-hypo group (P < 0.05 vs. CT) (Fig. 4a). PRP had recovered to 41.8 ± 7.1% of baseline (P < 0.05 vs. CT) in N₂O-hypo group and 58.0 ± 5.0% (P < 0.001 vs. CT) in sevo-hypo group, whereas it remained at 22.4 ± 5.3% in the CT group (Fig. 4b). In addition, after the first 2 min of reoxygenation, the amounts of GOT released in the N₂O-hypo (1.5 ± 0.3, P < 0.05) and sevo-hypo (1.2 ± 0.2, P < 0.001) groups were significantly lower than in the CT group (2.6 ± 0.2 IU g⁻¹ min⁻¹) (Fig. 4c). There were no significant differences in HR among the three groups (data not shown).

Effects of 50% N₂O or 4% sevoflurane on I_{Ca,L}

Sevoflurane and N₂O administered during hypoxia protected heart from injury induced by hypoxia-reoxygenation. These effects may occur through the inhibitory actions of the anesthetics on I_{Ca,L}, and reductions in cytosolic and mitochondrial Ca²⁺ overload. Therefore, we examined the effects of N₂O or sevoflurane on I_{Ca,L}. Isolated rabbit cardiomyocytes were superfused with 50% N₂O or 4% sevoflurane beginning about 8 min after initiation of the whole cell patch clamp, once the basal I_{Ca,L} had stabilized. Fifty percent N₂O reduced I_{Ca,L} from 5.2 ± 0.3 to 3.8 ± 0.2 pA pF⁻¹ (P < 0.001), while 4% sevoflurane reduced I_{Ca,L} from 7.2 ± 0.6 to 5.8 ± 0.4 pA pF⁻¹ (P < 0.05) (Fig. 5B). Normalizing effects to the CT group revealed that 50% N₂O and 4% sevoflurane significantly decreased I_{Ca,L} by 18.7 ± 2.3 and 26.3 ± 1.5%, respectively (P < 0.001), as compared to control (Fig. 5C). There was no significant difference in the reductions in I_{Ca,L} elicited by N₂O and sevoflurane.

Discussion

In this study, preconditioning with sevoflurane, but not N₂O, improved recoveries of LVDP and PRP, and reduced GOT release during reperfusion following hypoxia, suggesting sevoflurane, but not N₂O, exerts a protective effect against reoxygenation-induced injury (Fig. 3). In addition, application of either N₂O or sevoflurane during hypoxia suppressed GOT release and facilitated recoveries of LVDP and PRP, but the levels of recoveries at 35–40 min reoxygenation were not different from those of the control (Fig. 4). The increase in GOT release at the early phase of reperfusion or reoxygenation indicates the development of reperfusion injury on oxygen paradox. Thus, these anesthetics during hypoxia can protect heart from reperfusion-reoxygenation-induced injury.

Both 50% N₂O and 4% sevoflurane reversibly reduced PRP to a similar degree under normoxic conditions, and there was a corresponding reduction in I_{Ca,L}. Although the contractile depression induced by 50% N₂O could be due, in part, to a reduction in O₂, the contractile depression was significantly larger than that in the 50% N₂ group
are no similar reports on the effects of sevoflurane or N₂O. 

Reduces myocardial infarct size in adult rats [22], but there is no earlier report that desflurane administered during hypoxia protected heart from injury induced by hypoxia-reoxygenation. This is consistent with an earlier report that desflurane administered during ischemia attenuates myocardial stunning in dogs [21] and reduces myocardial infarct size in adult rats [22], but there are no similar reports on the effects of sevoflurane or N₂O administered during hypoxia or ischemia. Our study suggests that N₂O protects the heart when administered during the hypoxia in vitro, although the use of this anesthetic gas in patients with ischemic heart disease remains controversial [23].

![Fig. 5](image)

**Fig. 5** Effects of 4% sevoflurane or 50% N₂O on L-type calcium channel currents (I_{Ca,L}). A: Recordings showing the typical effects of sevoflurane (a) or N₂O (b) on peak amplitude of I_{Ca,L}. B: Group data showing the effects of N₂O or sevoflurane of peak of I_{Ca,L}. C: Ratio of sevoflurane- and N₂O-induced reductions in I_{Ca,L}. *P < 0.05 versus CT; ‡P < 0.001 versus CT. 

Volatile anesthetics reportedly depress Ca²⁺ currents (I_{Ca,L}) in atrial and ventricular myocytes [24, 25]. Our finding that 50% N₂O or 4% sevoflurane reduces I_{Ca,L} in isolated rabbit ventricular myocytes (Fig. 5) is consistent with those earlier reports. This suggests that N₂O and sevoflurane may mitigate Ca²⁺ overload in hypoxic hearts by inhibiting Ca²⁺-influx through L-type Ca²⁺ channels. Consistent with that idea, carbon monoxide and the L-type Ca²⁺ channel blockers nifedipine and diltiazem each alleviate Ca²⁺ overloading and cell death in ischemic H9c2 cells [26]. Sevoflurane has been shown to suppress sarcoplasmic reticulum Ca²⁺ release and depress myofilament Ca²⁺ sensitivity [27]. Administration of sevoflurane after ischemia also reduced cytosolic Ca²⁺ and myocardial damages [28]. Therefore, sevoflurane-induced protection may also occur through modulations of L-type Ca²⁺ channels and sarcoplasmic reticulum to reduce Ca²⁺ overload [29, 30].

Several limitations of this study should be noted. Firstly, we did not identify the area of necrosis or apoptosis. Secondly, the sevoflurane concentration used in this study was 4 vol%, other concentrations may have greater or lesser effects. Thirdly, further investigation is needed to identify essential components in these complex signal transduction cascades that mediate APC in our study.

In summary, our findings suggest that preconditioning with 4% sevoflurane, but not 50% N₂O, exerts a protective effect on the myocardium against hypoxia-reoxygenation injury, while administration of either 4% sevoflurane or 50% N₂O during hypoxia is cardioprotective. Both sevoflurane and N₂O reduced I_{Ca,L}. These results suggest that administration of 4% sevoflurane or 50% N₂O during hypoxia prevents Ca²⁺ overload, resulting in a protective effect. On the other hand, the absence of metabolic inhibition means that preconditioning with N₂O will not have a protective effect.

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**References**

1. Schlack W, Preckel B, Stunneck D, Thamer V (1998) Effects of halothane, enflurane, isoflurane, sevoflurane and desflurane on myocardial reperfusion injury in the isolated rat heart. Br J Anaesth 81:913–919
2. Preckel B, Thamer V, Schlack W (1999) Beneficial effects of sevoflurane and desflurane against myocardial reperfusion injury after cardioplegic arrest. Can J Anaesth 46:1076–1081
3. Preckel B, Schlack W, Comfere T, Ohal D, Barthel H, Thamer V (1998) Effects of enflurane, isoflurane, sevoflurane and desflurane on reperfusion injury after regional myocardial ischemia in the rabbit in vivo. Br J Anaesth 81:905–912
4. Ebel D, Preckel B, You A, Mullenheim J, Schlack W, Thamer V (2002) Cardioprotection by sevoflurane against reperfusion injury
after cardioplegic arrest in the rat is independent of three types of cardioplasia. Br J Anaesth 88:828–835. doi:10.1093/bja/88.6.828

5. Obal D, Preckel B, Schlack W et al (2001) One MAC of sevoflurane provides protection against reperfusion injury in the rat heart in vivo. Br J Anaesth 87:905–911. doi:10.1093/bja/87.9.905

6. Varadarajan SG, An J, Novalija E, Stowe DF (2002) Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca²⁺ loading in intact hearts. Anesthesiology 96:125–133. doi:10.1097/00000542-200201000-000025

7. Tanaka K, Ludwing LM, Kersten JR, Pagel PS, Warthier DC (2004) Mechanisms of cardioprotection by volatile anesthetics. Anesthesiology 100:707–721. doi:10.1097/00000542-200403000-00035

8. Weber NC, Toma O, Awab S, Frassdof J, Preckel B, Schlack W (2005) Effects of nitrous oxide on the rat heart in vivo: another inhalational anesthetic that preconditions the heart? Anesthesiology 103:1174–1182. doi:10.1097/00000542-200521000-00011

9. Riess ML, Kevin LG, McCormick J, Jiang MT, Rhodes SS, Stowe DF (2005) Anesthetic preconditioning: the role of free radicals in sevoflurane-induced attenuation of mitochondrial electron transport in Guinea pig isolated hearts. Anesth Analg 100:46–53. doi:10.1213/01.ANE.0000139346.76784.72

10. Nahrwold ML, Cohen PJ (1975) Anesthetics and mitochondrial function after cardioplegic arrest in the rat is independent of three types of ANE.0000081786.74722.DA

11. Kissin I, Aultman DF, Smith LR (1983) Effects of volatile anesthetics on myocardial oxidation-reduction status assessed by NADH fluorometry. Anesthesiology 59:447–452. doi:10.1097/00000542-198311000-00016

12. van Jaarsveld H, Kuyl JM, De Wet EH, Alberts DW, van der Westhuizen FD (1995) Effects of isoflurane, sevoflurane, and halothane on low molecular weight iron content and mitochondrial function of the rat myocardium. Free Radic Res Commun 334:661–668. doi:10.1016/j.bbrc.2005.02.150

13. Varadarajan SG, An J, Novalija E, Stowe DF (2002) Sevoflurane and isoflurane exert modest beneficial actions on left ventricular diastolic function during myocardial ischemia in dogs. Anesthesiology 83:1021–1035. doi:10.1097/00000542-199511000-00016

14. Haelewyn B, Zhu L, Hanouz JL, Persehaye E, Roussel S, Ducouret P, Gerard JL (2004) Cardioprotective effects of desflurane: effect of timing and duration of administration in rat myocardium. Br J Anaesth 92:552–557. doi:10.1093/bja/eah100

15. Becker GL, Pelligrino DA, Miletich DJ, Albrecht RF (1986) The role of the mitochondrial KATP channel in initiating cardioprotection. J Muscle Res Cell Motil 24:219–249. doi:10.1023/A:1026021430091

16. Davies LA, Gibson CN, Boyett MR, Hopkins PM, Harrison SM (2000) Effects of isoflurane, sevoflurane, and halothane on myofilament Ca²⁺ sensitivity and sarcoplasmic reticulum Ca²⁺ release in rat ventricular myocytes. Anesthesiology 93:1034–1044. doi:10.1097/00000542-200100000-00027

17. De Ruijter W, Musters RJP, Boer C, Stienen GJM, Simonides WS, de Lange JJ (2003) The cardioprotective effect of sevoflurane depends on protein kinase C activation, opening of mitochondrial K⁺TP channels and the production of reactive oxygen species. Anesth Analg 97:1370–1376. doi:10.1213/01.ANE.0000081786.74722.DA

18. Bains R, Moe MC, Larsen GA, Berg-Johnsen J, Vinje ML (2006) Sevoflurane reduces myocardial infarct size and decreases the time threshold for ischemic preconditioning in dogs. Anesthesiology 91:1437–1446. doi:10.1097/00000542-199911000-00037

19. Zaugg M, Schaub MC (2003) Signaling and cellular mechanisms in cardiac protection by ischemic and pharmacological preconditioning. J Muscle Res Cell Motil 24:219–249. doi:10.1023/A:1026021430091

20. Kawamura T, Kadosaki M, Nara N, Kaise A, Suzuki H, Endo S (2006) Effects of sevoflurane on cytokine balance in patients undergoing coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 20:503–508. doi:10.1053/j.jvca.2006.01.011

21. Pagel PS, Heutrck DA, Lowe D, Tessmer JP, Warltier DC (1995) Desflurane and isoflurane exert modest beneficial actions on left ventricular diastolic function during myocardial ischemia in dogs. Anesthesiology 83:1021–1035. doi:10.1097/00000542-199511000-00016

22. Sato M, Wu SC, Hu F, Hanouz JL, Persehaye E, Roussel S, Ducouret P, Gerard JL (2004) Cardioprotective effects of desflurane: effect of timing and duration of administration in rat myocardium. Br J Anaesth 92:552–557. doi:10.1093/bja/eah100

23. Silker D, Pagel PS, Pelc LR, Kampine JP, Schmeling WT, Warltier DC (1992) Nitrous oxide impairs functional recovery of stunned myocardium in barbiturate-anesthetized, acutely instrumented dogs. Anesth Analg 75:539–548

24. Hirotta K, Fujimura J, Wakasuki M, Ito Y (1996) Isoflurane and sevoflurane modulate inactivation kinetics of Ca²⁺ currents in single bullfrog atrial myocytes. Anesthesiology 84:377–383. doi:10.1097/00000542-199602000-00016

25. Hatakeyama N, Momose Y, Ito Y (1995) Effects of sevoflurane on contractile responses and electrophysiologic properties in canine single cardiac myocytes. Anesthesiology 82:559–565. doi:10.1097/00000542-199502000-00026

26. Uemura K, Adachi-Akahane S, Shintani-Ishida K, Yoshida K (2005) Carbon monoxide protects cardiomyogenic cells against ischemic death through L-type Ca²⁺ channel inhibition. Biochem Biophys Res Commun 334:661–668. doi:10.1016/j.bbrc.2005.06.142

27. Davies LA, Gibson CN, Boyett MR, Hopkins PM, Harrison SM (2000) Effects of isoflurane, sevoflurane, and halothane on myocardial Ca²⁺ sensitivitiy and sarcoplasmic reticulum Ca²⁺ release in rat ventricular myocytes. Anesthesiology 93:1034–1044. doi:10.1097/00000542-200100000-00027

28. Varadarajan SG, An J, Novalija E, Stowe DF (2002) Sevoflurane before or after ischemia improves contractile and metabolic function while reducing myoplasmic Ca²⁺ loading in intact hearts. Anesthesiology 96:125–133. doi:10.1097/00000542-200201000-00025

29. Zucchi R, Ronca F, Ronca-Testoni S (2001) Modulation of sarcoplasmic reticulum function: a new strategy in cardioprotection? Pharmacol Ther 89:47–65. doi:10.1016/S0163-7258(00)00103-0

30. Piper HM, Meuter K, Schäfer C (2003) Cellular mechanisms of ischemia-reperfusion injury. Ann Thorac Surg 75:S644–S648