

The Mechanisms of the Direct Vascular Effects of Sevoflurane on Saphenous Veins in Vitro

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Aim: The purpose of this study was to determine the mechanism of the direct effects of sevoflurane on human veins in vitro.

Methods: Dose-response curves were obtained for cumulative doses of sevoflurane (0.5, 1, 1.5 and 2 MAC) on saphenous vein strips precontracted with 5-hydroxytryptamine (5-HT 10⁻⁶ mol/L) incubated with either N^ω-nitro-L-arginine-methyl ester (L-NAME) (10⁻⁴ mol/L), indomethacine (10⁻⁵ mol/L), glibenclamide (10⁻⁶ mol/L) or tetraethylammonium (10⁻⁴ mol/L)

Results: Preincubation of vein strips with tetraethylammonium (TEA, Ca⁺⁺-activated K⁺ channel blocker) did not influence the relaxant responses to sevoflurane (p>0.05). L-NAME, indomethacine and glibenclamide reduced the relaxation response to sevoflurane (p<0.05).

Conclusion: Our results demonstrated that sevoflurane produces concentration dependent relaxation in human saphenous vein. The relaxant effects of sevoflurane are probably related with activation of nitric oxide synthase, cyclooxygenase and K_{ATP} channels pathways.

Key words: Sevoflurane, vascular effect, saphenous vein, in vitro

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INTRODUCTION

Sevoflurane, a fluorinated volatile anesthetic agent, reduces mean arterial pressure in a dose-dependent manner through several effects on the cardiovascular system (1). In vitro animal studies have suggested that sevoflurane act directly on vascular smooth muscle and endothelium (2,3). Akata et al., reported that anesthetic concentrations of sevoflurane have endothelium-independent inhibitory effects on vascular smooth muscle of isolated rabbit mesenteric artery (4). Other studies, sevoflurane decreases endothelium dependent vascular relaxations to acetylcholine in rat mesenteric artery and aorta (5,6). However, in these studies differences in the mechanisms of vascular effect of sevoflurane had been shown.

The peripheral veins such as saphenous vein play a role in regulation of systemic vascular resistance has been well known. However, there is no studies investigated the mechanisms of sevoflurane relaxation on human veins. The aim of this study was to assess the role of nitric oxide, prostanoids and potassium (K) channels in the relaxation of saphenous vein induced by sevoflurane.

MATERIAL AND METHODS

After institutional review board approval and written consent, human saphenous veins were obtained from 40 patients undergoing myocardial revascularization.

The discarded saphenous vein segments, were placed in cold Krebs-Henseleit Solution (KHS) (KHS, in mM NaCl 119, KCl 4.7, MgSO₄ 1.5, KH₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25 and glucose 11) and transferred to the laboratory in five minutes. After removal of the surrounding tissue, vein segments were cut helical into 2x15 mm strips and suspended in a 25 ml organ bath containing KHS at 37°C and continuously bubbled with 95 % O₂ and 5 % CO₂ gas mixture. Care was taken not to injure the endothelium during the preparation. At the beginning of the experiment, the strips were stretched to an initial tension of 1g and allowed to equilibrate for 90 min in the KHS, which was changed every 15 minutes. After the equilibration period, the endothelial cell integrity was determined in each strip before all experiments. Relaxation responses to acetylcholine (10⁻⁶ mol/L) in tissues precontracted with 5-hydroxytryptamine (5-HT, 10⁻⁶ mol/L) were used to test endothelial cell integrity. The tissues were washed several times after endothelium test, then allowed to equilibrate at resting tension for 20 min before one of the experimental groups.

Experiment 1: The tissue (n:8)

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Table 1. Percentage relaxant values of sevoflurane on contractions induced by 5-HT (10^{-6} mol/L) in human saphenous veins (All values are given as mean \pm SD).

	0,5 MAC	1 MAC	1,5 MAC	2 MAC
Control	32,2 \pm 3,1	56,5 \pm 5,1	72,4 \pm 3,9	85,3 \pm 4,3
TEA	33,6 \pm 2,9	63,4 \pm 4,9	80,4 \pm 6,3	87,8 \pm 4,6
Glibenclamide	22,3 \pm 3,7*	39,7 \pm 4,2*	54,3 \pm 5,2*	68,7 \pm 4,2*
L-NAME	6,3 \pm 1,2*	14,7 \pm 2,6*	33,8 \pm 2,1*	51,7 \pm 5,2*
Indomethacine	20,5 \pm 4,3*	34,7 \pm 5,2*	47,4 \pm 4,6*	55,2 \pm 4,9*

* $p < 0.05$ versus control

were contracted with 10^{-6} mol/L 5-hydroxytryptamine (5-HT, Sigma, St. Louis, MO). After the contraction had reached steady state, cumulative concentration response curves for sevoflurane (Sevorane Liquid, Abbott) at 0.5 MAC, 1 MAC, 1.5 MAC and 2 MAC were obtained with one curve each preparation. Each concentrations was maintained until the effect on the vein strip reached a plateau. The sustained contraction value with 5-HT (10^{-6} mol/L) was considered as the control contraction value. The vasodilator effect of sevoflurane was expressed as percentage decrease in tension compared with the plateau reached after 5-HT. This was done in order to standardize responses between the strips. Sevoflurane was delivered from a vaporizer in the circuit delivering the O_2 - CO_2 mixture to the bath.

Experiment 2: In order to analyze the role of nitric oxide or prostanoids in the vascular response, tissues were preincubated for 20 min with $N\omega$ -nitro-L-arginine-methyl ester (L-NAME, Sigma, St. Louis, MO) (10^{-4} mol/L) or indomethacine (Sigma, St. Louis, MO) (10^{-5} mol/L) after the maximal contractile response to 5-HT. After this incubation, sevoflurane was added and concentration-response curves were recorded.

Experiment 3: To determine whether the vasorelaxant effect of sevoflurane is mediated by K^+ channels, the strips ($n=8$) were preincubated with 10^{-6} mol/L glibenclamide (adenosine triphosphate-sensitive K^+ channel blocker) (Sigma, St. Louis, MO) or 10^{-4} mol/L tetraethylammonium (TEA, Ca^{++} -activated K^+ channel blocker) (Sigma, St. Louis, MO) following maximal contractile response to 5-HT. Sevoflurane (0.5, 1, 1.5, 2 MAC) was added 20 min later and concentration-response curves were recorded.

In all experimental procedures, the

isometric recording of tension changes was obtained with force transducers (Grass FTO4, Grass Instrument Co, W. Warwick, RI) connected through amplifiers to a polygraph (Grass 7D, Grass Instrument Co, W. Warwick, RI). Only one concentration-response curve was obtained in each experiment.

Statistical Analysis

The effects of sevoflurane are expressed as the percentage of the control contractile response elicited at 10^{-6} mol/L of 5-HT. The relaxant response of sevoflurane in the presence of antagonist were compared with the value obtained in the absence of antagonist. Results are expressed as means \pm SD. The significance of differences was evaluated by using Student's t test. A value of $p < 0.05$ was considered significant for all analyses.

RESULTS

5-HT (10^{-6} mol/L) produced contraction in human saphenous vein strips. Responses to 5-HT were reproducible and time-dependent changes were not observed. Sevoflurane does not alter basal tension at used concentration. In human saphenous vein strips precontracted with 5-HT (10^{-6} mol/L), sevoflurane (0.5, 1, 1.5, 2 MAC) produced concentration dependent relaxation. The effects of sevofluran on saphenous vein strips in the presence of L-NAME and indomethacine are summarized in Figure 1. Preincubation of saphenous vein strips with L-NAME (10^{-4} mol/L) and indomethacine (10^{-5} mol/L) markedly reduced the relaxation to 0.5, 1, 1.5, 2 MAC sevoflurane (Table 1, $p < 0.05$, $n=8$). Figure 2 shows the effects of K^+ channel blockers on sevoflurane induced relaxation in the saphenous vein strips. Preincubation of saphenous vein strips with (10^{-4} mol/L) TEA

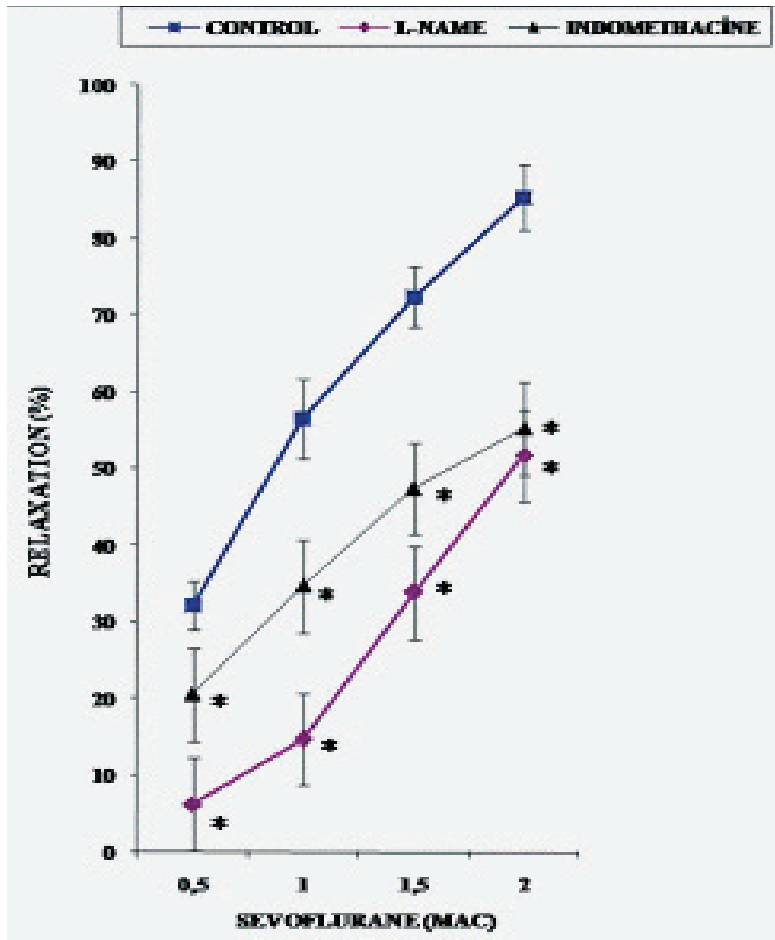


Figure 1. Concentration response curves for the vascular relaxation of human saphenous vein by sevoflurane. The preparations were precontracted with 10^{-6} mol/L 5-HT. Results are expressed as the percent of decrease in contraction induced by 5-HT. Data are shown as mean \pm SD of 8 experiments. * $p < 0.05$ versus control

did not influence relaxant responses to 0.5, 1, 1.5, 2 MAC sevoflurane (Table 1, $p > 0.05$, $n = 8$). Pretreatment of glibenclamide (10^{-6} mol/L) markedly reduced the relaxation to 0.5, 1, 1.5, 2 MAC sevoflurane (Table 1, $p < 0.05$, $n = 8$).

DISCUSSION

In this study, the mechanisms of sevoflurane induced vasodilatory effect was investigated in human saphenous vein. The vessels were studied *in vitro* to eliminate the potential confounding effects of sevoflurane on hemodynamics as well as organ metabolism or sympathetic output that may secondarily influence vascular tone.

Vascular endothelium appears to play a crucial role in the regulation of vascular smooth muscle tone by releasing vasoactive substances, such as nitric oxide and prostaglandins in response to various stimuli (4). Nitric oxide is generated from the metabolic conversion of L-arginine into L-citrulline by the activity of the endothelial nitric oxide synthase. It relaxes vessels by stimulating guanylate cyclase in underlying

smooth muscle cells (6). To assess the role of nitric oxide, we have compared the effect of sevoflurane between intact strips and strips incubated with L-NAME, a nitric oxide synthase inhibitor. The relaxant effects of sevoflurane were lower in the presence of L-NAME compared with control which indicates that nitric oxide mediate sevoflurane induced relaxation in human saphenous vein. In literature, several studies have been reported related to impairment of the nitric oxide mediated relaxation by sevoflurane. Akata et al., reported that isofluran, enfluran and sevoflurane inhibited acetylcholine induced relaxation in rabbit mesenteric arteries (4). Also, Yamaguchi and Okabe were confirmed the inhibitory effect of sevoflurane on acetylcholine induced relaxation in rabbit mesenteric artery (7). Recently, Ariero et al., have very similar results reported that sevoflurane attenuated endothelium mediated vasorelaxation induced by acetylcholine in isolated rat aorta (8). In these studies, the effects of sevoflurane were evaluated only on the acetylcholine relaxation in isolated animal arterial preparations. As detailed

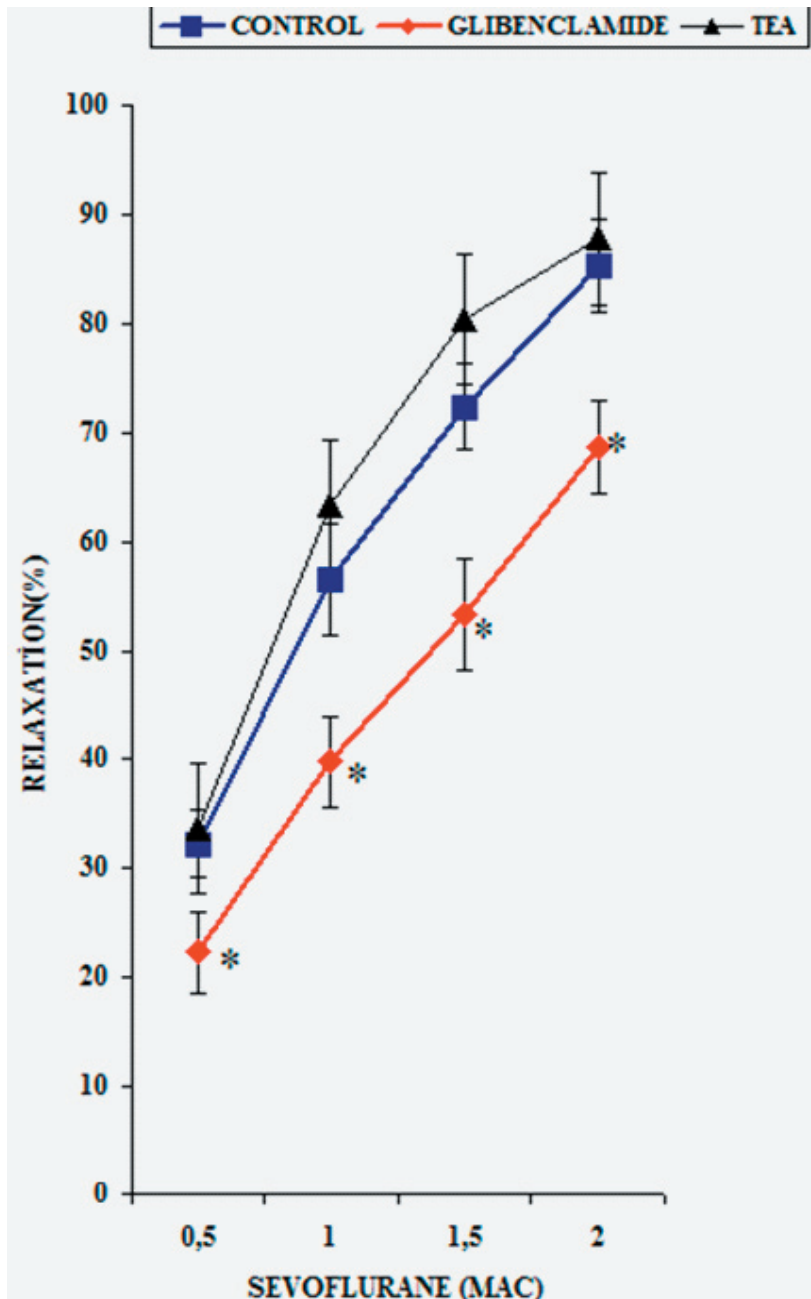


Figure 2. Concentration response curves for the vascular relaxation of human saphenous vein by sevoflurane. The preparations were precontracted with 10^{-6} mol/L 5-HT. Results are expressed as the percent of decrease in contraction induced by 5-HT. Data are shown as mean \pm SD of 8 experiments. * $p < 0.05$ versus control

in material and methods, we attempted to evaluate the effects of sevoflurane on 5-HT contractions in human saphenous vein strips. Most possible reasons in differences between our results and literatures are differences of the methodology and species. However, our results is supported by the study of Thorlacius et al., who suggested that sevoflurane, in contrast to its effect in animal models, promotes endothelium dependent relaxation in human omental arteries and veins (9).

Prostanoids are end products of arachidonic acid metabolism and are synthesized by endothelium via activation of

cyclooxygenase. Prostacyclin is a vasodilator whereas thromboxane A_2 is a vasoconstrictor (10). The prostanoids synthesis are blocked by indomethacin, a cyclooxygenase inhibitor. There is no study investigated the role of prostanoids in the sevoflurane induced relaxation on human veins. Present study revealed that indomethacin suppressed the relaxant effect of sevoflurane in human saphenous vein which suggest that one part of vasodilatation induced sevoflurane occur with release of vasodilatory prostanoid.

Potassium (K^+) channels play an important role in the regulation of vascular smooth

muscle cell membrane excitability and tonus (11). Activation of K^+ channels in vascular smooth muscle hyperpolarises cell membranes and closes voltage-dependent calcium channels. These actions decrease intracellular calcium leading vascular smooth muscle relaxation. On the contrary, inhibition of the channels produces membrane depolarization and vascular smooth muscle contraction (12). There is no study whether the potassium channels play a role in the effect of sevoflurane on human saphenous vein. Therefore, we prepared a study to clarify the mentioned mechanism of the sevoflurane on human vessels. We used TEA (Ca^{++} -activated K^+ channel blocker) and glibenclamide (adenosine triphosphate – sensitive K^+ channel blocker). TEA did not alter the relaxant effect of sevoflurane. But, glibenclamide reduced the sevoflurane induced relaxations, implying that K_{ATP} channels may play an important role in the relaxant effects of sevoflurane. This is consistent with the findings of Iida et al., who reported K_{ATP} channels are involved in the vasorelaxant effects of sevoflurane and isoflurane in dog cerebral arteries (13). However, a previous study has shown that sevoflurane exerts a cardioprotective effect that is mediate via activation of K_{ATP} channels in ischemic canine hearts (14).

In conclusion, these results suggest that sevoflurane can inhibit 5-HT mediated contraction of human saphenous vein; this effect was caused by the activation of nitric oxide synthase, cyclooxygenase and K_{ATP} channels pathways.

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