Effect of Sevoflurane and Desflurane on the Myogenic Constriction and Flow-induced Dilation in Rat Coronary Arterioles

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Background: Determinants of myocardial blood flow distribution include metabolic, myogenic, endothelial, and neurohumoral control mechanisms. The authors studied the effect of sevoflurane and desflurane on the myogenic and endothelial mechanisms.

Methods: Wistar rat subepicardial microvessels, approximately 100 μm in diameter, were monitored for diameter changes in vitro using a video detection system. Myogenic vasomotion was studied by varying the intraluminal pressure from 10 mmHg to 120 mmHg. Flow-induced, endothelium-dependent dilation was evaluated in U46619-preconstricted vessels by varying the pressure gradient across the isolated vessel from 10 mmHg to 80 mmHg, while maintaining the midpoint luminal pressure constant at 40 mmHg to avoid myogenic effects. Myogenic and flow-induced vasomotion both were studied in the presence of sevoflurane, 1 or 2 minimum alveolar concentration (MAC) (MAC is a unit of inhalational anesthetic potency), desflurane, 1 or 2 MAC, or no anesthetic (control).

Results: Myogenic constriction was shown above intraluminal pressures of 70 mmHg. Myogenic constriction was unchanged by sevoflurane, 1 MAC (P = 0.24), but was mildly enhanced by sevoflurane, 2 MAC (P < 0.05), or desflurane, 1 (P < 0.05) or 2 MAC (P < 0.01). Flow-induced dilation was shown over the pressure gradient range of 10–80 mmHg. Flow-induced dilation was not altered significantly by sevoflurane, 1 or 2 MAC (P > 0.3 each), but was significantly attenuated by desflurane, 1 or 2 MAC (P < 0.001 each).

Conclusions: Sevoflurane maintains myogenic and endothelial determinants of myocardial blood flow distribution. Conversely, desflurane attenuates endothelium-dependent flow-induced dilation while mildly enhancing myogenic constriction. (Key words: Blood flow; heart; inhalational anesthetic.)

MYOGENIC mechanisms and flow-induced dilation (FID) produce vasomotion in response to changes in intravascular pressure and in flow and shear stress, respectively, and are two of the important determinants of blood flow distribution in the coronary circulation.¹ ² These mechanisms play synergistic roles to the predominant role played by metabolic factors in matching coronary flow to tissue needs.³ We previously demonstrated in rat coronary microvessels that, whereas isoflurane 1–5% preserves myogenic constriction (MC), halothane, 1 and 2%, abolishes the response³ and that, whereas isoflurane attenuates FID, halothane preserves it and may even enhance it at a high concentration (2 minimum alveolar concentration [MAC]).⁴ We extend our investigation of MC and FID to sevoflurane and desflurane.

Methods

Vessel Preparation

In accordance with institutional Animal Care Committee standards. Wistar rats of either gender, weighing 100–150 g, were anesthetized by injecting ketamine, 40 mg/kg, and xylazine, 5 mg/kg, intraperitoneally. Subepicardial microvessels, fourth-generation branches in the left anterior descending artery distribution, were prepared as described previously.⁵ Each vessel was placed in a vessel chamber, cannulated with dual micropipettes (50–75 μm in diameter), and secured with 100 sutures. The vessel was continuously bathed with modified Krebs buffer (NaCl: 120 mm, KCl: 5.9 mm, dextrose: 11.1 mm, NaHCO₃: 25 mm,
Table 1. The Sizes and Number of Vessels Used for Each Experimental Group

<table>
<thead>
<tr>
<th></th>
<th>Myogenic Constriction</th>
<th>Flow-induced Dilation</th>
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<tr>
<td></td>
<td>Size (µm) N</td>
<td>Size (µm) N</td>
</tr>
<tr>
<td>Control</td>
<td>108 ± 7 8</td>
<td>100 ± 5 8</td>
</tr>
<tr>
<td>Sevoflurane 1 MAC</td>
<td>110 ± 5 8</td>
<td>95 ± 9 8</td>
</tr>
<tr>
<td>Sevoflurane 2 MAC</td>
<td>105 ± 9 6</td>
<td>103 ± 7 7</td>
</tr>
<tr>
<td>Desflurane 1 MAC</td>
<td>102 ± 9 8</td>
<td>101 ± 10 8</td>
</tr>
<tr>
<td>Desflurane 2 MAC</td>
<td>104 ± 7 8</td>
<td>104 ± 5 7</td>
</tr>
</tbody>
</table>

Vessel sizes are the baseline diameters achieved after equilibration at 37°C at an intraluminal pressure of 40 mmHg and are denoted as mean ± SD.

NaH₂PO₄: 1.2 mM, MgSO₄: 1.2 mM, CaCl₂: 2.5 mM), gassed with 95% oxygen–5% carbon dioxide mixture, and maintained at 37°C and a pH of 7.4. The pressure in the micropipettes was provided by connecting them to columns of Krebs buffer and was maintained constant or varied, as described herein, to provide distension. The vessel was visualized and its internal lumen diameter was measured and recorded using an optical density video detection system (Living Systems Instrumentation, Burlington, VT). Stability of endothelium intact and endothelium denuded vessels in our experimental preparation for at least 2.5 h was shown previously.

Vessel segments could be exposed to sevoflurane or desflurane, by adding the anesthetic to the 95% oxygen–5% carbon dioxide mixture bubbling the Krebs buffer solution, using an on-line, drug-specific vaporizer (Sigma Elite Penlon; Penlon Ltd, Abington, UK and Tec 6; Ohmeda, Madison, WI for sevoflurane and desflurane, respectively). We previously demonstrated by gas chromatographic analysis that, in our system, the millimolar concentrations of sevoflurane and desflurane remain proportional to their concentrations in the gas mixture bubbled into the buffer solution, and that it takes less than 10 min for either anesthetic to reach steady state concentrations after introduction of the anesthetic in the tissue chamber. The anesthetic content in the gas mixture was continuously monitored using an Ohmeda 5250 RGM monitor that was calibrated using industrial standards.

Effect of the Anesthetics on Myogenic Vasomotion
Each vessel was equilibrated in the vessel chamber at 37°C with an intraluminal pressure of 40 mmHg for 30 min. The 95% oxygen–5% carbon dioxide mixture bubbling the solution contained sevoflurane, 2.4 or 4.8% (1 or 2 MAC), desflurane 6.9 or 13.8% (1 or 2 MAC), or no anesthetic (control) (table 1). The luminal pressure was then increased from 10 mmHg to 120 mmHg in 10-mmHg increments. Lack of hysteresis of the myogenic response was previously demonstrated and, therefore, in the current study, the pressure–diameter relation was determined with pressure increments only. At each pressure, the vessel was allowed to reach a steady diameter for 3 min and the steady state diameter was recorded.

The vessel was then exposed to papaverine, 100 µM, to measure passive changes in diameter with pressure. The luminal pressure was then varied from 10 mmHg to 120 mmHg in 10-mmHg increments as above and the steady state diameter was recorded. Diameters recorded were normalized to the passive diameter with papaverine at 40 mmHg, using the following formula:

$$\text{normalized diameter} = \frac{100 \times (\text{diameter measured at each pressure})}{(\text{passive diameter measured at 40 mmHg})}.$$

At the end of each experiment, the anesthetic, if any, was discontinued and the vessel was flushed with fresh Krebs buffer and reequilibrated at 37°C with a luminal pressure of 40 mmHg. KCl was then added to a final concentration of 100 mM, followed by 10 µM of the endothelium-dependent dilator adenosine diphosphate. Only those vessels that constricted to KCl by 15% or more were considered to be viable and were included for data analyses. Preservation of endothelial function was assessed by the vessel response to adenosine diphosphate: endothelium-intact vessels dilated approximately 60% to adenosine diphosphate.

Effect of the Anesthetics on Flow-induced Dilation
To measure FID, we varied the pressure gradient (ΔP) across the vessel, while maintaining the intravascular pressure constant to avoid myogenic effects. By varying the heights of the columns of fluids connected to the pipettes simultaneously and equally in opposite directions, we could vary ΔP and, therefore, flow across the vessel, while maintaining the midpoint intravascular pressure constant. The system was arranged symmetrically with the vessel representing the midpoint. To further ensure symmetry of the system, each pipette was prepared using the Narishige automatic pipette maker (Narishige Scientific Instrument Laboratory, Tokyo, Japan) at constant settings and matched for size under the microscope on both sides of the vessel. As previously reported, symmetry of the setup was further verified by measuring FID with flow in one direction and then in the reverse direction (in random order) and noting no sig-
significant difference in FID with changes in the direction of flow. In addition, in seven vessels, the actual midpoint intravascular pressure was measured and found to vary less than 2 mmHg as the pressure gradient was varied from 0 mmHg to 80 mmHg.

After equilibration at an intraluminal pressure of 40 mmHg and ΔP of 0 mmHg for 30 min, the baseline ID (D_{baseline}) was measured. The vessel was then preconstricted with the thromboxane analogue U46619, 1 μM for 5 min. Into the 95% oxygen–5% carbon dioxide mixture bubbling the solution was introduced sevoflurane, 2.4 or 4.8% (1 or 2 MAC), desflurane, 6.9 or 13.8% (1 or 2 MAC), or no anesthetic (control) (table 1). Introduction of the anesthetic diluted the vessel up to 5 or 6% at 2 MAC desflurane or sevoflurane, respectively. The diameter obtained after preconstriction and anesthetic exposure was considered as the constricted diameter (D_{constr}). ΔP was then increased from 0 mmHg to 80 mmHg in 10-mmHg increments while maintaining the midpoint intraluminal pressure constant at 40 mmHg. We previously reported lack of hysteresis of the ΔP-diameter relation. Therefore, in the current study, all measurements were obtained during ΔP increments only.

At each pressure gradient, the vessel was allowed to reach a steady state diameter for 3 min and the steady state diameter was recorded (D_{relax}). Percent relaxation was calculated as follows:

\[
\text{% relaxation} = 100 \times \frac{D_{\text{relax}} - D_{\text{const}}}{D_{\text{baseline}} - D_{\text{const}}}
\]

At the end of each vessel run, the ΔP was returned to 0 mmHg and the vessel was flushed with fresh Krebs buffer and reequilibrated at 37°C. Viability of the vessel was assessed by its response to KCl 100 mm, and adenosine diphosphate, 10 μM, as noted before.

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Fig. 1. Normalized intraluminal diameter versus intraluminal pressure for rat coronary microvessels in the presence (passive response) and absence of papaverine (active control response). Data are the mean ± SD. The vessels showed significant myogenic constriction at intraluminal pressures 70 mmHg or more (P < 0.001). *P < 0.05 versus passive response.

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Fig. 2. Normalized intraluminal diameter versus intraluminal pressure for control rat coronary microvessels and sevoflurane-exposed vessels. Data are the mean ± SD. Only the active responses are shown. Myogenic constriction was not significantly altered by sevoflurane, 1 MAC (P = 0.24), but was mildly enhanced by sevoflurane, 2 MAC (P < 0.05). *P < 0.05 versus control active response.
Fig. 3. Normalized intraluminal diameter versus intraluminal pressure for control rat coronary microvessels and desflurane-exposed vessels. Data are the mean ± SD. Only the active responses are shown. Myogenic constriction was mildly enhanced by desflurane, 1 MAC ($P < 0.05$) or 2 MAC ($P < 0.01$). $P < 0.05$ versus control active response.

Statistical Analysis

Each animal contributed no more than one vessel to any one experimental group; therefore, N for each group represents the number of animals and the number of vessels. All data are presented as the mean ± SD.

The effect of volatile anesthetics on the myogenic response was evaluated by two-way analysis of variance with a repeated measures factor, with post hoc Newman–Keuls test for between-group comparison when the initial two-way analysis of variance yielded a significant $P$ value. To identify the range over which there was significant myogenic constriction, a best-fitting quadratic regression curve for the active myogenic response was obtained using a group indicator variable and a pressure range indicator variable, and an associated $t$ statistic for the coefficient of the quadratic term was computed.

Whether there is a $\delta P$-dependent dilation of the vessels was tested by one-way analysis of variance (Scheffe linear contrast). The effect of anesthetics on FID was evaluated by two-way analysis of variance with a repeated measures factor, with post hoc Newman–Keuls test for between-group comparison and stratified $z$ tests to identify the gradients for which the differences in responses were significant. Significance was considered as $P < 0.05$.

Results

Myogenic Constriction

Passive responses of all experimental groups after exposure to papaverine were not significantly different from one another. In the absence of papaverine, the control vessels showed significant MC, i.e., divergence from the passive response at 70 mmHg and above (fig. 1) ($P < 0.001$).
MC of the rat coronary microvessels was not significantly altered by sevoflurane, 1 MAC (P = 0.24), but was mildly enhanced by sevoflurane, 2 MAC (P < 0.05) (fig. 2). MC of the rat vessels was mildly enhanced by desflurane, 1 MAC (P < 0.05) or 2 MAC (P < 0.01) (fig. 3).

**Flow-induced Dilation**

FID was demonstrable in the rat coronary microvessels over the range of pressure gradients used, *i.e.*, 10–80 mmHg (P < 0.001) (fig. 4). FID in the rat vessels was not significantly altered by sevoflurane, 1 or 2 MAC (P > 0.3 each) (fig. 4). Conversely, FID was significantly attenuated by desflurane, 1 or 2 MAC (P < 0.001 each) (fig. 5).

**Discussion**

The most important findings of the current study in rat coronary microvessels are (1) that MC is maintained in the presence of sevoflurane, 1 MAC, and mildly enhanced by sevoflurane, 2 MAC, and by desflurane and (2) that FID is maintained in the presence of sevoflurane but is attenuated by desflurane at 1 or 2 MAC. Comparison of the anesthetic effects on MC and FID, including those of isoflurane and halothane, is tabulated in table 2.

Myogenic vasomotion occurs in response to changes in vessel transmural pressure and is traditionally thought of as having two components: myogenic dilation in the low-pressure ranges and MC in the high-pressure ranges. These responses tend to maintain flow relatively constant despite changes in pressure and thereby contribute to autoregulation. In our preparation, we were able to demonstrate only MC in the range of 70–120 mmHg, but did not observe any myogenic dilation. Previously we reported that MC in rat coronary microvessels is preserved by isoflurane but is abolished by halothane.

We now add the finding that MC is preserved or mildly enhanced by sevoflurane and is mildly enhanced by desflurane.

Although the exact nature of coupling between intraluminal pressure and vasomotion is understood incompletely, there is suggestive evidence that protein kinase C (PKC) may be involved in MC. Although the effect of halothane on PKC may vary depending on the tissue type studied, halothane has been shown to attenuate PKC action in vascular smooth muscle, whereas isoflurane maintains or enhances it. This difference on PKC action may explain the different effects of isoflurane and halothane on the myogenic response. To our knowledge, there has been no study of sevoflurane or desflurane effect on PKC.

FID is an important determinant of myocardial blood flow distribution and, dissimilar to MC, is endothelium dependent. FID plays a synergistic role in helping to match blood flow to tissue needs. Similar to isoflurane, we

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<tr>
<th>Anesthetic</th>
<th>Myogenic Constriction</th>
<th>Flow-induced Dilation</th>
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<tbody>
<tr>
<td>Halothane</td>
<td>↓ ↓ ↓</td>
<td>± or ↑</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>±</td>
<td>↓ ↓ ↓</td>
</tr>
<tr>
<td>Sevoflurane</td>
<td>± or ↑</td>
<td>±</td>
</tr>
<tr>
<td>Desflurane</td>
<td>↑</td>
<td>↓</td>
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Data for halothane and isoflurane are from our previous studies.↓ = attenuated; ↑ = enhanced; ± = maintained.
desflurane is found to attenuate FID, whereas sevoflu- 
rane and halothane do not.

Our findings of the effect of sevoflurane on FID point to a divergence of anesthetic effects on different types of endothelium-dependent dilation (EDD). Sevoflurane has been shown to attenuate agonist-induced EDD in both the resistance arteries and the larger arteries. However, sevoflurane does not attenuate flow-induced EDD. In this respect, sevoflurane resembles halothane, which also attenuates agonist-induced EDD, but not flow-induced EDD. This divergence of effects implies that the attenuating action of either halothane or sevoflurane on EDD is not likely to involve any steps downstream of endothelial nitric oxide synthesis in the coronary microvessels. Conversely, isoflurane has been shown to attenuate flow-induced and agonist-induced EDD in the coronary microvessels; isoflurane may therefore have an action downstream of the endothelial nitric oxide synthase in these vessels. Finally, although we have shown in the current study that desflurane attenuates FID, there have been no reports on its effect on agonist-induced EDD.

As noted previously, the other major determinant of myocardial blood flow distribution is metabolic. Metabolism-flow coupling may be the predominant factor matching blood flow to tissue needs and is believed to occur in the smallest arterioles (<50 μm). More studies are needed to directly show an effect of the volatile anesthetics on metabolism-flow coupling in the very small arterioles.

In summary, we demonstrated that MC and FID, two of the determinants of myocardial blood flow distribution, are preserved in the presence of sevoflurane. Conversely, desflurane, while mildly enhancing MC, attenuates FID.

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