Modulation of thiopental-induced vascular relaxation and contraction by perivascular adipose tissue and endothelium

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Editor’s key points
- Using thoracic aortic rings from rats, responses to thiopental compared with propofol were studied.
- Thiopental induced relaxation independent of the endothelium.
- Perivascular adipose tissue decreased this relaxation via angiotensin II.
- The endothelium decreased this relaxation via endothelin.
- Relaxation responses to thiopental and propofol have different mechanisms.

Background. Thiopental induces relaxation of vascular smooth muscle cells through its direct and/or indirect vasodilator effects. The perivascular adipose tissue (PVAT) and the endothelium are known to attenuate vascular contraction, and we have recently reported that PVAT potentiates the relaxation effect of propofol through endothelium-dependent and -independent mechanisms. Here, we studied the mechanisms of thiopental-induced vascular responses in relation to the involvement of PVAT and endothelium.

Methods. Thoracic aortic rings from male Wistar rats were prepared with or without PVAT (PVAT+ and PVAT−) and with an intact endothelium (E+) or with the endothelium removed (E−) for functional studies. The contraction and relaxation responses of these vessels to thiopental in the presence of agonists and various receptor antagonists and channel blockers were studied.

Results. In vessels pre-contracted with phenylephrine or KCl, thiopental-induced relaxation was highest in vessels denuded of both PVAT and the endothelium. PVAT attenuated the relaxation response to thiopental, and this attenuation effect was reduced by both angiotensin II (Ang II) type 1 receptor antagonists CV-11974 (2-n-butyl-4-chloro-5-hydroxymethyl-1-[2′-(1H-tetrazol-5-yl)biphenyl-methyl]-imidazole) or losartan and the angiotensin-converting enzyme inhibitor enalaprilat. Thiopental at high concentration (3×10−3 M) caused a contraction through an endothelin-dependent mechanism.

Conclusions. Thiopental induced relaxation in rat aorta through an endothelium-independent pathway and the presence of PVAT, endothelium, or both attenuated this relaxation response through Ang II-dependent and endothelin-dependent mechanisms, respectively.

Keywords: aorta; contraction; perivascular adipose tissue; rat; relaxation; thiopental

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Barbiturates such as thiopental (thiopentone) are known to depress the central nervous system to produce general anaesthesia. In the past, thiopental had been frequently used in clinical anaesthesia because of its rapid onset and short duration of action. However, propofol has now become the preferred anaesthetic for patients in the clinical setting as a result of its minimal side-effects, rapid elimination, short duration of action, low risk of postoperative vomiting and nausea, and rapid and complete recovery.

Thiopental is known to cause vasodilatation of isolated vessels from various vascular beds. However, depending on experimental conditions, thiopental can also induce vasoconstriction in some vessels. Perivascular adipose tissue (PVAT) is situated outside the adventitial layer and serves as the exterior covering layer of most of the systemic blood vessels. In the aorta, it is a highly vascularized tissue consisting of a mixture of brown and white adipocytes. Until recently, PVAT was thought to play a minor role in the modulation of vascular functions. We have reported that PVAT attenuates blood vessel contraction to various agonists through an endothelium-dependent pathway, which involves the release of nitric oxide, followed by the activation of K+ channels; and an endothelium-independent pathway involving the production of hydrogen peroxide by PVAT and the subsequent activation of soluble guanylyl cyclase. Most interestingly, PVAT enhances the relaxation effect induced by propofol in rat aorta through both endothelium-dependent and -independent pathways. Thiopental is a highly lipophilic anaesthetic similar to propofol, but its terminal elimination half-life time is longer than that with propofol, especially in obese patients. We therefore investigated whether these two anaesthetics differ in the mechanism of action of the modulation of vascular function by PVAT.
Methods

Animals
Male Wistar rats (300–350 g) were obtained from Harlan (Indianapolis, IN, USA). This protocol was in accordance with the guidelines of the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals (USA) and was approved by the Animal Research Ethics Board of McMaster University.

Preparation of aortic rings and contractility studies
The procedure for the preparation of aortic rings has been described in our previous reports. Briefly, rats were killed with an overdose of sodium pentobarbital (60 mg kg⁻¹, intraperitoneal), and the thoracic aorta was collected in an oxygenated physiological salt solution (PSS) at 4°C with the following composition (in 1×10⁻³ M): NaCl, 119; KCl, 4.7; KH₂PO₄, 1.2; MgSO₄, 1.2; NaHCO₃, 25; CaCl₂, 1.6; glucose, 11. Paired aortic rings with or without PV AT (PV AT⁺ or PV AT⁻, 4 mm long for each) were prepared with either an intact endothelium (E⁺) or the endothelium removed (E⁻) using the middle segment of the thoracic aorta. The removal of PV AT was carried out under microscopic observation and fine scissors, typically yielding 4–6 aortic rings. The endothelium of the aortic rings was mechanically removed by gently rubbing the internal surface with a fine wooden stick five or six times. Successful removal of the endothelium was confirmed by the absence of a relaxation response to carbamylcholine chloride (CCH, 1×10⁻⁶ M) in rings precontracted with phenylephrine (PHE, 1×10⁻⁶ M). Aortic rings were suspended on triangular-shaped stainless steel hooks in tissue baths containing PSS. A computerized myograph system was used to record the isometric tension of the aortic rings. After equilibration for at least 90 min at 3 g of preload, which is the optimal preload defined in our previous study, the arterial rings were challenged with KCl (6×10⁻² M) twice at an interval of 30 min. Contractile response to agonists was expressed as a percentage of the KCl contraction. Contraction induced by KCl was quite stable and returned back to the baseline within 15 min after wash out of KCl with PSS.

To study the direct relaxation, the contraction effect induced by thiopental, or both, a cumulative concentration-dependent response curve for thiopental was constructed in vessels precontracted with PHE (3×10⁻⁶ M). Thiopental was applied in a cumulative manner to obtain concentrations of 10⁻⁸ M, 3×10⁻⁵ M, 10⁻⁶ M, 3×10⁻⁶ M, 10⁻⁷ M, and 3×10⁻³ M. Each new dose was added after the relaxation had reached a steady state from the preceding dose, usually within 8–15 min. The relaxation and contraction responses recorded with increasing concentrations of the test drugs were expressed as the percentage relaxation from the precontraction load. Angiotensin II (Ang II) type 1 receptor antagonist losartan (3×10⁻⁶ M), or 2-n-butyl-4-chloro-5-hydroxy methyl-1-[2-(1H-tetrazol-5-yl) biphensyl-4-methyl]-imidazole (CV-11974 1×10⁻⁶ M); angiotensin-converting enzyme inhibitor (enalaprilat 1×10⁻⁵ M); soluble guanylyl cyclase inhibitor, 1H-(1,2,4) oxadiazolo (4,3-A) quinazoline-1-one (ODQ, 3×10⁻⁵ M); and endothelin-1 type A and B (ET₄A and ET₄B) receptor antagonist (bosentan 3×10⁻⁴ M) were incubated for 25–30 min to study the relaxation and/or contraction mechanisms induced by thiopental. The concentration chosen for these antagonists or inhibitors was based on our previous studies. In the case of losartan and bosentan, preliminary experiments were carried out to establish the effective dose. After incubation, a second cumulative concentration-dependent response curve was constructed, and the differences before and after incubation were noted. After experimentation, tissues were washed with PSS to return to baseline conditions (usually within 30 min) and re-challenged with KCl to ensure continued tissue variability.

Statistical analysis
Results are expressed as means (so), in which n represents the number of rats. Statistical analysis was performed by two-way repeated measurements or one-way analysis of variance (ANOVA) followed by post hoc t-test to determine any significant difference between the concentration-dependent response curves with the presence of PV AT or endothelium, or PV AT and/or endothelium removed. The difference was considered significant when P≤0.05.

Results

Contraction to KCl in aortic rings with or without PVAT and endothelium
The presence or absence of PVAT, endothelium, or both did not affect the maximal tension induced by KCl (6×10⁻² M, Fig. 1A). These results showed that the presence of PVAT did not pose a constraint on the ability of the aorta to contract, and the procedure to remove PVAT, endothelium, or both did not damage or affect the contractility of the aorta.

Effects of thiopental on aortic rings
In aortic vessels precontracted with PHE, PVAT⁻ vessels showed the most relaxation response to thiopental when compared with PVAT⁺ vessels (Fig. 1B), and the presence or absence of the endothelium had no effect. The EC₅₀ (10⁻⁶ M) was 0.81 (0.32) for the PVAT⁺ vessel, 0.43 (0.05) for the PVAT⁻ vessel, 0.59 (0.16) for the PVAT⁺E⁻ vessel, and 0.47 (0.10) for the PVAT⁻E⁻ vessel. There was no difference in EC₅₀ among these vessels. After the addition of the last dose of thiopental (3×10⁻³ M), where the maximum relaxation was observed, a contraction was noted in PVAT⁺ vessels instead of a relaxation response (Fig. 1C).

Involvement of endothelin in thiopental-induced vascular contraction
In vessels precontracted with PHE (3×10⁻⁶ M) and in the presence of both the PVAT and endothelium, endothelin-1 type A and B (ET₄A and ET₄B) receptor antagonist bosentan significantly increased thiopental-induced relaxation (Fig. 2A). This increase in thiopental-induced relaxation was not
observed in E− and PVAT− vessels. In PVAT−E+ vessels, thiopental-induced contraction was significantly inhibited by bosentan (Fig. 2B).

**Involvement of soluble guanylyl cyclase in thiopental-induced vascular relaxation**

In the four types of vessels precontracted with PHE (3×10⁻⁶ M), incubation with the soluble guanylyl cyclase inhibitor ODQ (3×10⁻⁵ M) did not affect the relaxation response induced by thiopental (Fig. 3A).

**Involvement of K⁺ channels in thiopental-induced vascular relaxation**

In vessels precontracted with KCl (6×10⁻² M), thiopental also induced a relaxation response. Vessels with PVAT− also induced a relaxation response. Vessels with PVAT E− or PVAT−E− vessels, the presence or absence of the endothelium did not affect the relaxation response to thiopental, indicating that the endothelium was not involved in this relaxation response induced by thiopental (Fig. 3A). Thiopental induced a higher relaxation in vessels precontracted with KCl or U46619 (3×10⁻⁷ M, a thromboxane mimetic) when compared with PHE-precontracted vessels in the presence of PVAT (Fig. 3B).

**Involvement of Ang II in thiopental-induced relaxation**

Aortae were precontracted with PHE (3×10⁻⁶ M). In vessels with an intact or denuded endothelium and in the presence of PVAT, incubation with either CV-11974 (Fig. 4) or enalaprilat (Fig. 5) significantly increased the thiopental-induced relaxation response. The EC₅₀ was higher in PVAT− control vessels than those treated with either CV-11974 (P=0.04).
or enalaprilat \((P=0.01)\). In the absence of PVAT and in both the presence and absence of the endothelium, incubation with either CV-11974 or enalaprilat did not affect the relaxation induced by thiopental (Figs 4 and 5).

### Involvement of Ang II in vessels precontracted with KCl

In vessels precontracted with KCl \((6 \times 10^{-2} \text{ M})\), relaxation induced by thiopental was significantly lower in vessels with intact PVAT when compared with those with PVAT removed, regardless of whether the endothelium was present or removed (Fig. 6). The EC\(_{50}\) was similar among these four types of vessels. In PVAT+E+ vessels, relaxation induced by thiopental was significantly higher in vessels preincubated with enalaprilat (Fig. 7A) or with losartan (Fig. 7B). The EC\(_{50}\) was higher in control vessels when compared with vessels treated with either enalaprilat \((P=0.04)\) or losartan \((P=0.03)\).

### Discussion

The novel findings of the present study are that thiopental induces a concentration-dependent relaxation response in rat aorta, which is not dependent on the presence of the endothelium, and that PVAT attenuates the relaxation response induced by thiopental through an Ang II-dependent mechanism involving PVAT. At high concentrations, thiopental induces the contraction of the aorta in the absence of PVAT through an endothelin-dependent mechanism. These
Thiopental and perivascular adipose tissue

Fig 4 Effect of CV-11974 (1×10⁻⁶ M) on the dose-dependent relaxation–response induced by thiopental in all four types of thoracic aortic vessels. Results are plotted as the per cent change from the contraction induced by PHE. Graphs show mean (SD), n=6. ANOVA, *P<0.05.

Fig 5 Effect of enalaprilat (1×10⁻⁵ M) on the dose-dependent relaxation–response induced by thiopental in all four types of thoracic aortic vessels. Results are plotted as the per cent change from the contraction induced by PHE. Graphs show mean (SD), n=6 rats/group. ANOVA, *P<0.05.
found that the relaxation response induced by thiopental decreased systemic arterial pressure. In this study, we evaluated the role of vasodilation of these two types of vessels. Results are different from those obtained with propofol, and thus highlighting the different vascular effects of these two highly lipophilic anaesthetics, especially with respect to their role in modulating PVAT and endothelium function.

Thiopental is known to cause a reduction in both systemic vascular resistance and cardiac output, resulting in decreased systemic arterial pressure. In this study, we found that the relaxation response induced by thiopental was similar both in the presence and in the absence of the endothelium, showing that this relaxation response was not dependent on the presence of the endothelium. In vessels precontracted with either PHE or KCl, vessels with intact PVAT showed less relaxation response when compared with those with PVAT removed. These results suggest that PHE or KCl stimulated the release of a constricting factor from PVAT, and we hypothesized that Ang II was involved. Our results showed that this indeed was the case, because in the presence of angiotensin-converting enzyme inhibitor enalaprilat, or angiotensin type 1 receptor blocker CV-11974 or losartan, the relaxation response induced by thiopental was increased, indicating that PHE or KCl stimulated the release of Ang II, which interfered with the direct relaxation effect of thiopental. The EC50 was not altered in response to thiopental, but maximal relaxation response was higher in PVAT−vessels (Figs 1a and 6) because Ang II released from PVAT did not affect the pathway through which thiopental caused the relaxation response. In PVAT+E−vessels precontracted with PHE and PVAT+E+ vessels precontracted with KCl, treatment with enalaprilat or with CV-11974 or losartan decreased the EC50 of thiopental in comparison with control vessels, thus showing the involvement of Ang II release from PVAT which attenuated the relaxation induced by thiopental. In PVAT+E+ vessels precontracted with PHE, however, a significant reduction in EC50 was not found in vessels pretreated with enalaprilat, or CV-11974. This was probably because of the release of endothelin from the endothelium induced by thiopental when vessels were precontracted with PHE.

To investigate whether other agonists besides PHE also stimulate the release of Ang II from PVAT, we studied the relaxation response of the vessels precontracted with U46619, a thromboxane mimetic. We found that the relaxation response was similar to the vessels precontracted with KCl, but higher than that precontracted with PHE. Taken together, these results indicate that α1 receptors are involved in stimulating the release of Ang II by PVAT in response to PHE. Electrical stimulation of rat aorta also caused the release of Ang II from PVAT which was mediated by α1 receptors. It is possible that the high concentration of KCl we used in this study might also cause the release of Ang II by depolarization of the nerves through the same mechanism as reported by Soltis and Cassis. Using enalaprilat and losartan, we found that these were effective in causing more relaxation of the vessels, therefore providing support for the results we have obtained using PHE that an Ang II-dependent mechanism associated with PVAT was involved in attenuating the relaxation action of thiopental.

We found that thiopental at low concentration caused a relaxation response in rat aorta, and a contraction response at high concentrations. This effect was dependent on the presence of the endothelium and the absence of PVAT. To investigate whether endothelin was involved, we used bosentan to block the endothelin receptors. Our results indeed showed that an endothelin-dependent mechanism was involved because bosentan was effective in blocking the
contractile response induced by a high concentration of thiopental in the PVAT−E+ aortic vessel.

Another important finding of our study is that although both thiopental and propofol can cause relaxation of the arteries, the mechanisms involved in relation to the effects of the endothelium and PVAT are quite different. (i) The relaxation effect of propofol is potentiated by the presence of the endothelium, while the relaxation effect of thiopental is not dependent on the presence of the endothelium. (ii) Propofol induced relaxation in the smooth muscle cells of rat aorta, via soluble guanylyl cyclase, but this is not involved in the relaxation response to thiopental. (iii) At high concentrations, thiopental but not propofol induces contraction through an endothelin-dependent mechanism. It is not known whether tissue levels of thiopental can reach this high level when used clinically in critical care. (iv) PHE and KCl attenuate the relaxation effect of thiopental through an Ang II-dependent mechanism involving PVAT. With propofol, even though Ang II released by PVAT may still be present in response to PHE, the release of relaxation factors from PVAT and endothelium induced by propofol predominated, so that more relaxation is present in PVAT+E+ vessels than PVAT+E− and PVAT−E+ vessels. Therefore, the predominant vascular effect of propofol is relaxation. In contrast, thiopental induces contraction of the blood vessel through endothelin-dependent and Ang II-dependent mechanisms involving the endothelium and PVAT, respectively. Previous studies have also found that thiopental is a potent vasoconstrictor and inhibits vasodilation. These differences may explain the difference in the haemodynamic effects of these two agents because greater decreases in arterial pressure and total peripheral resistance were observed with propofol than with thiopental.

In a study evaluating thiopental disposition in lean and obese patients, it was found that the volume of distribution was larger in obese than in lean control patients, and as a result, the elimination of half-life of thiopental was significantly longer in obese than in non-obese patients. The prevalence of obesity is rapidly increasing worldwide in both developed and developing countries. Body structure and function are altered in obese patients, which in turn affect the pharmacokinetic and pharmacodynamic properties of anaesthetic drugs, thus presenting dosing challenges to anaesthetists. Serum levels of thiopental can reach 3.5×10−4 M during induction, similar to concentrations used in our in vitro study, and is thus clinically relevant. It is possible that thiopental level in PVAT may be higher than in serum and other adipose tissue after redistribution, as a result of lipophilicity.

In conclusion, we found that thiopental induces both relaxation and contraction responses of the aortic blood vessels. The relaxation occurs through an endothelium-independent mechanism, while thiopental-induced contraction is related to an endothelin-dependent mechanism. We also found that PVAT attenuates the relaxation effect of thiopental through an Ang II-dependent mechanism stimulated by PHE or KCl. The effects of thiopental compared with propofol on PVAT and endothelium functions provide useful insight for the choice in the use of these agents in clinical anaesthesia.

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Declaration of interest

None declared.

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