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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-choloro-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.[1] 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.[2]

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.[3] 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.[4] 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.[5] Most interestingly, PVAT enhances the relaxation effect induced by propofol in rat aorta through both endothelium-dependent and -independent pathways.[6] 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.[7] 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.[8] 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; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; $CaCl_2$, 1.6; glucose, 11. Paired aortic rings with or without PVAT (PVAT+ and PVAT−, 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 PVAT was carried out under microscopic observation and fine scissors, typically yielding 4–6 aortic rings.[9] 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,[8] 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^{-5} M, 3×10^{-5} M, 10^{-4} M, 3×10^{-4} M, 10^{-3} 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 precontracted load. Angiotensin II (Ang II) type 1 receptor antagonist losartan (3×10^{-6} M), or 2-*n*-butyl-4-choloro-5-hydroxymehtyl-1-[2′-(1H-tetrazol-5-yl) biphenyl-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 (ET_A and ET_B) 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.[5 9 10] 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 (SD), 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 PVAT or endothelium, or PVAT and/or endothelium removed. The difference was considered significant when $P\leq0.05$.

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 EC_{50} (10^{-4} M) was 0.81 (0.32) for the PVAT+E+ vessel, 0.43 (0.05) for the PVAT−E+ 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 EC_{50} 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−E+ 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 (ET_A and ET_B) receptor antagonist bosentan significantly increased thiopental-induced relaxation (Fig. 2A). This increase in thiopental-induced relaxation was not