Thiopental attenuates relaxation and cyclic GMP production in vascular smooth muscle of endotoxin-treated rat aorta, independent of nitric oxide production

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Summary
As thiopental (thiopentone) suppresses cyclic GMP (cGMP) formation produced by nitric oxide donor drugs, we have tested if it suppresses cGMP formation and increases vascular tone after induction of calcium–calmodulin-independent nitric oxide synthase (iNOS). Rat aortic rings were treated with Escherichia coli lipopolysaccharide (LPS) 1 μg ml⁻¹ for 4 h, and the effects of thiopental on tension, cGMP concentrations and nitrite accumulation were determined. Thiopental 0.3 mmol litre⁻¹ reduced the tension of phenylephrine-precontracted aortic rings before LPS treatment, but caused no significant effects on tension in the presence of L-arginine 10 μmol litre⁻¹ after LPS treatment. L-Arginine 1 mmol litre⁻¹ to 1 mmol litre⁻¹ increased concentrations of cGMP in LPS-treated aorta in a concentration-dependent manner. This was reduced by thiopental 0.3–1 mmol litre⁻¹. Treatment with L-arginine 1 mmol litre⁻¹ increased concentrations of nitrite, the end product of nitric oxide; this was not affected by thiopental 1 mmol litre⁻¹. We conclude that thiopental suppressed cGMP formation in iNOS-induced vascular smooth muscle without affecting nitric oxide production. (Br. J. Anaesth. 1998; 81: 601–602.)

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Expression of calcium–calmodulin-independent nitric oxide synthase (iNOS) is one aspect of the pathogenesis of profound hypotension in septic patients. Constitutive nitric oxide synthase (cNOS), which includes endothelial (eNOS) and neuronal (nNOS) nitric oxide synthase, is activated only when cytosolic calcium is increased. However, iNOS converts L-arginine to nitric oxide, independent of calcium. Nitric oxide activates guanylate cyclase (GC) and increases production of cyclic GMP (cGMP), which then leads to vasorelaxation.

Until now, no anaesthetic has been shown to suppress the effect of iNOS. Kesler and colleagues showed that thiopental augmented iNOS induction in vascular smooth muscle, but the effect on cGMP production, when iNOS was induced, was not examined. As thiopental (thiopentone) strongly suppresses cGMP formation induced by a nitric oxide donor drug, in addition to that induced by activation of eNOS and nNOS, we anticipated that this anaesthetic may also suppress cGMP formation induced by iNOS. Therefore, we have tested this hypothesis by evaluating the effect of thiopental on tension and production of nitric oxide and cGMP in endotoxin-treated vascular smooth muscle.

Methods and results
In accordance with the standards of the Kyoto University Animal Use Committee, descending thoracic aortae were harvested from Wistar rats (weighing 250–350 g). Four to six endothelium-denuded ring segments were prepared from each aorta and placed in Krebs bicarbonate solution of the following composition (mmol litre⁻¹): NaCl 118.2, KCl 4.6, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 24.8, glucose 10 (pH 7.35–7.45), which was aerated with a mixture of 95% oxygen and 5% carbon dioxide and maintained at 36.5–37.5°C. The aortic rings, except for those for the time-controlled studies, were bathed in Krebs solution containing Escherichia coli lipopolysaccharide (LPS) 1 μg ml⁻¹ for 4 h, and then washed and equilibrated for 1 h.

Vascular rings for measurement of isometric tension were mounted under a resting tension of 3.0 g. After attainment of equilibrium, the rings were exposed to KCl 30 mmol litre⁻¹ to induce contraction, which was taken as the 100% level for each ring throughout the experiment. The absolute value for KCl-induced contractions was 585 (SEM 217) mg (n = 26). Rings were then exposed to acetylcholine 10 μmol litre⁻¹ to confirm endothelial denudation. None showed relaxation in response to acetylcholine.

After washing, the rings were exposed to phenylephrine 0.3 μmol litre⁻¹ to induce sustained contraction (189 (9.2)%, n = 26). Subsequent exposure to thiopental 0.3 and 1 mmol litre⁻¹ reduced the tension to 154 (8.2)% (P = 0.0030) and 33.2 (5.8)% (P < 0.0001), respectively (n = 13 each). After LPS treatment, addition of phenylephrine 0.3 μmol ml⁻¹ to

the bath produced sustained contraction (162 (10.7) %, n=26). The subsequent 10 min of exposure to L-arginine 10 μmol ml⁻¹ decreased this tension to 59.7 (9.6)% (P<0.0001) (n=12), while L-nitro-N-arginine (L-NNA) 30 μmol ml⁻¹ increased it to 231 (14.8) % (P<0.0001) (n=14). Exposure to thiopental 0.3 and 1 mmol litre⁻¹ did not affect the tension of L-arginine-exposed rings but the response of L-NNA-exposed rings to thiopental was similar to that of LPS-untreated rings.

The rings for cGMP and nitric oxide measurements were suspended in organ baths without tension, treated with LPS and, after washing, with thiopental 0.3 or 1 mmol litre⁻¹ or without (control) for 20 min. Those for cGMP measurement were exposed to L-arginine 1 μmol litre⁻¹ to 1 mmol litre⁻¹ for 12 min. cGMP content in each sample was assessed by radioimmunoassay. ¹⁴ cGMP production in LPS-treated aortae was dependent on the concentration of L-arginine, and was suppressed by thiopental 0.3–1 mmol litre⁻¹ (fig. 1).

Rings for assessment of nitric oxide formation were transferred to plastic microtubes containing 1 ml of Krebs solution with L-arginine 1 mmol ml⁻¹, with or without thiopental 1 mmol litre⁻¹, and incubated for 10 min. A sample of bath fluid was mixed with 8 vol% of Aspergillus nitrate reductase 5 IU ml⁻¹ and 40 vol% β-NADPH 500 mmol litre⁻¹, and equilibrated at room temperature for 1 h to allow conversion of nitrate to nitrite. ³ Nitrite concentration was measured using a nitric oxide analyser (NOA Model 270B, Sievers Research Inc., Boulder, CO, USA). The increases in nitrite in iNOS-induced aortae during 10 min of incubation in the absence and presence of thiopental 1 mmol litre⁻¹ were 49.2 (19.3) and 52.2 (14.0) μmol/g wet weight, respectively (P=0.9024) (n=6 each).

The effects of thiopental on tension were analysed using the Student’s t test for paired data and the effects on nitrite production were analysed using the Student’s t test for unpaired data. Other analyses were by factorial analysis of variance (ANOVA) with Scheff’s F test, or repeated measure ANOVA. Data are expressed as mean (SEM), n=number of animals tested and differences at P<0.05 were considered significant.

Comment
Our finding that thiopental decreased cGMP concentrations without altering nitric oxide formation clearly indicates that this anaesthetic decreases GC activity or augments cGMP breakdown in iNOS-expressed vascular smooth muscle. In spite of a marked decrease in cGMP concentration, thiopental at the concentrations tested (which were higher than clinically relevant concentrations⁶) did not alter the tension of iNOS-induced vascular smooth muscle.

This may be because thiopental, at these concentrations, has vasodilator effects, mainly as a result of suppression of calcium sensitivity of vascular smooth muscle.⁶ Thus our study failed to show that thiopental ameliorated vascular collapse caused by activation of iNOS. However, as anaesthetic management is mandatory in some septic cases and almost all anaesthetics have vasodilator effects, thiopental may be still more beneficial than other anaesthetics in some septic cases.

References