EFFECTS OF THIOPENTONE AND CHLORMETHIAZOLE ON HUMAN MYOMETRIAL ARTERIES FROM TERM PREGNANT WOMEN

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SUMMARY

We have investigated the effects of thiopentone and chlormethiazole on maternal intramyometrial arteries dissected from myometrial biopsies taken during Caesarean section at term. Ring preparations were mounted in organ baths and isometric tension was recorded. Thiopentone $10^{-4}$ to $10^{-3}$ mol litre$^{-1}$ inhibited responses to $K^+$ depolarization, noradrenaline and vasopressin. Chlormethiazole $3 	imes 10^{-5}$ to $3 	imes 10^{-3}$ mol litre$^{-1}$ inhibited responses to noradrenaline, while a concentration of $3 	imes 10^{-3}$ mol litre$^{-1}$ was required to attenuate responses to vasopressin and $K^+$ depolarization. Neither of the two agents affected relaxant responses to prosta- cyclin. The results did not yield evidence that clinical use of thiopentone and chlormethiazole should impair uteroplacental vascular perfusion by a direct effect.

KEY WORDS


Maintenance of uteroplacental perfusion is a major concern during anaesthesia for Caesarean section. Thiopentone is used currently for induction of anaesthesia in these patients, while chlormethiazole has been advocated in case of pre-eclampsia (n = 5) or active genital herpes infection (n = 4). The study was approved by the Ethics Committee of the University of Aarhus.

No premedication was given. Initially, tubocurarine 3 mg and atropine 0.6 mg were given i.v. and then anaesthesia was induced with thiopentone. Suxamethonium 100 mg was given to facilitate tracheal intubation and anaesthesia was maintained with 0.5% halothane and 50% nitrous oxide in oxygen. After delivery, biopsies were excised from the upper edge of the uterine incision.

The specimens were placed immediately in ice-cold physiological salt solution (PSS, see below). Myometrial arterial segments (outer diameter 400-500 μm, length 1.0 mm) were prepared by careful dissection under a stereomicroscope (Kyowoo). The vascular preparations were mounted in 5-ml organ baths as described by Hogestatt, Andersson and Edvinsson [13]. The baths contained physiological salt solution (37.0 ± 0.5 °C) bubbled with carbogen (5% carbon dioxide in oxygen), giving a final pH of 7.40 ± 0.05. The short parallel legs of two L-shaped hooks were inserted carefully through the lumen of the vessels. One of these hooks was attached to a displacement device which allowed fine adjustments of tension and the other was connected to a Grass Ft 03 transducer. Isometric tension was recorded and displayed on a Beckmann R 611 polygraph. The preparations were allowed to equilibrate for 1 h. During this period, passive tension of the vascular preparations was adjusted to 4 mN/mm vessel.
Length–tension studies have shown that near-optimum mechanical responses are achieved at this resting tension [7]. After the equilibration period, contractions were induced repeatedly by potassium (K⁺) 124 mmol litre⁻¹ until reproducible (less than 10% variation between two successive contractions).

Cumulative concentration–response relations were assessed for noradrenaline and vasopressin in control preparations and after pretreatment for 30 min with thiopentone or chlormethiazole. The maximum contractile response (Emax) was determined in each experiment and expressed as percent of the amplitude of the initial K⁺-induced response. In addition, the concentration of agonist producing half-maximum response (EC₅₀) was determined in each experiment by linear interpolation and expressed as pD₂ = −log(EC₅₀). The effects of chlormethiazole and thiopentone on relaxant vascular responses to prostacyclin (PGI₂) were tested in vessels precontracted with vasopressin 10⁻⁸ mol litre⁻¹ or noradrenaline 10⁻⁷ mol litre⁻¹ (producing maximum contractile responses to the peptide).

**Results**

**Statistics**

Statistical analyses were performed using GraphPAD InStat ver 1.13. Mean and se for Emax and pD₂ were calculated. After homogeneity of variance was ascertained, significance of difference was assessed by one-way analysis of variance or, if appropriate, by the two-tailed t test for groups of non-paired observations. P < 0.05 was considered significant.

**Solutions**

Physiological salt solution (PSS) (mmol litre⁻¹): NaCl 119, NaHCO₃ 15, KCl 4.6, CaCl₂ 1.5, NaH₂PO₄ 1.2, MgCl₂ 1.2, glucose 11.

High-K⁺ physiological salt solution (high-K⁺ PSS) (mmol litre⁻¹): KCl 124, NaHCO₃ 15, CaCl₂ 1.5, MgCl₂ 1.2, NaH₂PO₄ 1.2, glucose 11.

**Drugs**

Vasopressin (arginine-vasopressin, Sigma), noradrenaline ((−)-norepinephrine hydrochloride, Sigma), prostacyclin (PGI₂, UpJohn), chlormethiazole (Heminevrin, Astra) and thiopentone (Thiopental, Lovens kemiske Fabrik) were used in the study.

PGI₂ sodium salt was stored at −60°C. Immediately before use, the salt was dissolved in ice-cold Tris buffer 0.05 mol litre⁻¹ (pH 10.4); dilutions were made up in the same solvent. The solutions were kept on ice until added to the organ baths in amounts of 5 μl. This procedure did not change pH of the organ bath PSS.

None of the vascular preparations showed spontaneous contractile activity. Addition of thiopentone 10⁻⁵–10⁻³ mol litre⁻¹ or chlormethiazole 10⁻³–3 × 10⁻³ mol litre⁻¹ did not change resting tension, which remained constant during the experiments.

**Effects of thiopentone or chlormethiazole on responses to K⁺-induced depolarization**

Depolarization by K⁺ 124 mmol litre⁻¹ induced an initial fast increase in tension which continued into a maintained tonic contraction. The mean maximum amplitude of this response was 11.3 (SE 1.5) mN/mm vessel (n = 9). Pretreatment with thiopentone 10⁻⁴–10⁻³ mol litre⁻¹ produced concentration-dependent inhibition of the maximum amplitude of these contractions (fig. 1).

Pretreatment with chlormethiazole showed less effect in counteracting K⁺-induced responses. In concentrations up to 3 × 10⁻⁴ mol litre⁻¹, no significant effects were seen, while chlormethiazole 3 × 10⁻³ mol litre⁻¹ produced inhibition by about 50% (fig. 1).

**Effects of thiopentone or chlormethiazole on responses to noradrenaline**

Noradrenaline induced concentration-dependent contractile responses with mean Emax 111 (5.3)% and mean pD₂ 6.4 (0.25) (n = 9). Pretreatment with thiopentone up to 10⁻³ mol litre⁻¹ did not significantly change this response. At a concentration of 10⁻⁴ mol litre⁻¹, thiopentone almost abolished responses to noradrenaline (fig. 2).

Pretreatment with chlormethiazole up to 3 × 10⁻⁴ mol litre⁻¹ had no effects on noradrenaline-induced responses, while concentrations of 3 × 10⁻³ and 3 × 10⁻² mol litre⁻¹ produced inhibition by decreasing mean Emax to 84 (10)% (n = 9; P < 0.05), 86 (7)% (n = 9; P < 0.01) and 31 (11)% (n = 9; P < 0.0001), respectively (fig. 2). With chlormethiazole 3 × 10⁻³ mol litre⁻¹, a decrease in pD₂ to 5.5 (0.1) occurred (n = 9; P < 0.01).

**Fig. 1. Mean (SE) inhibitory effects of thiopentone and chlormethiazole on responses to K⁺ 124 mmol litre⁻¹ in human intramyometrial arteries. Responses expressed as percent of the initial response to K⁺ 124 mmol litre⁻¹. **P < 0.01; ***P < 0.0001.
Effects of thiopentone or chlormethiazole on responses to vasopressin

Vasopressin produced concentration-dependent contractions with mean $E_{\text{max}}$ 124 (4.0)% and $pD_2$ 10.2 (0.25) ($n = 8$). Pretreatment with thiopentone up to $10^{-6}$ mol litre$^{-1}$ had no effect, while thiopentone $10^{-4}$ mol litre$^{-1}$ decreased vasopressin-induced responses by depressing $E_{\text{max}}$ to 110 (5)% ($n = 8$; $P < 0.05$). Thiopentone $10^{-3}$ mol litre$^{-1}$ almost abolished responses to vasopressin (fig. 3).

Pretreatment with chlormethiazole up to $3 \times 10^{-5}$ mol litre$^{-1}$ did not affect vasopressin-induced responses, while chlormethiazole $3 \times 10^{-3}$ mol litre$^{-1}$ decreased mean $E_{\text{max}}$ to 54 (7)% ($n = 8$; $P < 0.0001$) and $pD_2$ to 9.0 (0.1) ($n = 8$; $P < 0.01$).

The inhibitory effects of thiopentone and chlormethiazole were reversed within 60 min after washout of the drugs.

Effects of thiopentone and chlormethiazole on responses to vasopressin

$E_{\text{max}}$ and $pD_2$ values for thiopentone and chlormethiazole are given in Table 1. The inhibitory effects of thiopentone and chlormethiazole were reversed within 60 min after washout of the drugs.

DISCUSSION

The present study on maternal intramyometrial arteries obtained from term pregnant women demonstrated consistent, inhibitory effects of thiopentone in various other types of vascular smooth muscle,
however, both enhancing and inhibitory effects on contractile activation have been reported. Thus thiopentone increased basal tension in isolated rat aorta [14] and rabbit aorta and pulmonary artery [4–6]. No such effects were observed in the present study, or with rat portal vein [14]. Furthermore, responses to transmural field stimulation were enhanced by thiopentone $10^{-5}$–$2.4 \times 10^{-4}$ mol l$^{-1}$ in the rabbit pulmonary artery. This seemed to reflect an effect on vascular alpha-adrenoceptor function, since thiopentone $10^{-4}$–$5 \times 10^{-4}$ mol l$^{-1}$ had no effect on neuronal release of $^{3}H$-noradrenaline, while responses to noradrenaline were potentiated [5, 6].

Pregnancy involves degeneration of the uterine sympathetic neuronal supply [15], and adrenergic neuronal responses to transmural field stimulation cannot be elicited in human intramymometrial arteries from term pregnant women [Svane and colleagues, unpublished observations]. However, no potentiating effects on responses to noradrenaline were found in the present study. In contrast, thiopentone $10^{-4}$–$10^{-3}$ mol l$^{-1}$ consistently produced unspecific inhibition of responses to noradrenaline, vasopressin and K$^+$ depolarization in human intramymometrial arteries, in common with previous findings of unspecific inhibition in human fetal stem villous arteries [8]. In rat aorta and portal vein, such inhibitory effects have been explained by interference with transmemebrane and intracellular mobilization of calcium for contractile activation [14]. The present study did not allow conclusions to be drawn on the site of action of thiopentone. In addition to the unspecific inhibition of responses to contractile agents, thiopentone did not interfere with vascular relaxation induced by prostacyclin. The present study on maternal intramymometrial arteries, and previous data in fetal stem villous arteries showed consistent inhibitory effects in both vascular beds.

Large concentrations of chlormethiazole inhibit contractile activation in human fetal stem villous arteries [8], but otherwise the data available on the vascular effects of this compound are sparse. However, only minor cardiovascular side effects are seen when the drug is used for induction of anaesthesia [2, 16, 17]. In the present study, chlormethiazole $3 \times 10^{-6}$–$3 \times 10^{-4}$ mol l$^{-1}$ inhibited contractions induced by noradrenaline, while a concentration of $3 \times 10^{-4}$ mol l$^{-1}$ was needed to affect responses to vasopressin and K$^+$ depolarization. This might reflect a specific effect of chlormethiazole on alpha-adrenoceptor-induced contractile activation, but further studies are needed to substantiate any assumption of such a site of action. Chlormethiazole did not interfere with relaxant responses to prostacyclin and, as with the findings for thiopentone, chlormethiazole seems consistently to inhibit vascular contractile activation in both fetal and maternal uteroplacental arteries.

The clinical relevance of the inhibitory effects of chlormethiazole and thiopentone demonstrated in the present study depends on the appropriateness of the vessels investigated in terms of uteroplacental resistance, and the actual plasma concentrations reached during induction and maintenance of anaesthesia by the two drugs. The distal part of the uterine myometrial arteries supplying the placenta are invaded by trophoblasts during pregnancy and are devoid of medial smooth muscle in late pregnancy. Active regulation of maternal uteroplacental resistance is exerted, therefore, more proximally, close to the radial arteries investigated in the present study [7]. The uterine vessels may show regional variations in functional characteristics related to the location of the placenta, and for ethical reasons the present study included preparations only from the uterine incision. However, a comparative study showed no functional differences between vessels from the lower segment taken from patients with or without low anterior insertion of the placenta/placenta previa [12]. Thus, with some precautions, the present data may be discussed in terms of uteroplacental resistance.

Both thiopentone and chlormethiazole produced inhibition of contractile activation only in large concentrations that probably caused mainly unspecific effects in the maternal intramymometrial arteries. Although peak concentrations of $7 \times 10^{-4}$ mol l$^{-1}$ (thiopentone) and $2 \times 10^{-4}$ mol l$^{-1}$ (chlormethiazole) are reached during Caesarean section, the binding to plasma proteins amounts to 85% and 64%, respectively [18], and major direct vascular effects during anaesthesia for Caesarean section therefore seem unlikely. Significant free plasma concentrations might be expected, however, in case of severely decreased concentrations of plasma albumin, as may occur in pre-eclampsia. According to the present results, uteroplacental vascular relaxation would be expected in such cases. The haemodynamic consequences of such effects depend on the overall systemic response, which includes several factors other than direct vascular effects, but the present results did not yield evidence that clinical use of chlormethiazole and thiopentone should impair placental perfusion.

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REFERENCES

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