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Thiopentone inhibits endothelium-dependent relaxations of rat aortas regulated by endothelial Ca^{2+}-dependent K^+ channels

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Abstract

The present study was designed to examine the mechanisms of inhibitory effect of barbiturates on endothelial function by determining whether thiopentone and phenobarbitone reduce relaxations to acetylcholine mediated by endothelial Ca^{2+}-dependent K^+ channels in rat aortas. Cumulative applications (10^{-9} to 10^{-5} M) of acetylcholine induced endothelium-dependent relaxations, which are abolished by inhibitors of nitric oxide synthase (N^G-nitro-L-arginine methyl ester, 10^{-4} M) and of soluble guanylate cyclase (1*H*-[1,2,4]oxadiazolo [4,3,-*a*]quinoxaline-1-one; ODQ, 5×10^{-6} M). Selective inhibitors of large-conductance Ca^{2+}-dependent K^+ channels (iberiotoxin, 5×10^{-8} M), but not of those with small-conductance (apamin, 5×10^{-8} M), significantly reduced the acetylcholine-induced vasorelaxation. ODQ, but neither iberiotoxin nor apamin, blocked the relaxations of arteries without endothelium induced by nitric oxide donors, sodium nitroprusside (10^{-9} to 10^{-5} M) and 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC-7; 10^{-10} to 10^{-5} M). Thiopentone (10^{-4} and 3×10^{-4} M) but not phenobarbitone (3×10^{-4} M) significantly impaired relaxations to acetylcholine, whereas thiopentone did not alter relaxations to sodium nitroprusside. Thiopentone (3×10^{-4} M) did not affect relaxations to acetylcholine in arteries treated with iberiotoxin (5×10^{-8} M), whereas it reduced these relaxations in arteries treated with apamin (5×10^{-8} M). These results suggest that in rat aortas, large-conductance, but not small-conductance, Ca^{2+}-dependent K^+ channels in endothelial cells, play a role in endothelium-dependent relaxations to acetylcholine, and that thiopentone, but not phenobarbitone, impairs relaxations to acetylcholine mediated by these channels.

Keywords: Acetylcholine; Aorta; K^+ channel, Ca^{2+}-dependent; Endothelium-dependent relaxation; Iberiotoxin; Thiopentone

1. Introduction

Previous studies demonstrated that anaesthetics impair the function of vascular endothelium, suggesting that this effect of anaesthetics may mediate haemodynamic changes during clinical anaesthesia (Johns, 1993). It has been shown that the most commonly used intravenous anaesthetics, barbiturates, including thiopentone and pentobarbitone, can reduce vasorelaxations via endothelial production of nitric oxide (Terasako et al., 1994). However, mechanisms of inhibitory effect of anaesthetics including barbiturates, on endothelial function, are unclear.

Nitric oxide is a potent vasodilator and plays a key role in endothelial control of vascular tone (Moncada et al., 1991). Formation of nitric oxide is dependent on enzymatic activity of nitric oxide synthase. The endothelial nitric oxide synthase, which is one of the constitutive isoforms, is activated by increased intracellular Ca^{2+} levels (Pollock et al., 1991). It has been suggested that endothelium-dependent agonists cause the release of intracellular Ca^{2+} and therefore a transient increase in cytosolic Ca^{2+} levels, which in turn activates endothelial Ca^{2+}-dependent K^+ channels, thereby inducing hyperpolarization of the endothelial cell membrane (Luckhoff and Busse, 1990). This hyperpolarization of cell membrane appears to provide the driving force for transmembrane Ca^{2+} influx into endothelial cells (Luckhoff and Busse, 1990). These results suggest that the activation of Ca^{2+}-dependent K^+ channels by endothelium-dependent agonists is an impor-

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tant regulator of production of nitric oxide in endothelial cells. Indeed, findings on porcine aortic endothelial cells, isolated rabbit aortas and cat hindquarters vascular beds suggest the role of endothelial Ca^{2+}-dependent K^+ channels in endothelial production of nitric oxide (Groschner et al., 1992; Demirel et al., 1994; Champion and Kadowitz, 1997).

Recent studies demonstrated that in isolated rat aortas without endothelium, thiopentone reduces relaxations to ATP-sensitive K^+ channel openers (Kinoshita et al., 1998), and that in cardiac myocytes, this anaesthetic suppresses delayed rectifier and inward rectifier K^+ currents (Sakai et al., 1996; Carnes et al., 1997). These studies suggest that thiopentone may reduce the activity of K^+ channels in vascular smooth muscle cells or cardiac myocytes. However, the modulator role of anaesthetics on Ca^{2+}-dependent K^+ channels, has not been demonstrated and the effects of anaesthetics on endothelial K^+ channels, including Ca^{2+}-dependent K^+ channels, have not been studied.

Therefore, the present study in isolated rat aortas was designed to determine the role of endothelial Ca^{2+}-dependent K^+ channels in the production of endothelium-derived nitric oxide and the mechanisms of inhibitory effect of intravenous anaesthetics on endothelium-dependent relaxations by examining: (a) whether an endothelium-dependent agonist, acetylcholine, augments nitric oxide synthesis via large-conductance or small-conductance Ca^{2+}-dependent K^+ channels in endothelial cells, and (b) whether barbiturates, including thiopentone and phenobarbitone, reduce relaxations to acetylcholine mediated by endothelial Ca^{2+}-dependent K^+ channels.

2. Materials and methods

2.1. Tissue preparation and experimental protocol

The experiments were performed on 3 mm thoracic aortic rings taken from male Wistar-Kyoto rats (300–350 g), anaesthetized with 50 mg kg^{-1} intraperitoneal pentobarbitone sodium. All procedures were conducted in accordance with institutional guidelines. Rings were studied in modified Krebs–Ringer bicarbonate solution (control solution) of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, calcium EDTA 0.026, and glucose 11.1. In certain rings, the endothelium was removed mechanically. Several rings cut from same artery were studied in parallel. Each ring was connected to an isometric force transducer and suspended in an organ chamber filled with 25 ml control solution (37°C, pH 7.4) bubbled with 94% O_2–6% CO_2 gas mixture. The artery was gradually stretched to the optimal point of its length-tension curve as determined by the contraction to phenylephrine (3×10^{-7} M). In most of the studied arteries, optimal tension was achieved approximately at 1.5 g. The functional integrity of endothelium was evaluated by the presence of relaxation induced by acetylcholine (10^{-5} M). Preparations were equilibrated for 90 min. All experiments were performed in the presence of indomethacin (10^{-5} M) to inhibit the possible production of prostanoids by the cyclooxygenase pathway. During submaximal contractions to phenylephrine (3×10^{-7} M), concentration–response curves to acetylcholine (10^{-9} to 10^{-5} M), sodium nitroprusside (10^{-10} to 10^{-5} M) or 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC-7; 10^{-10} to 10^{-5} M) were obtained in the absence or in the presence of N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M), 1*H*-[1,2,4]oxadiazolo [4,3,-*a*]quinoxaline-1-one (ODQ; 5×10^{-6} M), iberiotoxin (5×10^{-8} M), apamin (5×10^{-8} M), thiopentone (10^{-4}, 3×10^{-4} M), or phenobarbitone (3×10^{-4} M). Concentration–response curves were obtained in a cumulative fashion. Only one concentration–response curve was made from each ring. L-NAME, ODQ, iberiotoxin, apamin, thiopentone, and phenobarbitone were given 15 min before addition of phenylephrine (3×10^{-7} M). The relaxations were expressed as a percentage of the maximal relaxations to papaverine (3×10^{-4} M), which was added at the end of experiments to produce maximal relaxations (= 100%) of the arteries.

2.2. Drugs

The following pharmacological agents were used: apamin, dimethyl sulfoxide (DMSO), iberiotoxin, indomethacin, N^G-nitro-L-arginine methyl ester (L-NAME), papaverine hydrochloride, phenylephrine, sodium nitroprusside (Sigma, St. Louis, MO), 1*H*-[1,2,4]oxadiazolo [4,3,-*a*]quinoxaline-1-one (ODQ; ICN pharmaceuticals, Costa Mesa, CA), 1-hydroxy-2-oxo-3-(*N*-methyl-3-aminopropyl)-3-methyl-1-triazene (NOC-7; Dojindo Lab., Kumamoto, Japan), thiopentone sodium (Tanabe pharmaceutical, Osaka, Japan) and phenobarbitone sodium (Tokyo Kasei Kogyo, Tokyo, Japan). Drugs were dissolved in distilled water such that volumes of < 0.15 ml were added to the organ chambers. Stock solutions of ODQ (5×10^{-6} M) were prepared in DMSO (1.6×10^{-4} M). Stock solutions of indomethacin (10^{-5} M) or NOC-7 (10^{-5} M) were prepared in equal molar concentrations of Na_2CO_3 and 0.01 N NaOH solution, respectively. The concentrations of drugs are expressed as final molar (M) concentration.

2.3. Statistical analysis

The data are expressed as means ± S.E.; *n* refers to the number of rats from which the aorta was taken. Statistical analysis was performed using a one-way analysis of variance, followed by Fisher's test. Differences were considered to be statistically significant when P was < 0.05.

Table 1
Effect of N^G-nitro-L-arginine methyl ester (L-NAME; 10^{-4} M) and 1*H*-[1,2,4]oxadiazolo [4,3,-*a*]quinoxaline-1-one (ODQ; 5×10^{-6} M) on endothelium-dependent relaxations to acetylcholine or endothelium-independent relaxations to sodium nitroprusside (SNP) in rat aortas

Acetylcholine, log M											
	−9	−8.5	−8	−7.5	−7	−6.5	−6	−5.5	−5		
Control	0.0 ± 0.0	0.0 ± 0.0	−9.9 ± 3.6	−42.4 ± 6.5	−75.7 ± 3.9	−88.8 ± 1.7	−93.8 ± 0.6	−95.0 ± 1.4	−94.9 ± 1.8		
L-NAME	1.0 ± 1.0	1.9 ± 1.9	4.7 ± 2.0*	4.7 ± 2.0*	4.7 ± 2.0*	3.8 ± 1.8*	2.9 ± 1.2*	2.8 ± 1.8*	3.7 ± 1.6*		
Control	0.0 ± 0.0	0.0 ± 0.0	−3.1 ± 3.1	−18.8 ± 2.3	−56.7 ± 2.7	−75.6 ± 3.2	−80.9 ± 3.0	−86.3 ± 4.9	−87.9 ± 4.6		
ODQ	0.0 ± 0.0	0.0 ± 0.0	2.1 ± 1.2	3.2 ± 1.1*	0.7 ± 2.3*	−0.3 ± 2.0*	−1.1 ± 1.1*	−1.1 ± 1.1*	−1.1 ± 1.1*		
SNP, log M											
	−10	−9.5	−9	−8.5	−8	−7.5	−7	−6.5	−6	−5.5	−5
Control	0.0 ± 0.0	0.0 ± 0.0	−6.1 ± 2.6	−27.2 ± 7.5	−54.0 ± 8.9	−74.2 ± 4.9	−85.5 ± 4.0	−89.7 ± 2.3	−92.3 ± 1.5	−93.5 ± 0.9	−93.5 ± 0.9
ODQ	2.2 ± 2.2	2.2 ± 2.2	2.2 ± 2.2*	2.2 ± 2.2*	2.2 ± 2.2*	2.2 ± 2.2*	2.2 ± 2.2*	0.0 ± 1.8*	−4.7 ± 2.9*	−10.5 ± 4.2*	−13.7 ± 4.2*

Values are means ± S.E. Relaxation expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M; 100% = 760 ± 57 mg [$n = 5$] and 792 ± 54 mg [$n = 5$] for control rings and rings treated with L-NAME, 740 ± 50 mg [$n = 4$] and 880 ± 57 mg [$n = 4$] for control rings and rings treated with ODQ to acetylcholine, and 760 ± 85 mg [$n = 5$] and 728 ± 27 mg [$n = 5$] for control rings and rings treated with ODQ to sodium nitroprusside, respectively). * Differences between control rings and rings treated with L-NAME, and between control rings and rings treated with ODQ, are statistically significant ($P < 0.05$).

3. Results

During contractions to phenylephrine (3×10^{-7} M), a nitric oxide synthase inhibitor, L-NAME (10^{-4} M) abolished endothelium-dependent relaxations to acetylcholine (10^{-9} to 10^{-5} M) (Table 1). A selective soluble guanylate cyclase inhibitor, ODQ (5×10^{-6} M), abolished relaxations to acetylcholine (Table 1) and nitric oxide donors, sodium nitroprusside (10^{-9} to 10^{-5} M) (Table 1), NOC-7 (10^{-10} to 10^{-5} M) (data not shown) in arteries with or without endothelium, respectively.

In arteries with endothelium, a selective large-conductance Ca^{2+}-dependent K^+ channel inhibitor, iberiotoxin (5×10^{-8} M), significantly reduced relaxations to acetylcholine (Fig. 1a), whereas a selective small-conductance Ca^{2+}-dependent K^+ channel inhibitor, apamin (5×10^{-8} M), did not alter these relaxations (Fig. 1b). In arteries without endothelium, iberiotoxin (5×10^{-8} M) and apamin (5×10^{-8} M) did not affect relaxations to sodium nitroprusside ($-\log EC_{50} = 8.1 \pm 0.1$ [$n = 11$] and 7.9 ± 0.1 [$n = 11$] for control rings and rings treated with iberiotoxin, and maximal relaxation [%] = $-95.2 \pm 1.5\%$ [$n = 11$] and $-92.1 \pm 1.5\%$ [$n = 11$] for control rings and rings treated with iberiotoxin, respectively; $-\log EC_{50} = 8.3 \pm 0.1$ [$n = 6$] and 8.3 ± 0.1 [$n = 6$] for control rings and rings treated with apamin, and maximal relaxation [%] = $-91.8 \pm 3.9\%$ [$n = 6$] and $-98.2 \pm 1.9\%$ [$n = 6$] for control rings and rings treated with apamin, respectively) and NOC-7 (data not shown).

Thiopentone (10^{-4} and 3×10^{-4} M) (Fig. 2a), but not phenobarbitone (3×10^{-4} M) (Fig. 2b), significantly impaired endothelium-dependent relaxations to acetylcholine, whereas in arteries without endothelium, thiopentone did not affect relaxations to sodium nitroprusside ($-\log EC_{50} = 8.2 \pm 0.1$ [$n = 11$], 8.2 ± 0.1 [$n = 11$] and 8.0 ± 0.1 [$n = 11$] for control rings and rings treated with thiopentone [10^{-4} or 3×10^{-4} M], and maximal relaxation [%] = $-92.6 \pm 1.7\%$ [$n = 11$], $-91.4 \pm 2.0\%$ [$n = 11$] and $-88.2 \pm 2.2\%$ [$n = 11$] for control rings and rings treated with thiopentone [10^{-4} or 3×10^{-4} M], respectively). Thiopentone (3×10^{-4} M) did not affect relaxations to acetylcholine in arteries treated with iberiotoxin (5×10^{-8} M) (Fig. 3a), whereas it reduced these relaxations in arteries treated with apamin (5×10^{-8} M) (Fig. 3b).

All compounds used in the present study, did not affect contractions to phenylephrine (3×10^{-7} M) (data not shown) and relaxations to papaverine (3×10^{-4} M) (see figure legends).

4. Discussion

In the present study, a large-conductance Ca^{2+}-dependent K^+ channel inhibitor, iberiotoxin, but not a small-conductance Ca^{2+}-dependent K^+ channel inhibitor, apamin, impaired endothelium-dependent relaxations to

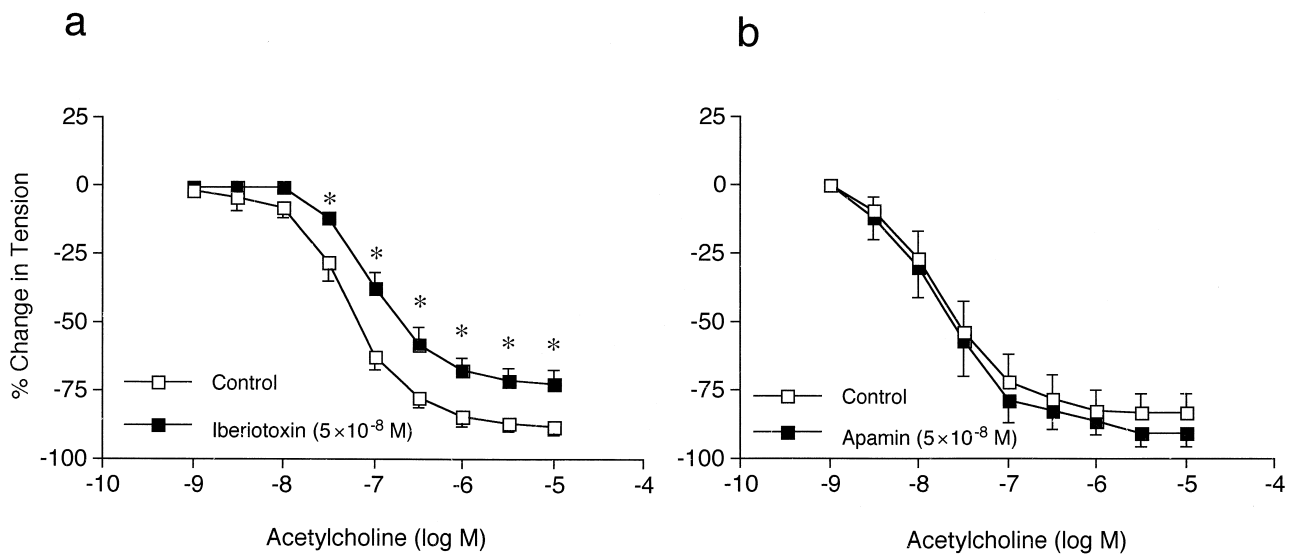

Fig. 1. Concentration–response curves to acetylcholine (10^{-9} to 10^{-5} M) in the absence and in the presence of (a) iberiotoxin (5×10^{-8} M) or (b) apamin (5×10^{-8} M), obtained in rat thoracic aortas with endothelium. Data are shown as mean ± S.E. and expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M; 100% = 673 ± 53 mg [$n = 6$], 735 ± 45 mg [$n = 6$] for control rings and rings treated with iberiotoxin, 616 ± 145 mg [$n = 5$], 528 ± 113 mg [$n = 5$] for control rings and rings treated with apamin, respectively). * Difference between control rings and rings treated with iberiotoxin or apamin is statistically significant ($P < 0.05$).

acetylcholine, whereas these inhibitors failed to alter relaxations to nitric oxide donors in arteries without endothelium. Thiopentone, but not phenobarbitone, reduced relaxations to acetylcholine, whereas thiopentone did not alter relaxations to a nitric oxide donor. Thiopentone did not affect relaxations to acetylcholine in arteries treated with iberiotoxin, whereas it reduced these relaxations in arteries treated with apamin. These results firstly suggest that in rat aortas, thiopentone, but not phenobarbitone, impairs endothelium-dependent relaxations to acetylcholine via its inhibitory effect on endothelial large-conductance Ca^{2+}-dependent K^+ channels.

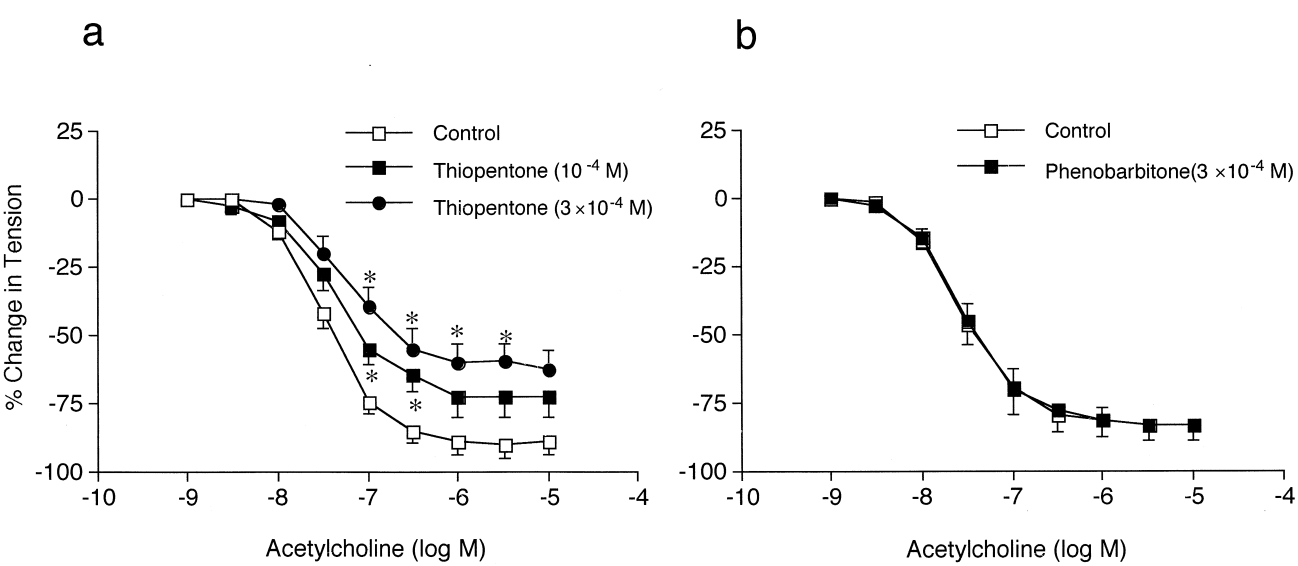

Fig. 2. Concentration–response curves to acetylcholine (10^{-9} to 10^{-5} M) in the absence and in the presence of (a) thiopentone (10^{-4}, 3×10^{-4} M) or (b) phenobarbitone (3×10^{-4} M) obtained in rat thoracic aortas with endothelium. Data are shown as means ± S.E. and expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M; 100% = 650 ± 44 mg [$n = 6$], 620 ± 88 mg [$n = 6$] and 647 ± 52 mg [$n = 6$] for control rings, rings treated with thiopentone (10^{-4} M) or thiopentone (3×10^{-4} M), 560 ± 55 mg [$n = 6$] and 573 ± 69 mg [$n = 6$] for control rings, rings treated with phenobarbitone [3×10^{-4} M], respectively). * Difference between control rings and rings treated with thiopentone (10^{-4} or 3×10^{-4} M) is statistically significant ($P < 0.05$).

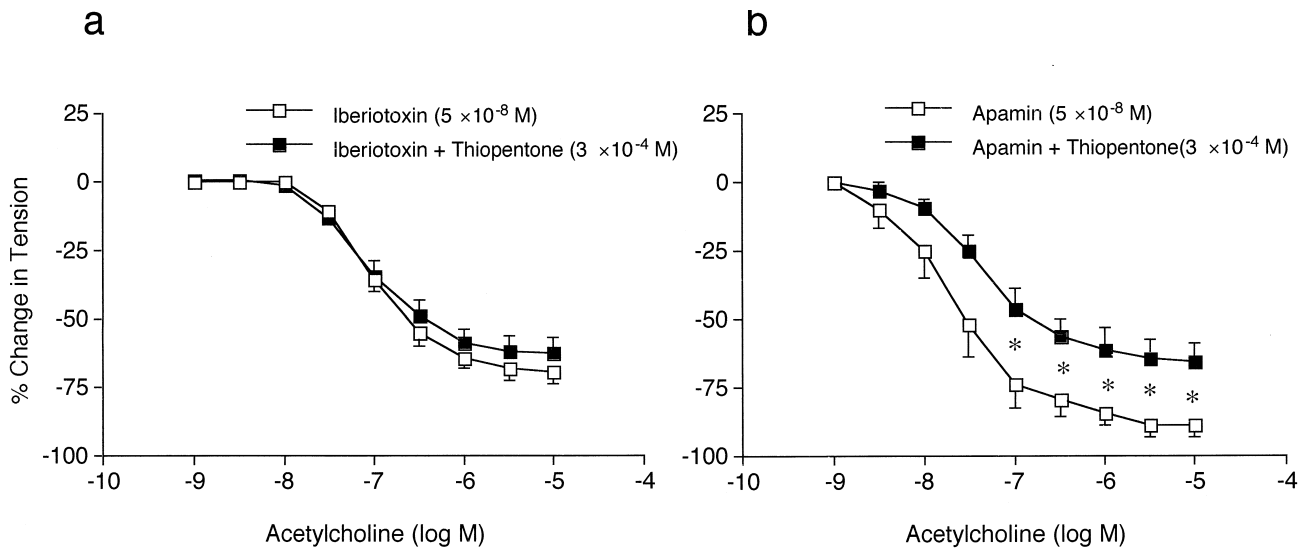

Fig. 3. Concentration–response curves to acetylcholine (10^{-9} to 10^{-5} M) in the presence of (a) iberiotoxin (5×10^{-8} M) or (b) apamin (5×10^{-8} M) with or without thiopentone (3×10^{-4} M), obtained in rat thoracic aortas with endothelium. Data are shown as means ± S.E. and expressed as percent of maximal relaxation induced by papaverine (3×10^{-4} M; 100% = 766 ± 36 mg [$n = 9$] and 720 ± 42 mg [$n = 9$] for rings treated with iberiotoxin or iberiotoxin in combination with thiopentone, 547 ± 94 mg [$n = 6$], 580 ± 82 mg [$n = 6$] for rings treated with apamin or apamin in combination with thiopentone, respectively). * Difference between rings treated with apamin and rings treated with apamin in combination with thiopentone is statistically significant ($P < 0.05$).

In rat aortas, acetylcholine induced endothelium-dependent relaxations which are abolished by a nitric oxide synthase inhibitor L-NAME, demonstrating that nitric oxide produced by the activation of L-arginine metabolism in endothelial cells, is the sole mediator of these relaxations (Moore et al., 1990; Kinoshita and Katusic, 1997). In cerebral arterioles, some of nitric oxide synthase inhibitors reduce relaxations to ATP-sensitive K^+ channel openers, suggesting that L-arginine analogues may impair directly the function of K^+ channels (Kontos and Wei, 1996). However, since in rat aorta, L-NAME did not affect relaxations to an ATP-sensitive K^+ channel opener, pinacidil, it is unlikely that in our experimental condition, L-NAME may alter directly vasodilator effects mediated by the K^+ channels (Ishida et al., unpublished observations). ODQ is a selective inhibitor of soluble guanylate cyclase, with neither effect on nitric oxide synthase activity nor interaction with nitric oxide (Garthwaite et al., 1995; Moro et al., 1996; Olson et al., 1997). ODQ completely abolished relaxations to acetylcholine and nitric oxide donors, suggesting that both endothelium-derived and exogenous nitric oxide may be capable of inducing vasorelaxations via increased production of cytosolic cyclic GMP in smooth muscle cells, and that the direct activation of K^+ channels in smooth muscle cells by nitric oxide, may not mediate these relaxations (Bolotina et al., 1994). In the present study, thiopentone impaired relaxations to acetylcholine, whereas it did not affect those to a nitric oxide donor. Considering these results, it is likely that thiopentone can reduce relaxations to an endothelium-dependent agonist, mediated by nitric oxide released from endothelial cells, and that it does not affect relaxing process of vascular smooth muscle cells, including the enzymatic activity of soluble guanylate cyclase in these cells (Garthwaite et al., 1995).

Lischke et al. (1995) and Kessler et al. (1996) demonstrated that in rabbit carotid arteries and human renal arteries, thiopentone reduces relaxations to the endothelium-derived hyperpolarizing factor via decreased production of this relaxing factor in endothelial cells. However, since endothelium-dependent relaxations to acetylcholine were completely abolished by L-NAME, it is unlikely that in our preparations, endothelium-derived hyperpolarizing factor is involved in the relaxations to acetylcholine (Cohen et al., 1997).

A thiobarbiturate thiopentone, but not an oxybarbiturate phenobarbitone, impaired relaxations to acetylcholine. A previous study demonstrated that an oxybarbiturate pentobarbitone reduces endothelium-dependent relaxations (Terasako et al., 1994). Therefore, it is unlikely that the structural difference between thiobarbiturates and oxybarbiturates, are responsible for the differential inhibitory effects of barbiturates on endothelium-dependent relaxations. In addition to these structural differences, phenobarbitone differs from pentobarbitone only by the substitution of a phenyl group for the methylbutyl side chain, and thiopentone does not contain a phenyl group side chain, suggesting that the phenyl group may play a role in the effects of barbiturates on endothelial function (Piatt and Schiff, 1984). A previous study demonstrated that thiopentone favors induction of a cytochrome *P*-450-dependent monooxygenase, compared with phenobarbitone, indicat-

ing that the potency of barbiturates to induce this enzyme is dependent on the degree of lipophilicity of these drugs (Kim and Fulco, 1983). Therefore, the differential effect of barbiturates on endothelium-dependent relaxations may also be due, in part, to the differential lipophilicity of these compounds.

Ca^{2+}-dependent K^+ channels are classified into several subtypes, including iberiotoxin-sensitive large-conductance and apamin-sensitive small-conductance Ca^{2+}-dependent K^+ channels (Latorre et al., 1989). In endothelial cells, these subtypes of Ca^{2+}-dependent K^+ channels have been identified in several blood vessels including rat aortas (Marchenko and Sage, 1996; Muraki et al., 1997). It has been demonstrated that iberiotoxin and apamin are the selective antagonists of large-conductance and small-conductance Ca^{2+}-dependent K^+ channels, respectively (Galvez et al., 1990; Kuriyama et al., 1995; Nelson and Quayle, 1995; Marchenko and Sage, 1996). Iberiotoxin, but not apamin, reduced relaxations to acetylcholine, whereas these inhibitors did not affect relaxations to nitric oxide donors, suggesting that in rat aortas, endothelial large-conductance, but not small-conductance, Ca^{2+}-dependent K^+ channels play a role in endothelium-dependent relaxations to acetylcholine.

Thiopentone reduced relaxations to acetylcholine in control arteries and arteries treated with apamin, whereas it did not affect these relaxations in arteries treated with iberiotoxin. In addition, thiopentone did not affect relaxations to a nitric oxide donor. These results suggest that thiopentone selectively impairs relaxations to endothelium-derived nitric oxide produced in mediation of large-conductance Ca^{2+}-dependent K^+ channels. This is the first study which illustrates the inhibitory effect of an intravenous anaesthetic on relaxations mediated by endothelial Ca^{2+}-dependent K^+ channels. Precise mechanisms of inhibitory effect are unclear. Muscarinic receptors are coupled with the G-protein (Magyar and Szabo, 1996), and in endothelial cells, Ca^{2+}-dependent K^+ channels coupled with the G-protein(s), play a role in increased intracellular levels of Ca^{2+} (Vaca et al., 1992). However, since thiopentone also reduces endothelium-dependent relaxations to calcium ionophore (Terasako et al., 1994), it is unlikely that this intravenous anaesthetic inactivates endothelial Ca^{2+}-dependent K^+ channels via the direct inhibition of the G-protein-mediated mechanism.

Previous studies in isolated rat aortas (Kinoshita et al., 1998) and in cardiac myocytes (Baum, 1993; Sakai et al., 1996; Carnes et al., 1997) and oocytes (Heath and Terrar, 1997) illustrate the similar inhibitory effect of thiopentone on the activity of K^+ channels other than Ca^{2+}-dependent K^+ channels. In rat aortas, this anaesthetic reduced relaxations to ATP-sensitive K^+ channel openers, cromakalim and pinacidil (Kinoshita et al., 1998). In cardiac myocytes and oocytes, thiopentone suppressed delayed rectifier and inward rectifier K^+ currents (Baum, 1993; Sakai et al., 1996; Carnes et al., 1997; Heath and Terrar, 1997). These results may suggest that thiopentone is capable of producing inhibitory effect of K^+ channels in a number of preparations.

The plasma concentrations of thiopentone during induction of anaesthesia in humans, have been reported as up to 4.5×10^{-4} M, suggesting that serum concentrations of thiopentone may be reached to the concentrations used in the present study only transiently during induction as redistribution of the drug to fatty reservoirs occurs (Morgan et al., 1981; Burch and Stanski, 1983). But in the field of neurosurgery and intensive care, high dose barbiturates are used as a tool of cerebral protection for brain ischaemic patients, and in these cases, serum levels of barbiturates (thiopentone, up to 3×10^{-4} M; phenobarbitone, up to 10^{-4} M) are compatible to or rather lower than the concentrations in the present study (Piatt and Schiff, 1984). Therefore, our results demonstrating the role of endothelial large-conductance Ca^{2+}-dependent K^+ channels in production of nitric oxide, suggest that thiopentone, but not phenobarbitone, may modify vasorelaxations mediated by endothelial Ca^{2+}-dependent K^+ channels in the clinical situation. However, it is still unclear whether the results in conduit arteries have also relevance to the endothelial function in resistance blood vessels such as cerebral arterioles.

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