 © 1995 Stockton Press All rights reserved 0007-1188/95 $12.00

Selective inhibition by barbiturates of the synthesis of endothelium-derived hyperpolarizing factor in the rabbit carotid artery

*Volker Lischke, Rudi Busse & [1]Markus Hecker

Centre of Physiology and *Department of Anaesthesiology, Johann Wolfgang Goethe University Clinic, Theodor-Stern-Kai 7, D-60590 Frankfurt am Main, Germany

1 Several lines of evidence suggest that both volatile and intravenous anaesthetics may interfere with the synthesis and release of endothelium-derived vasoactive factors. We have investigated the effects of three different barbiturates on the release of nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) in phenylephrine (1 μM)-preconstricted, endothelium-intact ring segments of the rabbit carotid artery. The segments were pretreated with the cyclo-oxygenase inhibitor, diclofenac (1 μM), to prevent the formation of vasoactive prostanoids, such as prostacyclin (PGI_2).

2 Acetylcholine (ACh) elicited a concentration-dependent relaxation (EC_{50} 0.15 μM) in control segments which was not significantly different from the relaxant responses of segments pretreated with methohexitone (0.03-0.3 mM), phenobarbitone (0.1-0.3 mM) or thiopentone (0.1-0.3 mM).

3 Inhibition of NO synthesis with N^G-nitro-L-arginine (0.1 mM) significantly reduced the maximum relaxant response to ACh from 96 to 40%. This NO/PGI_2-independent relaxation appeared to be mediated by the release of EDHF, since it was strongly diminished in the presence of the K^+_{Ca} inhibitors, tetrabutylammonium (1-3 mM) and charybdotoxin (10 nM), following preconstriction with potassium calcium (40 mM) or removal of the endothelium. Thiopentone or methohexitone markedly attenuated the EDHF-mediated relaxant response to ACh, while phenobarbitone had no effect. The endothelium-independent relaxation elicited by sodium nitroprusside (0.01-10 μM), on the other hand, was only marginally affected by these anaesthetics.

4 The cytochrome P450 inhibitor, clotrimazole (3-100 μM), mimicked the inhibitory effect of thiopentone and methohexitone on the NO/PGI_2-independent relaxant response to ACh. Moreover the cytochrome P450-catalyzed *O*-dealkylation of 7-ethoxycoumarin by rabbit liver microsomes was inhibited in the presence of thiopentone or methohexitone (0.3-1 mM), while phenobarbitone was without effect.

5 These findings suggest that thiopentone and methohexitone selectively attenuate the EDHF-mediated relaxant response to ACh in the rabbit carotid artery, presumably by interfering with its synthesis from arachidonic acid via the cytochrome P450 epoxygenase pathway.

Keywords: Endothelium-derived hyperpolarizing factor; nitric oxide; acetylcholine; barbiturates; carotid artery; cytochrome P450

Introduction

The vascular endothelium appears to play a pivotal role in mediating some of the haemodynamic effects of anaesthetics (Johns, 1993). Previous investigations have focussed primarily on the interaction of anaesthetics with the synthesis of nitric oxide (NO) by the endothelium and/or its effect on vascular smooth muscle. From these studies it would appear that volatile anaesthetics are potent inhibitors of the NO-dependent vasodilatation at clinically relevant concentrations (Uggeri *et al.*, 1992). Moreover intravenous anaesthetics, such as barbiturates, have been shown to inhibit the endothelium-dependent dilator response to acetylcholine in certain vascular beds (Gerkens, 1987; Terasako *et al.*, 1994). It remains to be elucidated how this barbiturate effect is brought about.

In principle the vascular endothelium is capable of releasing at least three different vasoactive autacoids in response to receptor-dependent agonists: NO, prostacyclin (PGI_2), and an endothelium-derived hyperpolarizing factor (EDHF). By opening K^+_{Ca} channels this factor hyperpolarizes the underlying smooth muscle cells, hence causing a relaxation of the entire vessel wall (Chen *et al.*, 1991; Cowan & Cohen, 1991; Holzmann *et al.*, 1994; Nakashima *et al.*, 1993; Suzuki *et al.*, 1992). Moreover recent experimental evidence suggests that EDHF is a cytochrome P450-derived arachidonic acid metabolite, presumably an epoxide or a mixture thereof (Hu & Kim, 1993; Rosolowsky & Campbell, 1993; Bauersachs *et al.*, 1994; Hecker *et al.*, 1994a; Fulton *et al.*, 1995).

The aim of the present study was to identify the mechanism by which barbiturates interfere with the release of NO and EDHF in isolated segments of the rabbit carotid artery as a vascular model.

Methods

Organ bath studies

New Zealand White rabbits of either sex (1.4-3.2 kg body weight) were anaesthetized with pentobarbitone sodium (Nembutal, Sanofi, 60 mg kg^{-1}, i.v.) and exsanguinated by cutting through both the aorta and vena cava. The left and right carotid artery were dissected, cleaned of adventitial adipose and connective tissue, and cut into rings of 3-4 mm width which were mounted between K30 force transducers (Hugo Sachs Elektronik, March, Germany) and a rigid support for measurement of isometric force. Four rings were simultaneously incubated in 10 ml-organ baths (kindly made available by Hugo Sachs Elektronik), containing warmed (37°C), oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solu-

[1] Author for correspondence.

tion, pH 7.4 (composition in mM: Na^+ 144.0; K^+ 5.9; Cl^- 126.9; Ca^{2+} 1.6; Mg^{2+} 1.2; $H_2PO_4^-$ 1.2; SO_4^{2-} 1.2; HCO_3^- 25.0; D-glucose 11.1) to which the cyclo-oxygenase inhibitor, diclofenac, was added at a concentration of 1 μM. Passive tension was adjusted over a 30 min equilibration period to 2 g, and the Krebs-Henseleit solution exchanged in 10 min intervals. Thereafter the segments were preconstricted with phenylephrine (1 μM) to ~2 g, and the integrity of the endothelium was tested by briefly applying 1 μM acetylcholine; segments showing <60% relaxation were discarded.

Experimental protocol

After washout of phenylephrine and acetylcholine, the rings were allowed to equilibrate for 20 min and then preconstricted again with phenylephrine (1 μM). When a stable constriction was obtained, the barbiturates or other test compounds were administered to three rings for 15 min, and a cumulative concentration-response curve to acetylcholine (0.03 – 10 μM) was established in the presence of the barbiturates followed by a washout period of 30 min. One segment received no barbiturate, but was exposed to acetylcholine at the same time as the other three rings (time control).

To study the effects of the anaesthetics on the NO/PGI_2-independent relaxant response to acetylcholine, in addition to diclofenac the segments were treated with the NO synthase inhibitor, N^G-nitro-L-arginine (0.1 mM), for 30 min followed by the same experimental protocol as described before (i.e. three rings received the test compounds, the fourth segment did not). After another 30 min washout period, the segments were again constricted with phenylephrine, and the effects of the test compounds on the endothelium-independent relaxation induced by sodium nitroprusside (SNP, 0.01 – 10 μM) were investigated in the same manner.

Despite the repeated exposure to acetylcholine no tachyphylaxis was observed in any of the control segments throughout the study (n = 59).

Cytochrome P450 assay

To elucidate the potential cytochrome P450-inhibitory effect of the barbiturates, a sensitive spectrofluorometric assay was employed in which the *O*-dealkylation of 7-ethoxycoumarin to the highly fluorescent 7-hydroxycoumarin (umbelliferon) is monitored over time (Ullrich & Weber, 1972; Prough *et al.*, 1978). As a source of cytochrome P450 the microsomal fraction from the liver of non-induced New Zealand White rabbits was prepared essentially as previously described for NO synthase (Hecker *et al.*, 1994b). An aliquot of the microsomal protein (0.5 mg) was stirred in a quartz glass cuvette with 0.1 M Tris-HCl buffer (pH 7.6), containing 20 μM 7-ethoxycoumarin. After 2 min equilibration at 37°C, in the presence or absence of the anaesthetics, the reaction was initiated by the addition of NADPH (0.1 mM) and monitored over a period of 10 min in a dual wavelength spectrofluorometer (PTI, Wedel, Germany) with the excitation and emission wavelengths set to 370 and 455 nm respectively. Calibration of the assay was performed by adding known concentrations of umbelliferon (0.1 – 10 μM) to a cuvette containing heat-denatured microsomal protein. The specificity of the fluorimetric assay was confirmed by using the cytochrome P450 inhibitor, metyrapone (1 mM), which almost completely abrogated the microsomal de-ethylase activity (93.3 ± 1.4% inhibition, n = 6).

Statistics

Unless indicated otherwise, all data in the figures and text are expressed as means ± s.e.mean of n observations with ring segments from different arteries. Statistical evaluation was performed by two-sided Fischer-Pitman analysis followed by a Bonferroni post test for multiple comparisons with a P-value < 0.05 considered statistically significant.

Materials

Diclofenac (Voltaren) was obtained from Ciba-Geigy (Wehr, Germany); apamin and charybdotoxin from Alomone Laboratories (Jerusalem, Israel); N^G-nitro-L-arginine (free acid) from Serva (Heidelberg, Germany); acetylcholine, clotrimazole, 7-ethoxycoumarin, NADPH, metyrapone (2-methyl-1,2-di-3-pyridyl-1-propanone), phenylephrine, sodium nitroprusside and umbelliferon from Sigma (Deisenhofen, Germany); methohexitone sodium (Brevimytal) from Lilly (Bad Homburg, Germany); phenobarbitone sodium (Luminal) from Bayer (Leverkusen, Germany); and thiopentone sodium (Trapanal) from Byk Gulden (Konstanz, Germany).

Results

Acetylcholine-induced NO-dependent relaxation

Exposure of endothelium-intact carotid artery segments to a half-maximally effective concentration of phenylephrine (1 μM) produced an active tension of 2.4 ± 0.1 g (n = 47). The subsequent addition of acetylcholine resulted in a concentration-dependent relaxation in endothelium-intact (Figures 1 – 3), but not in endothelium-denuded rings (n = 4). In the presence of phenobarbitone (Figure 1a), methohexitone (Figure 2a) or thiopentone (Figure 3a) at concentrations up to 0.3 mM, neither the level of preconstriction (Table 1) nor the relaxant response to acetylcholine was significantly different from the corresponding control segments, suggesting that the barbiturates did not affect basal or agonist-stimulated NO release. In agreement with this finding, all three barbiturates (0.01 – 1 mM) failed to affect the activity of a semi-purified constitutive NO synthase preparation from rabbit brain and freshly isolated porcine aortic endothelial cells (n = 6 – 8), as determined by monitoring the N^G-nitro-L-arginine-sensitive conversion of radiolabelled L-arginine to L-citrulline (Hecker *et al.*, 1994b).

Acetylcholine-induced EDHF-mediated relaxation

After incubation of the segments with N^G-nitro-L-arginine for 30 min, the vasoconstrictor effect of phenylephrine was significantly enhanced (3.9 ± 0.1 g, n = 59, P < 0.01; cf. Table 1), indicative of a loss of basal NO release. The concentration-response curve to acetylcholine was significantly shifted to the right (from 0.15 ± 0.03 to 0.45 ± 0.07 μM, n = 47 – 59, P < 0.01), while its maximum relaxant effect was reduced from 95.5 ± 0.4 to 40.0 ± 4.8% (P < 0.01).

This NO/PGI_2-independent relaxation was markedly attenuated when the segments were preconstricted with potassium chloride (40 mM, Figure 4a) to the same level of tone as attained with phenylephrine and N^G-nitro-L-arginine (Table 2). The non-selective K^+_{Ca} channel inhibitor tetrabutylammonium (1 – 3 mM, Figure 4b) and charybdotoxin

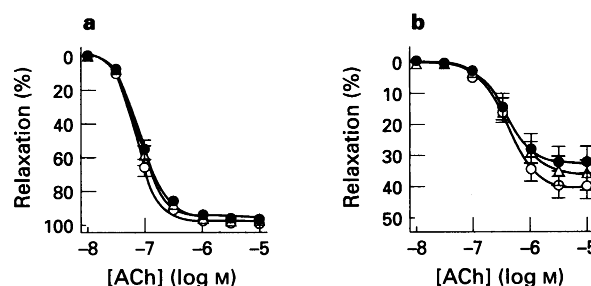

Figure 1 Effects of phenobarbitone (○, 0.1 mM; △, 0.3 mM) on the relaxant response to acetylcholine (●) in the absence (**a**) and presence (**b**) of N^G-nitro-L-arginine (n = 8 – 11).

Table 1 Effects of phenobarbitone, methohexitone and thiopentone on contractions to phenylephrine (1 μM) in the absence (untreated) and presence (treated) of 0.1 mM N^G-nitro-L-arginine

Compound	*Concentration* (mM)	*Force* (g) *Untreated*	*Treated*	n
	0	2.4±0.2	3.8±0.2**	11
Phenobarbitone	0.1	2.4±0.2	3.7±0.2**	11
	0.3	2.3±0.1	3.7±0.2**	10
	0	3.2±0.2	5.1±0.3**	14
Methohexitone	0.03	2.5±0.2•	3.3±0.1*,•	7
	0.1	2.8±0.2	4.1±0.2**,•	11
	0	2.4±0.3	3.6±0.3*	8
Thiopentone	0.1	2.4±0.2	3.4±0.2**	12
	0.3	2.3±0.2	3.0±0.2*	13

*$P<0.05$, **$P<0.01$ vs. untreated; •$P<0.05$ vs. time control, i.e. 0 mM methohexitone.

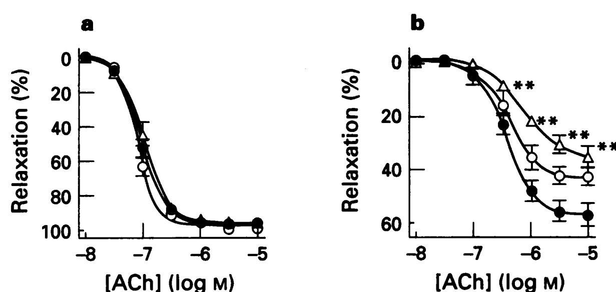

Figure 2 Effects of methohexitone (○, 0.03 mM; △, 0.1 mM) on the relaxant response to acetylcholine (●) in the absence (a) and presence (b) of N^G-nitro-L-arginine (n=7–14, **$P<0.01$ vs. segments not exposed to methohexitone).

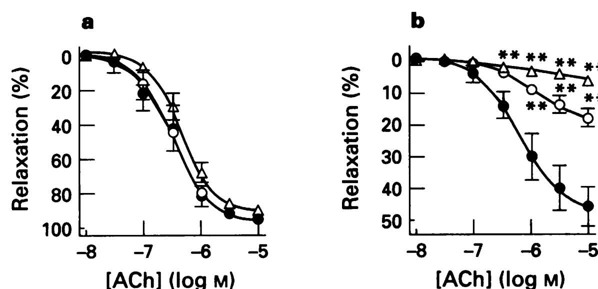

Figure 3 Effects of thiopentone (○, 0.1 mM; △, 0.3 mM) on the relaxant response to acetylcholine (●) in the absence (a) and presence (b) of N^G-nitro-L-arginine (n=8–13, **$P<0.01$ vs. segments not exposed to thiopentone).

(10 nM, Figure 4c), a specific inhibitor of large conductance (35–250 pS) K^+_{Ca} channels (Miller *et al.*, 1985), also strongly diminished the relaxant response to acetylcholine in segments pretreated with N^G-nitro-L-arginine, while apamin (1 μM), a specific inhibitor of small conductance (6–14 pS) K^+_{Ca} channels (Blatz & Magleby, 1986), had no effect (Figure 4d). Similarly, the K^+_{ATP} channel inhibitor, glibenclamide (3 μM; Cowen & Cohen, 1991) did not attenuate the N^G-nitro-L-arginine-resistant relaxant response to acetylcholine (n=4), suggesting that this NO/PGI_2-independent relaxation was mediated by the release of EDHF from the endothelium and the activation of medium to high conductance K^+_{Ca} channels in the smooth muscle.

Phenobarbitone in concentrations up to 0.3 mM failed to affect the acetylcholine-induced EDHF-mediated relaxation (Figure 1b) which, on the other hand, was significantly attenuated by 0.1 mM methohexitone (Figure 2b). At 0.3 mM the inhibitory effect of methohexitone was virtually identical (n=9), suggesting that 0.1 mM represents the maximally effective concentration of this barbiturate. In the presence of thiopentone, the NO/PGI_2-independent relaxant response to acetylcholine was strongly reduced at 0.1 mM and abolished at 0.3 mM (Figure 3b). Moreover pentobarbitone, which was used to anaesthetize the rabbits before the removal of the carotid arteries, had no significant effect on the acetylcholine-induced release of NO or EDHF in concentrations up to 0.3 mM (n=6).

Role of cytochrome P450

The inhibitory effect of methohexitone and thiopentone on the EDHF-mediated acetylcholine-induced relaxation was mimicked by the cytochrome P450 inhibitor, clotrimazole, with an IC_{50}- value of ~5 μM (Figure 5a). Clotrimazole on the other hand did not affect the endothelium-dependent relaxant response to acetylcholine in the absence of N^G-nitro-L-arginine (n=6–9).

In a separate series of experiments, clotrimazole also potently (IC_{50}~10 μM) inhibited the cytochrome P450 activity present in rabbit liver microsomes which was determined by monitoring the *O*-dealkylation of 7-ethoxycoumarin to umbelliferon (Figure 5b). In this assay system, phenobarbitone was virtually inactive and methohexitone produced only a moderate inhibitory effect, while thiopentone was as effective as, but less potent than clotrimazole in attenuating the microsomal cytochrome P450 activity (Figure 5c).

Sodium nitroprusside-induced relaxation

Phenobarbitone had no inhibitory effect on the endothelium-independent relaxant response to sodium nitroprusside which on the other hand was slightly enhanced by methohexitone and modestly attenuated by thiopentone (Figure 6a). Similar findings were obtained at a barbiturate concentration of 0.3 mM (n=5–32). Moreover phenobarbitone and thiopentone (0.3 mM) did not significantly affect the stimulation by sodium nitroprusside of a purified soluble guanylyl cyclase preparation isolated from bovine lung (n = 3), as determined by monitoring the conversion of radiolabelled GTP to cyclic GMP (Hecker *et al.*, 1994b). Clotrimazole did not alter the relaxant response to sodium nitroprusside (Figure 6b).

Discussion

The present findings demonstrate that in the carotid artery of the rabbit barbiturates, such as phenobarbitone, pentobarbitone, methohexitone or thiopentone, have no significant effect on the acetylcholine-induced release of NO from the en-

Table 2 Effects of high K^+ (isotonic replacement of Na^+ by K^+), the $K^+{}_{Ca}$ channel antagonists and clotrimazole (treated) on contractions to phenylephrine (1 μM) in the presence of 0.1 mM N^G-nitro-L-arginine

Compound	*Concentration*	*Force* (g) *Untreated*	*Treated*	n
K^+	40 mM	3.5±0.3	3.5±0.1	6
Apamin	1 μM	4.0±0.2	4.9±0.6	4
Charybdotoxin	10 nM	4.0±0.2	4.5±0.2	4
Tetrabutylammonium	1 mM	3.7±0.8	4.3±0.6	6
	3 mM		3.2±0.5	7
Clotrimazole	3 μM	4.1±0.2	3.5±0.2	17
	10 μM		4.5±0.2	9
	100 μM		3.3±0.3*	9

*$P<0.05$ vs. N^G-nitro-L-arginine alone, i.e. untreated.

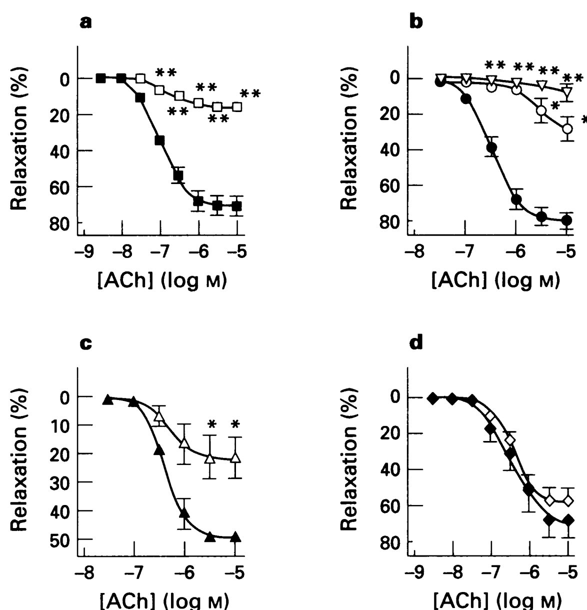

Figure 4 Effects of (**a**) preconstriction with potassium chloride (□, 40 mM), and exposure to (**b**) tetrabutylammonium (○, 1 mM; ▽, 3 mM), (**c**) charybdotoxin (△, 10 nM) or (**d**) apamin (◇, 1 μM) on the relaxant response to acetylcholine in the presence of N^G-nitro-L-arginine ($n=4-7$; *$P<0.05$, **$P<0.01$, vs. segments not exposed to high K^+ (■) or the $K^+{}_{Ca}$ inhibitors; ●, ▲, ◆).

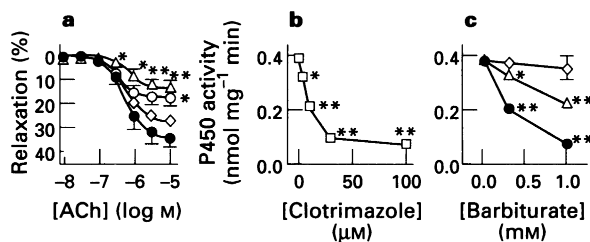

Figure 5 (**a**) Effects of clotrimazole (◇, 3 μM, ○, 10 μM, △, 100 μM) on the relaxant response to acetylcholine in the presence of N^G-nitro-L-arginine ($n=6-9$; *$P<0.05$, **$P<0.01$ vs. segments not exposed to the cytochrome P450 inhibitor, ●). (**b,c**) Effects of (**b**) clotrimazole (□) and (**c**) phenobarbitone (◇), methohexitone (△) or thiopentone (●) on the *O*-dealkylation of 7-ethoxycoumarin by rabbit liver microsomes ($n=3-5$; *$P<0.05$, **$P<0.01$ vs. control). Where no error bar is shown, s.e.mean falls within the dimensions of the symbol.

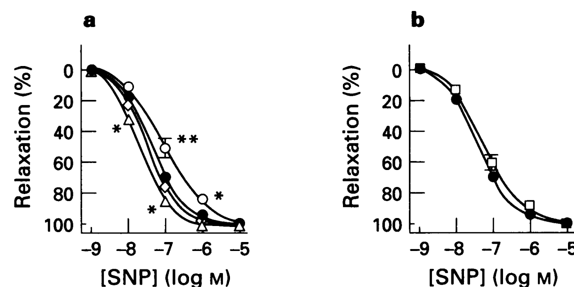

Figure 6 Effects of (**a**) phenobarbitone (◇, 0.1 mM), methohexitone (△, 0.1 mM) and thiopentone (○, 0.1 mM; $n=9-32$) or (**b**) clotrimazole (□, 0.1 mM, $n=9$) on the endothelium-independent relaxant response to sodium nitroprusside (SNP; *$P<0.05$, **$P<0.01$ vs. segments not exposed to the barbiturates or the cytochrome P450 inhibitor, ●).

dothelium and/or its effect on soluble guanylyl cyclase in the smooth muscle. In contrast two of these anaesthetics, methohexitone and thiopentone, markedly attenuated the NO/PGI_2-independent relaxant response to acetylcholine which was mediated by the release of EDHF, as shown by the lack of effect of acetylcholine on N^G-nitro-L-arginine-treated segments in the presence of depolarizing concentrations of K^+ or after $K^+{}_{Ca}$ channel blockade. This effect of the two barbiturates was mimicked by the cytochrome P450 inhibitor, clotrimazole, reinforcing the notion that EDHF is a cytochrome P450-derived arachidonic acid metabolite (Pinto *et al.*, 1987; Bauersachs *et al.*, 1994; Hecker *et al.*, 1994a; Fulton *et al.*, 1995). Moreover the finding that clotrimazole was a potent inhibitor of the NO/PGI_2-independent relaxant response to acetylcholine provides further indirect evidence that EDHF is an arachidonic acid epoxide (Hu & Kim, 1993; Rosolowsky & Campbell, 1993; Hecker *et al.*, 1994a), since this cytochrome P450 inhibitor is thought to block specifically the epoxygenase pathway of arachidonic acid metabolism (Capdevilla *et al.*, 1988; Fulton *et al.*, 1995).

Although barbiturates are well known for their stimulatory effect on cytochrome P450 gene expression, information concerning their effect on cytochrome P450 activity is rather scarce. Unfortunately the amount of cytochrome P450 present in the endothelium of the rabbit carotid artery is too small to allow a determination of its activity by conventional fluorimetric methods. We have therefore investigated the potential cytochrome P450-inhibitory effect of the barbiturates by monitoring the *O*-dealkylation of 7-ethoxycoumarin to umbelliferon in rabbit liver microsomes. Thiopentone and methohexitone, but not phenobarbitone inhibited this cytochrome P450-dependent de-ethylase reaction, suggesting that in principle these anaesthetics can interfere with the cytochrome P450-dependent synthesis of EDHF rather than with its effect on smooth muscle $K^+{}_{Ca}$ channel activity. This hypothesis was strengthened by the relative inhibitory potency of the two

barbiturates in the cytochrome P450 assay which matched the extent of their inhibitory effect on the acetylcholine-induced release of EDHF. In this context, however, it is important to note that the de-ethylase reaction in the microsomes and the epoxygenase-like formation of EDHF are likely to be catalyzed by two very different cytochrome P450 isoenzymes.

Thiopentone, but not pentobarbitone, causes a moderate inhibition of the endothelium-dependent relaxant response to acetylcholine in the canine coronary artery and in the rat aorta, and this effect has been attributed to an interaction of the anaesthetic with the release and/or effect of NO (Terasako *et al.*, 1994). However at 0.3 mM thiopentone had no significant effect on the acetylcholine-induced increase in intravascular cyclic GMP (an index of NO release), while at this concentration the barbiturate clearly attenuated the acetylcholine-induced relaxation. Moreover the relaxant response to acetylcholine in these arteries is known to involve the release of EDHF (Pinto *et al.*, 1987; Chen *et al.*, 1991; Suzuki *et al.*, 1992). The findings of Terasako *et al.* (1994) may therefore be interpreted similarly to ours as an inhibition by thiopentone of the acetylcholine-induced EDHF release.

The lack of effect of the barbiturates on the NO-mediated relaxant response to acetylcholine was substantiated by the finding that these anaesthetics have no direct inhibitory effect on endothelial NO synthase activity. Moreover their marginal effect on the endothelium-independent relaxation elicited by sodium nitroprusside as well as their lack of effect on the stimulation of purified soluble guanylyl cyclase by this NO donor suggest that they do not interfere with the NO-induced formation of cyclic GMP in the vascular smooth muscle at concentrations which markedly attenuate the acetylcholine-induced synthesis of EDHF.

When compared to NO, the physiological importance of EDHF release, e.g. in isolated coronary (Holzmann *et al.*, 1994) and carotid arteries (this study) where 40–60% of the relaxant response to bradykinin or acetylcholine is resistant to inhibition of either NO or PGI_2 formation, may have been overlooked. In the coronary microcirculation of the rat heart only the duration, but not amplitude, of the dilator response to bradykinin is affected by the combined treatment with an inhibitor of NO synthase and cyclo-oxygenase (Bauersachs *et al.*, 1994; Fulton *et al.*, 1995). Similarly, the duration, but not amplitude, of the systemic hypotensive response to acetylcholine, ATP or bradykinin in the anaesthetized rat *in vivo* is reduced following application of an NO synthase inhibitor (O'Shaughnessy *et al.*, 1992). Taken together these findings imply that the release of EDHF may play an important role in the control of local vascular tone under physiological conditions.

Moreover it is conceivable that under conditions of reduced NO synthesis, i.e. in atherosclerosis, hypertension or ischaemia, the release of this autacoid represents a compensatory or reserve mechanism for the maintenance of vascular tone. Indeed, in the carotid artery of hypercholesteroleamic rabbits, the NO-independent, charybdotoxin-sensitive relaxant response to acetylcholine is preserved, while the acetylcholine-induced NO release is strongly impaired (Najibi *et al.*, 1994). The adverse effect of thiopentone and methohexitone on the synthesis of EDHF may therefore pose a haemodynamic problem during the induction of anaesthesia with these barbiturates in patients with atherosclerosis.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (Bu 486/4-3, He 1587/5-1) and the European Commission (BMH1-CT92-1893). M.H. is the recipient of a Heisenberg fellowship from the Deutsche Forschungsgemeinschaft. We thank Dr Agnieszka T. Bara for her help with the organ bath studies and Gabriele Hoch for expert technical assistance.

References

BAUERSACHS, J., HECKER, M. & BUSSE, R. (1994). Display of the characteristics of endothelium-derived hyperpolarizing factor by a cytochrome P450-derived arachidonic acid metabolite in the coronary microcirculation. *Br. J. Pharmacol.*, **113**, 1548–1553.

BLATZ, A.L. & MAGLEBY, K.L. (1986). Single apamin-blocked Ca^{2+}-activated K^+ channels of small conductance in cultured rat skeletal muscle. *Nature*, **323**, 718–720.

CAPDEVILA, J., GIL, L., ORELLANA, M., MARNETT, L.J., MASON, J.I., YODAGIRI, P. & FALCK, J.R. (1988). Inhibitors of cytochrome P-450 dependent arachidonic acid metabolism. *Arch. Biochem. Biophys.*, **261**, 257–263.

CHEN, G.F., YAMAMOTO, Y., MIWA, K. & SUZUKI, H. (1991). Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. *Am. J. Physiol.*, **260**, H1888–H1892.

COWEN, C.L. & COHEN, R.A. (1991). Two mechanisms mediate the relaxation by bradykinin of pig coronary artery: NO-dependent and -independent responses. *Am. J. Physiol.*, **261**, H380–H385.

FULTON, D., MAHBOUBI, K., MCGIFF, J.C. & QUILLEY, J. (1995). Cytochrome P450-dependent effects of bradykinin in the rat heart. *Br. J. Pharmacol.*, **114**, 99–102.

GERKENS, J.F. (1987). Barbiturate inhibition of endothelium-dependent dilatation of blood- and Krebs-perfused rat tail arteries. *Eur. J. Pharmacol.*, **134**, 293–301.

HECKER, M., BARA, A.T., BAUERSACHS, J. & BUSSE, R. (1994a). Characterization of the endothelium-derived hyperpolarizing factor released from coronary arteries as a cytochrome P450-derived arachidonic acid metabolite. *J. Physiol.*, **481**, 407–414.

HECKER, M., MÜLSCH, A., BASSENGE, E., FÖSTERMANN, U. & BUSSE, R. (1994b). Subcellular localisation and characterization of nitric oxide synthase(s) in endothelial cells - physiologic implications. *Biochem. J.*, **299**, 247–252.

HOLZMANN, S., KUKOVETZ, W.R., WINDISCHHOFER, W., PASCHKE, E. & GRAIER, W.F. (1994). Pharmacologic differentiation between endothelium-dependent relaxations sensitive and resistant to nitro-L-arginine in coronary arteries. *J. Cardiovasc. Pharmacol.*, **23**, 747–756.

HU, S. & KIM, H.S. (1993). Activation of K^+ channel in vascular smooth muscles by cytochrome P450 metabolites of arachidonic acid. *Eur. J. Pharmacol.*, **230**, 215–221.

JOHNS, R.A. (1993). Endothelium, anaesthetics, and vascular control. *Anesthesiology*, **79**, 1381–1391.

MILLER, C., MOCZYDLOWSKI, E., LATORRE, R. & PHILLIPS, M. (1985). Charybdotoxin, a protein inhibitor of single Ca^{2+}-activated K^+ channels from mammalian skeletal muscle. *Nature*, **313**, 316–318.

NAJIBI, S., COWAN, C.L., PALACINO, J.J. & COHEN, R.A. (1994). Enhanced role of potassium channels in relaxations to acetylcholine in hypercholesterolemic rabbit carotid artery. *Am. J. Physiol.*, **266**, H2061–H2067.

NAKASHIMA, M., MOMBOULI, J.-V., TAYLOR, A.A. & VANHOUTTE, P.M. (1993). Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J. Clin. Invest.*, **92**, 2867–2871.

O'SHAUGHNESSY, K.O., NEWMAN, C.M. & WARREN, J.B. (1992). Inhibition in the rat of nitric oxide synthesis in vivo does not attenuate the hypotensive action of acetylcholine, ATP or bradykinin. *Exp. Physiol.*, **77**, 285–292.

PINTO, A., ABRAHAM, N.G. & MULLANE, K.M. (1987). Arachidonic acid-induced endothelial-dependent relaxations of canine coronary arteries: contribution of a cytochrome P-450-dependent pathway. *J. Pharmacol. Exp. Ther.*, **240**, 856–863.

PROUGH, R.A., BURKE, M.D. & MAYER, R.T. (1978). Direct fluorometric methods for measuring mixed-function oxidase activity. *Methods Enzymol.*, **52**, 372–377.

ROSOLOWSKY, M. & CAMPBELL, W.B. (1993). Role of PGI_2 and epoxyeicosatrienic acids in relaxation of bovine coronary arteries to arachidonic acid. *Am. J. Physiol.*, **264**, H327–H335.

SUZUKI, H., CHEN, G. & YAMAMOTO, Y. (1992). Endothelium-derived hyperpolarizing factor (EDHF). *Jpn. Circ. J.*, **56**, 170–174.

TERASAKO, K., NAKAMURA, K., TODA, H., KUKUYAMA, M., HATANO, Y. & MORI, K. (1993). Barbiturates inhibit both endothelium-dependent and independent relaxation mediated by cyclic GMP. *Anesth. Analg.*, **78,** 823–830.

UGGERI, M.J., PROCTOR, G.J. & JOHNS, R.A. (1992). Halothane, enflurane, and isoflurane attenuate both receptor and non-receptor mediated EDRF production in rat thoracic aorta. *Anesthesiology*, **76,** 1012–1017.

ULLRICH, V. & WEBER, P. (1972). The O-dealkylation of 7-ethoxycoumarin by liver microsomes. *Hoppe-Seyler's Z. Physiol. Chem.*, **353**, 1171–1177.

(Received February 9, 1995
Revised March 27, 1995
Accepted April 7, 1995)