Br. J. Pharmacol. (1992), **105**, 424–428

Endothelium-dependent contractile responses to 5-hydroxytryptamine in the rabbit basilar artery

Joanna M. Seager, Angela H. Clark & [1]C.J. Garland

Department of Physiology and Pharmacology, University of Southampton, Bassett Crescent East, Southampton SO9 3TU

1 5-Hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) stimulated additional, endothelium-dependent contractions in rabbit isolated basilar arteries which had been submaximally contracted with either histamine or potassium chloride.

2 The additional contractions to 5-HT were not altered by the 5-HT_2 antagonist, ketanserin (1 μM), but were abolished in the presence of the cyclo-oxygenase inhibitor indomethacin (3 μM).

3 The additional smooth muscle contraction stimulated by 5-HT was increased in the presence of the competitive substrate inhibitor for nitric oxide synthase, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM).

4 Neither of the selective 5-HT agonists, 8-hydroxy-dipropylaminotetralin (8-OH DPAT) or α-methyl 5-HT stimulated endothelium-dependent contraction, but these agonists did reduce the rate at which histamine-induced tension spontaneously declined. This effect represented a direct action on the smooth muscle cells, as it was independent of the presence of endothelial cells.

5 Smooth muscle relaxation was not obtained in response to 5-HT, whether or not indomethacin was present to block endothelium-dependent contraction. None of the other selective 5-HT agonists, 5-CT, 8-OH DPAT or α-methyl 5-HT produced endothelium-dependent smooth muscle relaxation, when applied against a background of contraction.

6 These data show that endothelium-dependent smooth muscle contraction can be produced by stimulating 5-HT receptors in the partially contracted rabbit basilar artery. Similar contraction to 5-CT indicates an involvement by 5-HT_1 receptors. The susceptibility of the contractions to indomethacin suggest they are mediated by a metabolite of arachidonic acid.

Keywords: Vascular smooth muscle; 5-hydroxytryptamine; basilar artery; endothelial cells; endothelium-derived contractile factors (EDCF)

Introduction

The overall action of 5-hydroxytryptamine (5-HT) on blood vessels is complex. Its main action is to stimulate contraction in vascular smooth muscle cells, although a direct relaxant action has been demonstrated (Feniuk *et al.*, 1983). 5-HT can also influence smooth muscle tone via the vascular endothelium. The majority of the evidence which is available has resulted from investigations into the ability of the endothelium to attenuate the constrictor action of 5-HT.

In pre-contracted coronary arteries and jugular veins, 5-HT induces endothelium-dependent relaxation of the smooth muscle cells, which reflects the release of endothelium-derived relaxing factor (EDRF, Cocks & Angus, 1983; Leff *et al.*, 1987). This action of 5-HT is not blocked by ketanserin, and appears to be mediated by a 5-HT_1 receptor. In a number of other arteries, including the canine basilar artery, although removal of the endothelium has been found to potentiate the contractile action of 5-HT, it has not been possible to demonstrate relaxation in pre-contracted arteries with an intact endothelium (Martin *et al.*, 1986; Connor & Feniuk, 1989). One possibility, is that the increased contraction to 5-HT which follows removal of the endothelium in these arteries can be explained by a high spontaneous release of EDRF. In the rabbit basilar artery, removal of the endothelium increases the contractile response to 5-HT, indicating that part of the response to 5-HT in this artery is mediated via the endothelium (Garland, 1987).

In addition to an endothelium-dependent inhibitory action on vascular smooth muscle cells, there is some evidence to suggest that 5-HT can stimulate endothelium-dependent smooth muscle contraction in the aorta of spontaneously hypertensive rats, and *in vivo*, in mouse cerebral arteries (Luscher & Vanhoutte, 1988; Rosenblum & Nelson, 1988).

[1] Author for correspondence.

We have now investigated the cerebrovascular action of 5-HT and some related agonists, using rabbit isolated, pre-contracted basilar arteries, to show to what extent constrictor responses to 5-HT are mediated by the endothelium. Some of these results have been presented in preliminary form to the British Pharmacological Society (Clark & Garland, 1990).

Methods

Preparation of isolated arteries

New Zealand white rabbits (2–4 kg) of either sex were anaesthetized with an intravenous injection of sodium pentobarbitone (60 mg kg^{-1}), and killed by rapid exsanguination. The brain was removed, placed in physiological salt solution (PSS; see Garland, 1987 for composition) at room temperature, and the basilar artery carefully dissected and removed. In some cases, the endothelial cells were destroyed at this stage, by carefully rubbing the intimal surface with a blunt-ended syringe needle. This procedure abolished relaxation to the subsequent addition of acetylcholine (1–100 μM). After some experiments, artery segments were examined histologically, to confirm that the endothelial cells had been destroyed. The artery was then cut into 2 mm cylindrical segments, which were placed in individual organ baths (5 ml) containing PSS bubbled with 95% O_2:5% CO_2. The segments were each suspended between 2 L-shaped stainless steel wire supports (each of 0.14 mm diameter) inserted into the lumen. One support was connected to an isometric force transducer (Grass, FT03), the other to a micrometer. Segments were then equilibrated at a predetermined optimal resting tension of 500 mg for at least 1 h (Garland, 1987). The response to a submaximal concentration of histamine (1 μM) or potassium chloride (30 mM) was then determined in each tissue. These concentrations of histamine and potassium gave contractions of around 50% tissue maximum.

Concentration-response determinations

Cumulative concentration-response curves to agonists were obtained by direct addition of agonists to the bathing fluid, and allowing sufficient time for the effects of each concentration to become fully established before adding a higher concentration. In experiments on pre-contracted segments, four preparations from the same animal were mounted in parallel and precontracted with submaximal concentrations of either histamine or potassium chloride. In three of the baths, cumulative concentration-response curves to agonists were established on top of the background contraction. The fourth segment served as a time control to detect 'fade' of background contraction. No more than two concentration-response curves were determined in each segment. The concentration-response curves were reproducible under these conditions. In the experiments studying the effect of indomethacin and N^G-nitro-L-arginine methyl ester (L-NAME), these compounds were added 30 and 5 min before contraction was induced with histamine, respectively.

Drugs

The compounds used in this study were: 5-hydroxytryptamine creatinine sulphate (5-HT), histamine dihydrochloride, N^G-nitro-L-arginine methyl ester (Sigma). 5-Carboxamidotryptamine (5-CT), 8-hydroxy-dipropylaminotetralin hydrobromide (8-OH DPAT), α-methyl 5-hydroxytryptamine (α-methyl 5-HT) Research Biochemicals. Ketanserin tartrate was a generous gift from Janssen U.K.

Statistical analysis

Data are expressed as the mean ± s.e.mean. The significance of differences between curves and mean values was calculated with either one or two-way analysis of variance or Students *t* test. Values of $P < 0.05$ were taken as significant.

Results

Effect of 5-hydroxytryptamine on precontracted arteries

In segments of basilar artery submaximally contracted with 1 μM histamine (13.8 ± 0.8 mN; $n = 44$ arteries) and with a functional endothelium, both 5-HT and 5-CT (1 nM–100 μM) produced additional, concentration-dependent increases in smooth muscle tension (Figures 1 and 2). The additional contractions were stimulated with agonist concentrations in excess of 1 nM, and reached a maximum with 1 μM in the case of 5-HT, and 100 μM with 5-CT. The contraction in response to 5-HT represented an increase above the histamine contraction of 22.3 ± 3.6% ($n = 6$), and with 5-CT an additional contraction of 19.1 ± 5.5% ($n = 9$). In pre-contracted arteries in which the endothelial cells had been destroyed, additional contraction was not produced by the addition of 5-HT (Figure 1). Removal of the endothelium did not significantly affect the contraction produced in response to 1 μM histamine (12.8 ± 2.0 mN; $n = 40$), nor did it alter the time-dependent fade in the histamine-induced contraction, indicating that the smooth muscle cells were not damaged by the procedure employed to remove the endothelium. In the absence of endothelial cells, only the highest concentration of 5-CT, 100 μM, induced any additional contraction. This contraction had a similar maximum (19.1 ± 5.1%; $n = 9$) to the experiments on segments with a functional endothelium (Figure 2). Although neither agonist produced any additional contraction in the absence of functional endothelial cells, apart from 100 μM 5-CT, the spontaneous decline in the histamine contraction was reduced, indicating direct smooth muscle stimulation. In the presence of contraction produced by raised extracellular potassium (30 mM; 14.0 ± 1.2 mN, $n = 9$) rather than histamine, the additional contraction produced in response to 5-HT was not significantly altered (Figure 3). Smooth muscle relaxation was not obtained with either 5-HT or 5-CT in any of the precontracted artery segments.

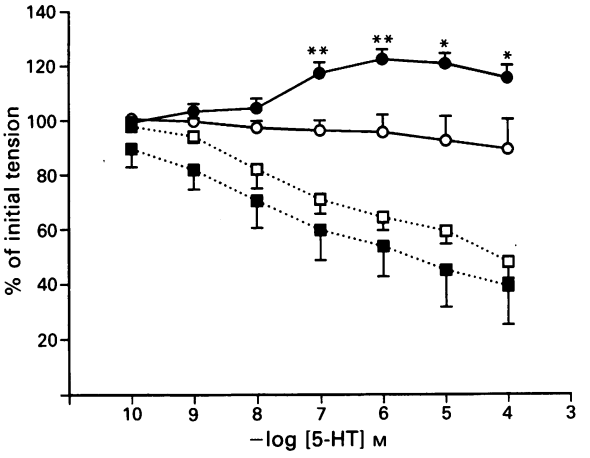

Figure 1 Additional contraction to 5-hydroxytryptamine (5-HT) in segments of basilar artery submaximally contracted with 1 μM histamine. Additional changes in tension are expressed relative to the histamine response (100%). Points are the mean from 6 experiments with s.e.mean shown by vertical bars. Arteries with (●) and without (○) a functional endothelium; time controls from segments with (■) and without (□) an endothelium. $*P < 0.05$; $**P < 0.01$: differences between arteries with and without an endothelium.

Effect of ketanserin, indomethacin and N^G-nitro-L-arginine methyl ester

The additional contraction produced in response to 5-HT was not modified by the presence of ketanserin, in concentrations ranging from 10 nM to 1 μM ($n = 4$ separate experiments with each concentration of ketanserin). With each of these concentrations of ketanserin, the concentration-response curves for additional contraction to 5-HT were superimposable. In some of these experiments, ketanserin did slightly reduce the initial contraction to histamine. However, in these tissues the concentration of histamine was increased, to ensure that the level of smooth muscle contraction was similar in control segments and segments equilibrated with either 10 nM, 100 nM or 1 μM ketanserin. The level of contraction induced with histamine

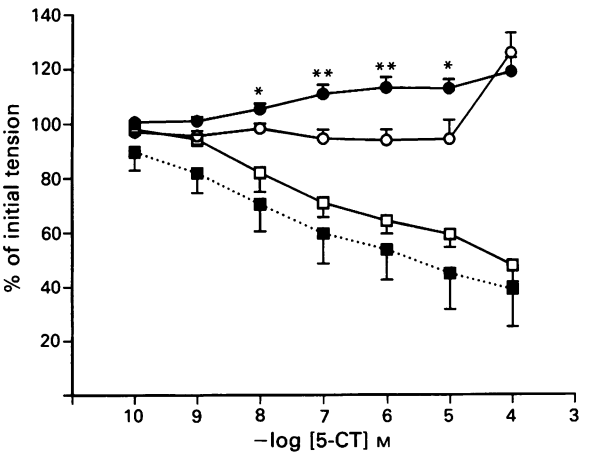

Figure 2 Additional contraction to 5-carboxamidotryptamine (5-CT) in segments of basilar artery submaximally contracted with 1 μM histamine. Additional changes in tension are expressed relative to the histamine response (100%). Points are the mean from 9 experiments with s.e.mean shown by vertical bars. Arteries with (●) and without (○) a functional endothelium; time controls from segments with (■) and without (□) an endothelium. $*P < 0.05$; $**P < 0.01$; differences between arteries with and without an endothelium.

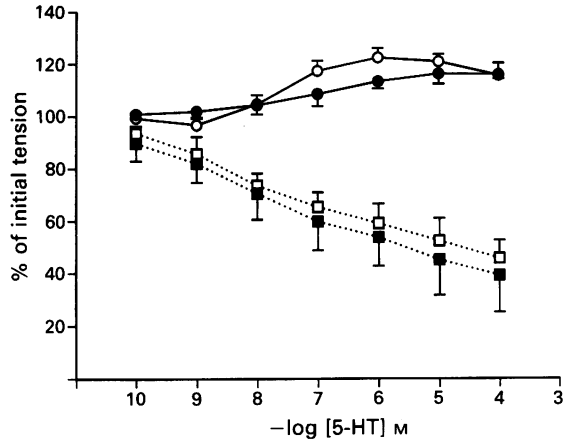

Figure 3 Additional contraction produced in response to 5-hydroxytryptamine (5-HT) in arteries submaximally contracted with either 1 μM histamine (○) or 30 mM potassium (●). In both series the endothelium was functional, and the additional contractions in the presence of either histamine or potassium were not significantly different: $P > 0.05$; (□) and (■) represent the corresponding time controls. Values represent the mean from 7 experiments with s.e.mean shown by vertical bars.

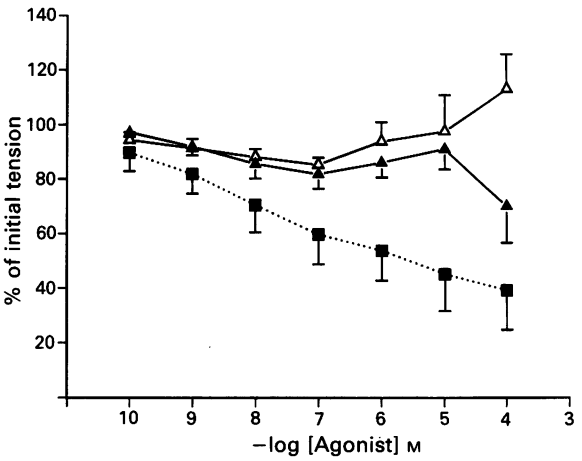

Figure 5 Effect of α-methyl 5-hydroxytryptamine (△, $n = 8$) and 8-hydroxy dipropylaminotetralin (▲, $n = 7$) in segments of basilar artery submaximally contracted with 1 μM histamine; Points are means (s.e.mean shown by vertical bars) from arteries with a functional endothelium, and are expressed relative to the contraction produced with histamine (100%); (■) represent the values of the corresponding time controls.

immediately before the addition of 5-HT was 12.8 ± 1.9 mN, 12.7 ± 1.6 mN, 11.1 ± 1.5 mN and 12.2 ± 2.1 mN, respectively ($n = 4$ in each case).

Indomethacin (3 μM) abolished the additional contractions to 5-HT, across the entire concentration-range (Figure 4). Although no additional smooth muscle contraction was produced to 5-HT when indomethacin was present, the background contraction was maintained. This indicated that the direct constrictor action of 5-HT had not been altered—compare Figure 4 with the time control shown in Figure 1. Indomethacin did not modify the initial contraction in response to histamine. In this series, 1 μM histamine induced a contraction of 12.4 ± 1.4 mN, which was not different from control values of 13.8 ± 0.8 mN.

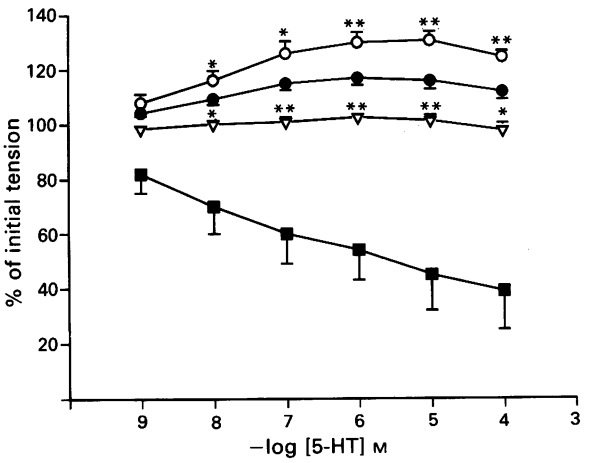

Figure 4 Responses to 5-hydroxytryptamine (5-HT) in segments of basilar artery submaximally contracted with 1 μM histamine. Indomethacin (3 μM) abolished additional contraction to 5-HT (▽), whereas the nitric oxide synthase substrate inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) significantly increased the contractions (○). Control responses in the absence of inhibitor (●); time control showing spontaneous fade of contraction (■). The inhibitors did not significantly modify the time control. Changes in tension are expressed relative to the histamine response (100%). Points are the mean from 5 separate experiments in artery segments with a functional endothelium; vertical bars show s.e.mean. $*P < 0.05$; $**P < 0.01$; differences from control values in the absence of either indomethacin or L-NAME.

L-NAME, which is a competitive substrate inhibitor for nitric oxide synthase (Rees *et al.*, 1990), had the opposite effect to indomethacin. In the presence of L-NAME, the additional contractions to 5-HT were increased (Figure 4). As with indomethacin, L-NAME was effective over the range of 5-HT concentrations tested. L-NAME was applied before histamine, and did not increase smooth muscle tone.

Effect of α-methyl 5-HT and 8-hydroxy-dipropylaminotetralin on precontracted arteries

The other agonists tested on precontracted basilar arteries were 8-OH DPAT and α-methyl 5-HT (Figure 5). Only α-methyl 5-HT produced any significant additional contraction, but in much higher concentrations than was the case with either 5-HT or 5-CT. Although the contraction in response to 100 μM α-methyl 5-HT (13.0 ± 3.0%) was similar to the maximum contraction with both 5-HT and 5-CT, this action of α-methyl 5-HT was not dependent on the presence of an intact endothelium, as very similar responses occurred in artery segments in which the endothelium had been destroyed ($n = 7$). Both 8-OH DPAT and α-methyl 5-HT significantly reduced the spontaneous fade of contraction to histamine, indicating a direct constrictor action on the vascular smooth muscle cells. Both α-methyl 5-HT and 8-OH DPAT failed to induce endothelium-dependent relaxation in the basilar artery, although concentrations of 8-OH DPAT in excess of 10 μM did, in some experiments, directly stimulate smooth muscle relaxation. The response of precontracted arteries to 8-OH DPAT was not modified in the presence of either indomethacin ($n = 3$) or L-NAME ($n = 4$).

Discussion

The finding that 5-HT and 5-CT, but not the other 5-HT agonists, 8-OH DPAT and α-methyl 5-HT, produced additional, endothelium-dependent smooth muscle contraction, indicates that the contraction is specifically receptor linked. Further, the results suggest that the receptor which mediates this response is probably of the 5-HT_1 type, but not 5-HT_{1A}, as endothelium-dependent contraction was stimulated with 5-CT but not 8-OH DPAT. The lack of effect with both α-methyl 5-HT and ketanserin also argues against the involvement of 5-HT_2 receptors. The most likely explanation for the contraction, is that the endothelial cells release a vasoconstrictor agent in response to stimulation with either 5-HT or 5-CT.

The release of endothelium-derived contractile factors (EDCF), stimulated in various ways, has been reported in a number of vascular preparations. Studies with cerebral arteries have concentrated on arteries from the dog, cat and sheep, and show that noradrenaline, acetylcholine, arachidonic acid, ATP, ADP, stretch, hypoxia and increases in transmural pressure can all produce endothelium-dependent contraction (Usui *et al.*, 1987; Shirahase *et al.*, 1987; Katusic *et al.*, 1988; Shirahase *et al.*, 1988a,b; Katusic *et al.*, 1987; Katusic & Vanhoutte, 1986; Klaas & Wadsworth, 1989; Harder, 1987). Reports that 5-HT can produce endothelium-dependent contraction are limited to two observations, the first in the aorta of spontaneously hypertensive rats and the second in mouse cerebral arteries *in vivo* (Luscher & Vanhoutte, 1986; Rosenblum & Nelson, 1988). In the latter the action of 5-HT on pial arteries was reversed from contraction to vasodilatation by localized, laser-induced damage to the endothelium.

However, it is of interest to note that additional contraction to 5-HT has been reported in partially contracted canine basilar and guinea-pig iliac arteries with 5-HT and 5-HT_1 agonists (Connor & Feniuk, 1989; Sahin-Erdemli *et al.*, 1991). In neither case was the endothelium-dependence of the contraction assessed. In addition, in human umbilical arteries, under some conditions, a component of the contraction to 5-HT mediated by 5-HT_1 receptors, involved a product of cyclo-oxygenase activity (MacLennan *et al.*, 1989). Although the endothelial cells were apparently not functional in this study, the experimental basis for this conclusion was not reported. It is therefore not clear whether these responses to 5-HT depended in any way on the endothelium.

In only a small number of studies has the release of a diffusible constrictor factor(s) from endothelial cells been demonstrated directly (Rubanyi & Vanhoutte, 1985; Harder *et al.*, 1989). However, the use of various inhibitors indicates that in the majority of cases the endothelium-mediated constrictor action involves one or more metabolites of arachidonic acid. In some cases this metabolite appears to be thromboxane A_2. The ability of indomethacin to abolish the endothelium-dependent contraction to 5-HT in the present study, conforms with the observations made with other agonists and vascular preparations. If the additional contraction to 5-HT in the basilar artery does involve the release of a constrictor factor, the mechanism of release apparently does not require a change in the membrane potential of the endothelial cells, as similar responses to 5-HT followed sub-maximal contraction induced with either histamine or potassium. Not all endothelium-dependent contractions can be abolished with inhibitors of cyclo-oxygenase. The contraction of cerebral arteries, which follows an increase in transmural pressure, although endothelium-dependent and involving a diffusible factor, is resistant to cyclo-oxygenase inhibitors. This suggests that in cerebral arteries at least, the release of different factors, each of which can cause smooth muscle contraction, can be controlled independently (Harder *et al.*, 1989).

In addition to an endothelium-dependent constrictor action in the basilar artery, these cells also in some way reduce the direct smooth muscle contractile action of 5-HT (Garland, 1987). This attenuation is similar to the influence exerted by endothelial cells in the canine basilar artery, where it was suggested the attenuation may reflect a high basal release of EDRF (Connor & Feniuk, 1989). The fact that additional contraction to 5-HT was increased following inhibition of the arginine-nitric oxide pathway, provides evidence that nitric oxide has a role in responses of the basilar artery to 5-HT, but does not indicate whether contraction is reduced by basal or stimulated release of nitric oxide. Likewise, failure to demonstrate relaxation to 5-HT, in the presence of sub-maximal contraction and indomethacin to block endothelium-dependent contraction, does not mean that 5-HT cannot stimulate the release of EDRF/NO. To determine this we would need to block selectively the direct action of 5-HT on the vascular smooth muscle cells. Despite using a variety of available 5-HT antagonists (methiothepin, spiperone, cyanopindolol, ketanserin, mianserin and 1-(1-naphthyl)piperazine) we were unable to find one that was selective. In fact only methiothepin depressed contractions to 5-HT, but was not sufficiently selective to allow investigation of a possible relaxant action of 5-HT.

In summary, the present work is the first demonstration of endothelium-dependent contraction to 5-HT in isolated cerebral arteries, and indicates that the response is mediated by a 5-HT_1-like receptor. Whether or not 5-HT has a similar action in cerebral arteries from other species requires clarification.

This work was supported by a grant from the Wellcome Trust. C.J.G. is a Nuffield Foundation Science Research Fellow. J.M.S. was supported by a SERC studentship.

References

CLARK, A.H. & GARLAND, C.J. (1990). Endothelium-dependent contraction to 5-HT in rabbit isolated cerebral arteries. *Br. J. Pharmacol.*, **100**, 453P.

COCKS, T.M. & ANGUS, J.A. (1983). Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature*, **305**, 627–630.

CONNOR, H.E. & FENIUK, W. (1989). Influence of the endothelium on contractile effects of 5-hydroxytryptamine and selective 5-HT agonists in canine basilar artery. *Br. J. Pharmacol.*, **96**, 170–178.

FENIUK, W., HUMPHREY, P.P.A. & WATTS, A.D. (1983). 5-Hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. *Eur. J. Pharmacol.*, **96**, 71–78.

GARLAND, C.J. (1987). The role of membrane depolarization in the contractile response of the rabbit basilar artery to 5-hydroxytryptamine. *J. Physiol.*, **392**, 333–348.

HARDER, D.R. (1987). Pressure-induced myogenic activation of cat cerebral arteries is dependent on intact endothelium. *Circ. Res.*, **60**, 102–107.

HARDER, D.R., SANCHEZ-FERRER, C.F., KAUSER, K., STEKIEL, W.J. & RUBANYI, G.M. (1989). Pressure releases a transferable endothelial contractile factor in cat cerebral arteries. *Circ. Res.*, **65**, 193–198.

KATUSIC, Z.S., SHEPHERD, J.T. & VANHOUTTE, P.M. (1987). Endothelium-dependent contractions to stretch in canine basilar arteries. *Amer. J. Physiol.*, **252**, H671–H673.

KATUSIC, Z.S., SHEPHERD, J.T. & VANHOUTTE, P.M. (1988). Endothelium-dependent contractions to calcium ionophore A23187, arachidonic acid and acetylcholine in canine basilar arteries. *Stroke*, **19**, 476–479.

KATUSIC, Z.S. & VANHOUTTE, P.M. (1986). Anoxic contractions in isolated canine cerebral arteries. Contribution of endothelium-derived factors, metabolites of arachidonic acid and calcium entry. *J. Cardiovasc. Pharmacol.*, **8** (suppl), S97–S101.

KLAAS, M. & WADSWORTH, R. (1989). Contraction followed by relaxation in response to hypoxia in the sheep isolated middle cerebral artery. *Eur. J. Pharmacol.*, **168**, 187–192.

LEFF, P., MARTIN, G.R. & MORSE, J.M. (1987). Differential classification of vascular smooth muscle and endothelial cell 5-HT receptors by the use of tryptamine analogues. *Br. J. Pharmacol.*, **91**, 321–331.

LUSCHER, T.F. & VANHOUTTE, P.M. (1986). Endothelium-dependent contractions to platelets and serotonin in sponteneously hypertensive rats. *Hypertension 8*, (suppl II), II55–II60.

MACLENNAN, S.J., WHITTLE, M.J. & MCGRATH, J.C. (1989) 5-HT_1-like receptors requiring functional cyclo-oxygenase and 5-HT_2 receptors independent of cyclo-oxygenase mediate contraction of the human umbilical artery. *Br. J. Pharmacol.*, **97**, 921–933.

MARTIN, W., FURCHGOTT, R.F., VILLANI, G.M. & JOHIANANDAN, D. (1986). Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J. Pharmacol. Exp. Ther.*, **237**, 529–538.

REES, D.D., PALMER, R.M.J., SCHULTZ, R., HODSON, H.F. & MONCADA, S. (1990). Characterization of three inhibitors of endo-

thelial nitric oxide synthase *in vitro* and *in vivo*. *Br. J. Pharmacol.*, **101**, 746–752.

ROSENBLUM, W.I. & NELSON, G.H. (1988). Endothelium-dependent constriction demonstrated *in vivo* in mouse cerebral arteries. *Circ. Res.*, **63**, 837–843.

RUBANYI, G.M. & VANHOUTTE, P.M. (1985). Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. *J. Physiol.*, **364**, 45–56.

SAHIN-ERDEMLI, I., HOYER, D., STOLL, A., SEILER, M.P. & SCHOEFFTER, P. (1991). 5-HT_1-like receptors mediate 5-hydroxytryptamine-induced contraction of guinea-pig isolated iliac artery. *Br. J. Pharmacol.*, **102**, 386–390.

SHIRAHASE, H., FUJIWARA, M., USUI, H. & KURAHASHI, K. (1987). A possible role of thromboxane A_2 in endothelium in maintaining resting tone and producing contractile response to acetylcholine and arachidonic acid in canine cerebral arteries. *Blood Vessels*, **24**, 117–119.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988a). An endothelium-dependent contraction induced by A-23187, a Ca^{2+} ionophore in canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 701–705.

SHIRAHASE, H., USUI, H., MANABE, K., KURAHASHI, K. & FUJIWARA, M. (1988b). Endothelium-dependent contraction and -independent relaxation induced by adenine nucleotides and nucleoside in the canine basilar artery. *J. Pharmacol. Exp. Ther.*, **247**, 1152–1157.

USUI, H., KURAHASHI, K., SHIRAHASE, H., FUKUI, K. & FUJIWARA, M. (1987). Endothelium-dependent vasoconstriction in response to noradrenaline in the canine cerebral artery. *Jpn. J. Pharmacol.*, **44**, 228–231.

(Received June 11, 1991
Revised August 19, 1991
Accepted October 1, 1991)