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Influence of the endothelium on contractile effects of 5-hydroxytryptamine and selective 5-HT agonists in canine basilar artery

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1 We have investigated the influence of endothelial damage on the cerebrovascular reactivity to 5-hydroxytryptamine (5-HT) and some selective 5-HT agonists in canine basilar artery.

2 5-HT, α-methyl 5-HT, GR 43175 (3-[2-dimethyl amino] ethyl-N-methyl-1H-indole-5-methane sulphonamide) and 5-carboxamidotryptamine (5-CT) produced concentration-dependent contractions of untreated dog basilar artery with a functional endothelium. Following endothelial damage by perfusion with Triton X-100 (0.1%), which abolished the relaxant response to substance P, the maximum contractile effect of 5-HT, α-methyl 5-HT, GR 43175 and 5-CT was markedly enhanced although there was little change in the EC_{50} values. Endothelial damage did not modify the vasoconstrictor effect of the thromboxane agonist, U46619, or potassium chloride.

3 Neither 5-HT nor 5-CT caused relaxation of untreated canine basilar arteries contracted with prostaglandin $F_{2\alpha}$, U46619, uridine triphosphate or potassium chloride.

4 These results suggest that canine basilar artery spontaneously releases endothelium-derived relaxing factor which can attenuate the vasoconstrictor effect of 5-HT and selective 5-HT agonists. This effect appeared to be specific since the vasoconstrictor response to U46619 was not modified.

5 These results demonstrate that the cerebrovascular endothelium can markedly influence the reactivity of the vascular smooth muscle of canine basilar artery to 5-HT and 5-HT_1-like receptor agonists. However we could find no evidence that 5-HT receptor activation stimulates endothelial cell function as it does in some other blood vessels.

Introduction

Over recent years it has been realised that the vascular endothelium has an important influence on blood vessel tone via release of 'endothelium-derived relaxing factor' (EDRF). Thus in many isolated blood vessels, the response to a variety of agonists is dependent on the integrity of the endothelium (Furchgott, 1983). This can reflect interaction of an agonist with specific receptors located on the endothelium cell to cause EDRF release (Furchgott, 1983). However, it has recently been established that certain blood vessels release EDRF spontaneously (Martin *et al.*, 1986). 5-Hydroxytryptamine (5-HT) is a highly potent spasmogen in isolated cerebral arteries (Muller-Schweinitzer & Engel, 1983; Peroutka *et al.*, 1986). In canine and primate basilar artery, the contractile effects of 5-HT appear to be mediated through stimulation of a mixed population of 5-HT_1-like and 5-HT_2 receptors (Connor *et al.*, 1987). However, in those experiments no attempt was made to remove the endothelium and, indeed, studies in our laboratories have demonstrated that such vessels retain a functional endothelium (Connor & Feniuk, 1987). We have therefore investigated the influence of the cerebrovascular endothelium on 5-HT-induced contractions of dog isolated basilar artery. A preliminary account of these findings has been presented to the British Pharmacological Society (Connor & Feniuk, 1988).

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Methods

Preparation of artery segments

Beagle dogs (7–10 kg, either sex) were killed with pentobarbitone sodium (100 mg kg^{-1}, i.v.) and basilar arteries were removed and stored overnight in modified Krebs solution (Apperley *et al.*, 1976) at 4°C. A segment of artery was perfused intraluminally with Triton X-100 (0.1%, 0.5 ml min^{-1} for 1 min) to abolish endothelial function as described in detail by

Connor & Feniuk (1987); control preparations were taken from an adjacent segment of unperfused artery.

Ring segments (3–4 mm) of untreated or Triton X-100-perfused basilar artery were placed in separate 10 ml organ baths containing modified Krebs solution (Apperley *et al.*, 1976) at 37°C, bubbled with 95% O_2, 5% CO_2. The segments were suspended between two L-shaped steel wire supports (0.2 mm diameter) inserted into the lumen to record isometric tension changes. Preparations were maintained at an initial resting tension of 200–300 mg for 1 h and then tension was increased to 1 g. Preparations were allowed to equilibrate for a further hour and then the response to a submaximal concentration of potassium chloride (30 mM) was determined in each tissue.

Assessment of endothelial integrity

At least 30 min later, after frequent washing, the functional integrity of the endothelium was assessed in each preparation by examining the response to the endothelium-dependent vasodilator agent substance P (1–10 nM) (Furchgott, 1983) after vascular tone had been increased by a submaximal concentration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 1 μM). Untreated preparations consistently responded to substance P with marked concentration-related relaxations (about 100% reduction in $PGF_{2\alpha}$ tone), whilst in Triton X-100-perfused segments, substance P caused little or no relaxation (less than 15% reduction in $PGF_{2\alpha}$ tone) and, in many preparations, caused small increases in tone (Connor & Feniuk, 1987). Occasionally, a preparation that had been perfused with Triton X-100 would still respond to substance P with relaxation, suggesting that some endothelial function remained; these preparations (less than 10% of total) were rejected.

Determination of agonist potency

Agonists were administered to the bathing fluid of paired untreated and Triton X-100-perfused preparations according to a cumulative concentration schedule, allowing sufficient time for the effects of each concentration to become fully established before adding the next concentration, until a maximum response was obtained or when relaxation started to occur. Contractions were expressed as % response obtained to the initial challenge with potassium chloride (30 mM). No more than 2 agonists were examined in any one preparation.

Determination of antagonist potency

Concentration-effect curves for 5-HT were constructed in two untreated or Triton X-perfused segments. Antagonist was added to one bath, whilst the second preparation served as control to assess any spontaneous change in tissue sensitivity to 5-HT. Thirty minutes equilibration time was allowed for contact of the antagonist with the tissue before the agonist concentration-effect curve was redetermined. Results were expressed in terms of % maximum response to 5-HT obtained in the first curve. In experiments in which the antagonist caused a parallel rightward displacement of the agonist concentration-effect curve, the agonist concentration-ratio (CR) was calculated at the level of 50% maximum 5-HT response. Corrections were made for any spontaneous changes in sensitivity to 5-HT. In some experiments (see below) ketanserin (1 μM) was continually present in the Krebs solution.

Histology

Histological examination was performed either via scanning electron microscopy (EM) or light microscopy (haematoxylin and eosin staining on serial transverse sections) on untreated and Triton X-100-perfused dog basilar artery segments to assess the integrity of the endothelium.

Statistical analysis

Significance was calculated by a paired Student's t test; $P < 0.05$ was considered as statistically significant.

Drugs

The following drugs and compounds were used in this study: 5-hydroxytryptamine creatinine sulphate (Sigma), ketanserin (Janssen), methiothepin maleate (Roche), methylene blue (Sigma), phenoxybenzamine hydrochloride (SKF), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, dinoprost tromethamine, Upjohn), substance P (Bachem), Triton X-100 (Sigma) and uridine triphosphate (Sigma).

5-Carboxamidotryptamine (5-CT), (±)-α-methyl 5-hydroxytryptamine (α-Me 5-HT), GR 43175 (3-[2-dimethyl amino]ethyl-N-methyl-1H-indole-5-methanesulphonamide) and U46619 (11,9-epoxymethano PGH_2) were synthesized by members of the Chemistry Research Department, Glaxo Group Research Ltd., Ware.

Ketanserin was initially dissolved in 0.1 M tartaric acid and U46619 was initially dissolved in 1% sodium bicarbonate; dilutions were made in distilled water. Dilutions of $PGF_{2\alpha}$ were made in 0.9% saline. All other drugs were dissolved and diluted in distilled water.

Results

Responses to potassium chloride

The initial submaximal challenge concentration of potassium chloride (30 mM) produced similar contractile responses in paired untreated and Triton X-100-perfused dog basilar artery (1.68 ± 0.12 g and 1.58 ± 0.10 g, respectively, $n = 20$). Subsequent contractile responses to agonists were expressed as a % of the response to potassium chloride.

Effects of 5-HT and selective 5-HT agonists

After confirming that Triton X-100 had either abolished or markedly attenuated substance P-induced relaxation (see Methods), the contractile effects of 5-HT or selective 5-HT agonists were examined, and compared to responses obtained in paired untreated preparations with a functional endothelium. 5-HT produced contractions of Triton X-100-perfused artery segments which were much greater in magnitude than responses obtained in corresponding untreated controls; the maximum contraction obtained for 5-HT appeared to be approximately doubled by Triton X-100 perfusion (Figure 1, Table 1). However, there was no change in sensitivity of the tissue to 5-HT since EC_{50} values were similar in untreated and Triton X-100 perfused preparations (Table 1). Interestingly in some experiments, the nature of the 5-HT-induced contraction seemed to be modified by perfusion with Triton X-100. In untreated preparations, contractions tended to be rapid in onset but transiently maintained whilst, in contrast, preparations that had been perfused with Triton X-100 responded to 5-HT consistently with slower, more maintained increases in tension. A representative trace which illustrates this difference is shown in Figure 2 (a and b). In different experiments, the influence of methylene blue (10 μM) on 5-HT-induced contractions of preparations with a functional endothelium was examined. Although methylene blue itself caused a marked, sustained

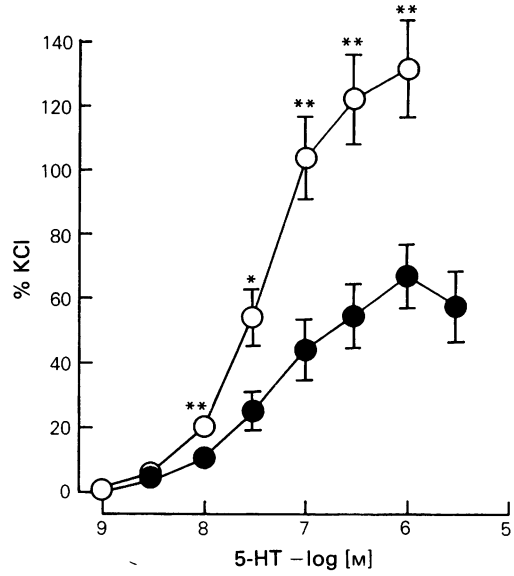

Figure 1 Contractile effects of 5-HT in control (●) and Triton X-100 perfused (○) canine basilar artery ($n = 7$). Responses are expressed in terms of the contraction obtained to 30 mM KCl; values are means (s.e.mean shown by vertical bars) from n experiments. * $P < 0.05$, ** $P < 0.01$ (paired Student's t test) compared to controls.

Table 1 EC_{50} values and maximum responses produced for 5-hydroxytryptamine (5-HT) and selective 5-HT agonists in control (untreated) and Triton X-100-perfused canine basilar artery

	Control		*Triton-X-perfused*	
Agonist	EC_{50} (nM)	*Max response* (% KCl)	EC_{50} (nM)	*Max response* (% KCl)
5-HT	62 (30–126)	67 ± 10	40 (30–54)	130 ± 15**
α-Me 5-HT	320 (150–670)	72 ± 3	310 (160–630)	140 ± 16*
GR 43175	94 (19–462)	26 ± 5	107 (19–588)	66 ± 3**
5-CT	4.2 (2.6–6.8)	20 ± 7	4.5 (3.9–5.1)	41 ± 7**

Values are geometric mean (95% confidence limits) or arithmetic mean ± s.e.mean, $n = 4$–7. * $P < 0.05$; ** $P < 0.01$ significantly different from control (paired Student's t test). Responses are expressed in terms of the contraction obtained to 30 mM KCl.

α-Me 5-HT = (±)-α-methyl 5-hydroxytryptamine; GR 43175 = 3-[2-dimethyl amino]ethyl-N-methyl-1H-indole-5-methane sulphonamide; 5-CT = 5-carboxamidotryptamine.

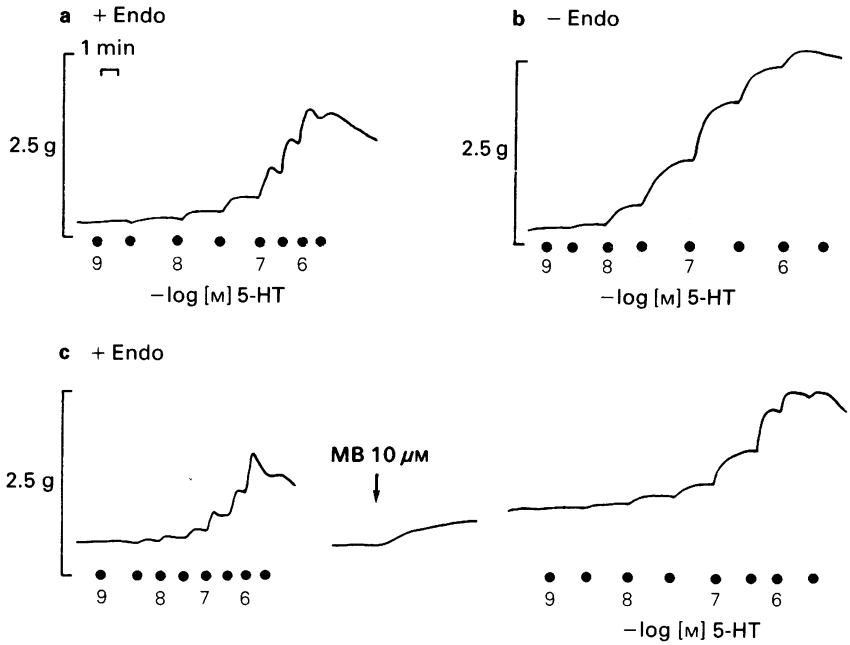

Figure 2 Representative tracing to illustrate the effects of 5-hydroxytryptamine (5-HT) in (a) untreated, endothelium-intact, and (b) Triton X-100-perfused canine basilar artery. Responses are time matched from paired preparations. In (c) the effects of 5-HT are shown before and 30 min after methylene blue, (MB, 10 μM), in untreated canine basilar artery. Note the contractile effect of methylene blue alone. 5-HT was added to the bath at 0.5 log unit increments at points indicated; figures indicate cumulative concentration (−log M).

contraction of dog basilar artery (0.79 ± 0.16 g, $n = 4$), which made an analysis of its effects on 5-HT more complex, 5-HT contractions, in the presence of methylene blue, appeared to be larger and more sustained (Figure 2c).

The selective 5-HT agonists, α-methyl 5-HT, GR 43175 and 5-CT each caused contraction of control dog basilar artery segments. These agonists differed markedly in the magnitude of contraction produced with a rank order of α-methyl 5-HT > GR 43175 > 5-CT. However, the effects of each agonist appeared to be greatly enhanced by Triton X-100 perfusion (see Table 1) and, furthermore, responses appeared to be enhanced by a similar extent (about 100%). There was, however, no evidence for a change in sensitivity of the tissue, since the respective agonist EC_{50} values were similar in untreated and Triton X-100-perfused tissues (Table 1). The concentration-effect curves for α-methyl 5-HT, GR 43175 and 5-CT in untreated and treated dog basilar artery are shown in Figure 3.

In separate experiments, attempts were made to demonstrate a relaxant effect of 5-HT or 5-CT in endothelium-intact dog basilar artery. Tone was increased with submaximal concentrations of a variety of spasmogens, (e.g. U46619 (10–30 nM), $PGF_{2\alpha}$ (1–3 μM), uridine triphosphate (10–100 μM), potassium chloride (20–30 mM)) but 5-HT and 5-CT caused only further contractions. Furthermore, even in the presence of antagonists to block contraction (ketanserin 1 μM or phenoxybenzamine 30 μM), no relaxation to either 5-HT or 5-CT could be revealed (results not shown).

Effects of antagonists

The contractile effects of 5-HT were antagonized by ketanserin (1 μM) to a similar degree in untreated and Triton X-100-treated dog basilar artery; there was little shift in the curve but a depression of the maximum response (Figure 4a and b). Furthermore, the antagonist potency of methiothepin (0.1 μM, in the continued presence of ketanserin 1 μM) did not appear to be modified by Triton X-100; calculated CRs were 33 (20–49) and 39 (29–65) (geometric mean (range) $n = 3$) in untreated and Triton X-100-treated dog basilar artery, respectively (Figure 4c and d).

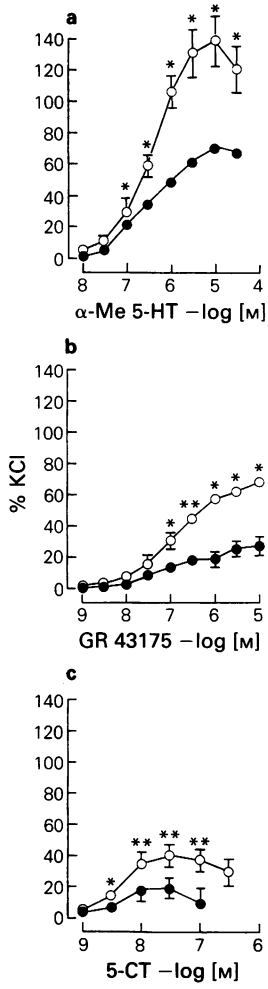

Figure 3 Effects of selective 5-hydroxytryptamine (5-HT) agonists in control (●) and Triton X-100-perfused (○) canine basilar artery; (a) α-methyl 5-HT, (b) GR 43175 (3-[2-dimethyl amino] ethyl-N-methyl-1H-indole-5-methane sulphonamide) and (c) 5-carboxamidotryptamine (5-CT). Responses are expressed in terms of the contraction obtained to 30 mM KCl. Values are means (s.e.mean shown by vertical bars) from 4 experiments. * $P < 0.05$, ** $P < 0.01$ (paired Student's t test) compared to controls.

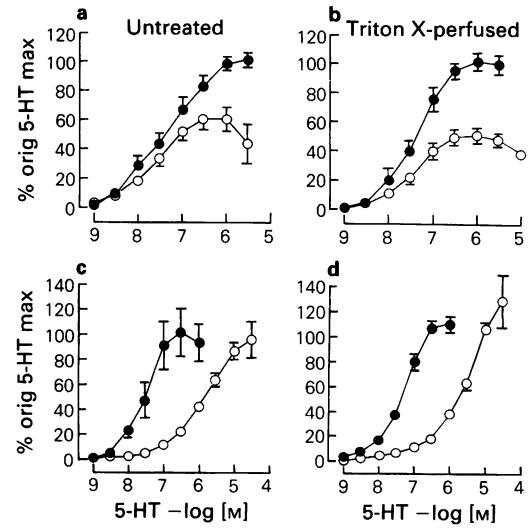

Figure 4 Antagonist effect of ketanserin, 1 μM (a,b, $n = 5$–7) or methiothepin, 0.1 μM (in the presence of ketanserin 1 μM; c,d, $n = 3$) against 5-HT-induced contraction of endothelium-intact (untreated) or endothelium denuded (Triton X-perfused) canine basilar artery. Responses shown are the 2nd 5-HT control curve (●) and 5-HT in the presence of antagonist (○) expressed in terms of the maximum response for the 1st 5-HT curve in each tissue. Values shown are means (with s.e.mean shown by vertical bars) from n experiments.

Effects of other agonists

The contractile effects of some other agonists were also examined in untreated and Triton X-100-perfused dog basilar artery. $PGF_{2\alpha}$ produced larger responses in preparations without a functional endothelium although this difference did not attain statistical significance (Figure 5a); the EC_{50} value was 2(0.3–11) and 0.6(0.4–0.7) μM and the maximum response was 126 ± 14 and 205 ± 44% of the contraction obtained to 30 mM KCl for untreated and Triton X-perfused preparations respectively ($n = 3$). The effects of the thromboxane A_2-mimetic, U46619, were unchanged (Figure 5b); the EC_{50} value was 4(2–6) and 3(2–6) nM and the maximum response was 151 ± 22 and 156 ± 12% of the contraction obtained to 30 mM KCl respectively ($n = 4$, values are means (95% confidence limits) or means ± s.e.mean). Potassium chloride also produced largely similar contractions of untreated and Triton X-100-perfused preparations (Figure 5c).

Histology

Using scanning EM, untreated artery segments were seen to have a relatively intact layer of endothelial cells over the intimal surface. In marked contrast, examination of artery segments that had been perfused with Triton X-100 showed large areas from which endothelial cells were absent. Remaining endothelial cells were damaged or fragmented.

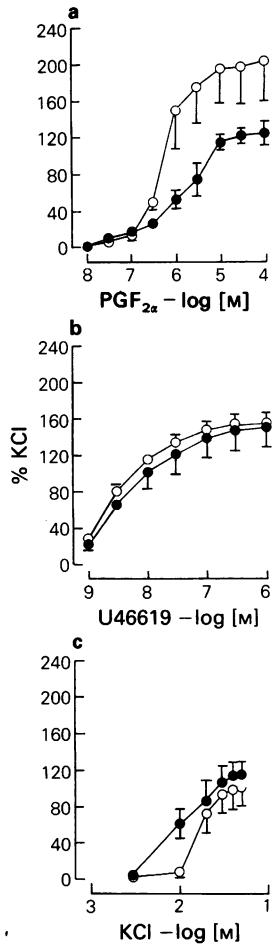

Figure 5 Contractile effects of (a) prostaplandin $F_{2\alpha}$ ($PGF_{2\alpha}$) ($n = 3$), (b) U46619 ($n = 4$) and (c) potassium chloride (KCl, $n = 4$) in untreated (●) and Triton X-100-perfused (○) canine basilar artery. Responses are expressed in terms of the contraction obtained to 30 mM KCl. Values are means (with s.e.mean shown by vertical bars) from n observations.

Similar evidence for the ability of Triton X-100 perfusion to cause loss of endothelial cells was obtained from light microscopy examination of serial transverse sections of control and Triton X-perfused basilar artery segments (see Connor & Feniuk, 1987).

Discussion

These experiments were performed in order to determine whether the endothelium can modify the contractile effects of 5-HT and selective 5-HT agonists in canine basilar artery. It is now well established that, in many isolated blood vessels, the endothelium can have an important influence on responses produced by a variety of agonists. This is attributed to the ability of the endothelium to produce EDRF, which acts directly on the vascular smooth muscle to cause relaxation and therefore offsets any direct contractile effect of the agonist (Furchgott, 1983). Release of EDRF can be produced through the action of an agonist at specific receptors thought to be located on the endothelial cell membrane. More recently it has become evident that, in certain blood vessels, there is a continuous basal release of EDRF from the endothelium. This exerts a depressant influence on the ability of a wide variety of spasmogens to increase tone in the vessel (Martin *et al.*, 1986).

Removal of the endothelium from isolated blood vessels is usually performed by gentle mechanical abrasion. However, in small vessels such as cerebral arteries, this technique can also cause some degree of smooth muscle damage. Therefore in the present studies, the endothelium of canine basilar artery was removed by a method described by Verrechia *et al.* (1986) in which the vessel was perfused with Triton X-100 (0.1%). The integrity of the endothelium was assessed pharmacologically, by examining the relaxant effects of the endothelium-dependent vasodilator substance P (Furchgott, 1983), and histologically. We have previously found that Triton X-100-perfusion of canine basilar artery will abolish the relaxant effect of substance P, whilst relaxations produced by sodium nitroprusside are unchanged (Connor & Feniuk, 1987). This indicates that the capacity of the vascular smooth muscle to relax is not impaired by this procedure. In the present studies, we have compared the effects of 5-HT in canine basilar artery with an intact endothelium, with responses obtained in Triton X-100-perfused basilar artery which lacked a functional endothelium. In these preparations, potassium chloride (30 mM) produced similar responses, suggesting that Triton X-100 had not caused smooth muscle damage. Triton X-100 perfusion markedly enhanced the magnitude of contractions produced by 5-HT. However, since the EC_{50} value for 5-HT was similar in untreated and Triton X-100-perfused preparations, it appeared that damage to the endothelium did not modify the sensitivity of the cerebrovascular smooth muscle to 5-HT. Interestingly, in some experiments there was a clear difference in the nature of the 5-HT-induced contractions in endothelium-intact compared to the endothelium-damaged preparations. In untreated preparations, the effects of 5-HT were rapid in onset and transiently maintained, whilst in contrast, after Triton X-100 perfusion, 5-HT produced slower, more maintained contractions (see Figure 2). These results are

in contrast to a previous study in cat cerebral arteries in which, after rubbing to remove the endothelium, responses to 5-HT appeared unchanged (Young *et al.*, 1986). However, in those experiments, contractions produced by potassium chloride were reduced in denuded preparations, compared to unrubbed preparations, suggesting that the rubbing procedure had caused some degree of smooth muscle damage. A recent report by Garland (1987) is in agreement with our findings: 5-HT-induced contractions of rabbit basilar artery were increased by removal of the endothelium although 5-HT-induced depolarization was unchanged.

A subclassification of 5-HT receptors into 5-HT_1-like, 5-HT_2 and 5-HT_3 receptor types has recently been proposed (Bradley *et al.*, 1986a). It is now apparent that 5-HT_1-like receptors are a heterogeneous group and can be further subdivided (Humphrey *et al.*, 1988). There is a lack of agreement in the literature on the 5-HT receptor type which mediates contraction of cerebral arteries (Muller-Schweinitzer & Engel, 1983; Bradley *et al.*, 1986b; Peroutka *et al.*, 1986; Connor *et al.*, 1987); this is probably due, at least in part, to the involvement of a mixed population of 5-HT_1-like and 5-HT_2 receptors. In the present studies, to assess the influence of endothelial damage on cerebrovascular reactivity, the effects of α-methyl 5-HT, 5-CT and GR 43175 were compared in endothelium-intact and Triton X-100-perfused canine basilar artery. These 5-HT receptor agonists show very different selectivities for the various 5-HT receptor subtypes (Richardson *et al.*, 1985; Bradley *et al.*, 1986a; Humphrey *et al.*, 1988). However, despite these differences each agonist, like 5-HT, produced significantly larger contractions in preparations without an intact endothelium, with no accompanying change in their respective EC_{50} values. The maximum response produced by each of the selective agonists and 5-HT was increased by a similar extent ($\simeq 100\%$). Therefore, there was no indication that selectivity for a particular 5-HT receptor subtype resulted in a greater, or lesser, degree of enhancement. Furthermore, analysis of the influence of either the 5-HT_2 antagonist ketanserin or the 5-HT_1 antagonist methiothepin (in the presence of a high concentration of ketanserin) on 5-HT-induced contractions of canine basilar artery revealed that endothelial damage did not modify the potency of either antagonist.

A possible explanation for these findings is that 5-HT and selective 5-HT agonists can stimulate the release of EDRF from the endothelium of canine basilar artery, through an action at a specific 5-HT receptor. In a few peripheral isolated blood vessels, such as dog and pig coronary artery (Cocks & Angus, 1983), rabbit jugular vein (Leff *et al.*, 1987), and chick jugular vein (Imaizumi *et al.*, 1984), 5-HT has been reported to stimulate release of EDRF through an atypical 5-HT (non 5-HT_2) receptor. However in dog basilar artery, either alone or in the presence of ketanserin or phenoxybenzamine to prevent the contractile effects, we could not reveal a relaxant effect for 5-HT or 5-CT when tone in the preparation was increased. This would suggest that 5-HT does not directly stimulate release of EDRF through a specific 5-HT receptor, although the possibility that the same receptors mediate contraction and relaxation cannot be discounted. Further evidence against there being an endothelium-dependent 5-HT receptor which mediates relaxation in this tissue comes from the finding that endothelium removal potentiates the effects of 5-HT and a variety of 5-HT receptor agonists showing varying degrees of selectivity for different 5-HT receptor subtypes by a remarkably similar extent. If a specific 5-HT receptor were involved, the selective agonists might have been expected to be differentially affected by removal of the endothelium.

5-HT can stimulate prostacyclin synthesis in smooth muscle cells (Coughlin *et al.*, 1984). Since prostacyclin is a potent dilator agent of cerebral arteries (Boullin, 1980), it could be hypothesized that our findings might be explained by a 5-HT-induced release of prostacyclin from the cerebrovascular endothelium. However, this is unlikely since flurbiprofen, at a concentration (0.5 μM) which inhibits prostaglandin synthesis (Nozu, 1978), did not enhance the contractile effects of 5-HT in endothelium-intact canine basilar artery. Indeed, although in the presence of flurbiprofen, there was no shift in the concentration-effect curve to 5-HT (at EC_{50} level), there appeared to be a small reduction (20% compared to 1st curve) in the maximum contraction obtained (our own unpublished observations).

It could be argued that our findings are due to some effect of Triton X-100 on the cerebrovascular smooth muscle which was unrelated to endothelium removal. Responses to $PGF_{2\alpha}$ were also smaller in the untreated preparations. However, in contrast, the thromboxane-A_2-mimetic, U46619, and potassium chloride produced contractions which were similar in untreated and Triton X-perfused preparations, making such a suggestion seem unlikely.

A more probable explanation for our findings is that there is a spontaneous release of EDRF in dog basilar artery, which attenuates contractions produced by certain spasmogens. Although we have not investigated why the cerebrovascular endothelium appears able to reduce responses to some spasmogens but not others, it is interesting that Martin *et al.* (1986) reported that the endothelium of rat aorta can differentially modify the effects of various spasmogens, probably via spontaneous release of EDRF.

They found that for partial agonists, the magnitude of the response was increased and for full agonists, tissue sensitivity was increased, by endothelium removal. Our findings may suggest that the influence of endothelial removal on the contractile response to a given agonist is dependent upon its relative intrinsic efficacy. Thus for agonists with a high intrinsic efficacy, (U46619), endothelial removal has little influence, whilst for agonists with a low intrinsic efficacy, the contractile response is markedly enhanced. However, unlike Martin *et al.* (1986), we were unable to detect an increase in tissue sensitivity to agonists with high intrinsic efficacy following endothelial removal. The reason for this difference is unclear. A possible receptor-mediated release of EDRF by agonists with low intrinsic efficacy has already been discounted (see above).

Although we have not provided direct evidence for 'EDRF' being responsible for attenuating influence of the endothelium, this is the most likely candidate. Our attempts to characterize this attenuating factor as 'EDRF' by the use of haemoglobin (Martin *et al.*, 1986) were hampered by the fact that haemoglobin caused considerable foaming in the tissue baths and also caused contraction of the isolated cerebral arteries (Connor & Feniuk, 1987). However, our preliminary studies using methylene blue lend some support to this proposal. Methylene blue alone caused marked contraction of the cerebral artery, which might reflect blockade of spontaneous release of EDRF. Indeed, this concentration of methylene blue (10 μM) has been shown previously to block endothelium-dependent relaxation (Martin *et al.*, 1985). Furthermore, it is noteworthy that the effects of 5-HT in the presence of methylene blue appeared to be remarkably similar to the effects of 5-HT in endothelium-denuded dog basilar artery (compare Figure 2b and 2c).

In conclusion, these results suggest that the endothelium can have an important influence on the responsiveness of isolated canine basilar artery to a variety of spasmogens. Of particular interest is the finding that 5-HT caused contractions of the isolated cerebral artery which were markedly depressed in the presence of a functional endothelium, possibly through spontaneous release of EDRF. Whether or not the endothelium of human cerebral blood vessels has the ability to attenuate contractions to spasmogens, like 5-HT remains to be determined. However our findings raise the possibility that in conditions where there is damage to the cerebrovascular endothelium, either in the form of a reduced capacity for EDRF release or actual loss of endothelial cells, the vasoconstrictor effects of endogenous 5-HT on cerebral blood vessels could be markedly enhanced. This could have important implications in cerebrovascular disorders such as cerebral vasospasm or transient ischaemic attacks, in which diminished blood flow to certain brain areas is a consequence of cerebral artery constriction, and where 5-HT, along with other spasmogens, may be involved.

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