Role of the endothelium in modulating functional responses of isolated bovine anterior ciliary arteries to vasoconstrictor agonists

Christine H Buckley, Patrick W F Hadoke, Colm J O'Brien

Abstract

Background/aims—Endothelium dependent vasodilatation is an important regulator of blood flow to the eye but its role has not been investigated in vessels supplying the ciliary body. This study assessed the role of the endothelium in modulating vasoconstrictor responses of the intraocular bovine anterior ciliary artery.

Methods—Bovine anterior ciliary arteries (n=33) were mounted in a myograph, containing physiological salt solution at 37°C, for isometric force measurement. Cumulative concentration-response curves were obtained to the constrictor agonists 5-hydroxytryptamine (5-HT), noradrenaline, phenylephrine, prostaglandin F$_2$α, endothelin-1, and KCl in both endothelium intact and denuded arteries.

Results—All vasoconstrictors produced sustained contractile responses which were unaffected by the removal of the endothelium. Responses to 5-HT were also unaffected by inhibition of nitric oxide synthase.

Conclusion—These results indicate that neither agonist stimulated nor basal release of nitric oxide from the endothelium modulates responses to vasoconstrictor agonists in the isolated bovine anterior ciliary artery when measured in a no flow isometric system.

(Br J Ophthalmol 1998;82:826–829)

Basal and/or agonist induced release of endothelium derived nitric oxide (NO) is a significant factor in the regulation of ocular blood flow. Consequently endothelial cell dysfunction in ocular vessels may account for the ophthalmic complications of diseases associated with dysfunction of the endothelium in the systemic vasculature (for example, diabetes, hypertension). Similar alterations may also occur in some patients with glaucoma who have ocular or generalised vasospastic sequelae.

The role of endothelium derived mediators in the control of vascular function in the eye varies in different arteries. Their contribution to tone in vessels supplying the ciliary body has not been investigated, although high levels of constitutive NO synthase (eNOS) are located in the human outflow pathway and ciliary muscle. This may be relevant to the development of glaucoma as patients with this condition, which is associated with an increase in both outflow resistance and intraocular pressure (IOP), have a reduced distribution of NO containing cells in the outflow pathway. Furthermore, nitrovasodilators, which mimic the actions of NO, reduce IOP through a mechanism involving alterations in the resistance to aqueous humour outflow.

The limited data available on normal physiological control of ocular blood vessels make it difficult to describe the exact vascular alterations which occur during disease progression. The development of the small vessel myograph by Mulvany and Halpern has provided a technique which can be used to investigate vascular function in vitro using vessels with an internal diameter of as little as 100 μm. The aim of the present study was to investigate the role of the endothelium in mediating vasoconstriction in the intraocular bovine anterior ciliary artery.

Materials and methods

Tissue preparation

Bovine eyes were obtained from the abattoir and transported in cold physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.7, MgSO$_4$ 1.17, KH$_2$PO$_4$ 1.18, glucose 5.5, K$_2$EDTA 0.026, NaHCO$_3$ 25, CaCl$_2$ 2.5. The posterior segment of the eye together with the vitreous was removed, intraocular anterior ciliary arteries were dissected from beneath the choroid and transferred to the chamber of a wire myograph (Model 400A, JP Trading, Aarhus, Denmark).

Arterial rings, approximately 2 mm in length, were mounted on two 40 μm intraluminal wires for measurement of isometric force development. The vessels were allowed to equilibrate in PSS at 37°C and gassed with 95% oxygen, 5% carbon dioxide for 30 minutes before undergoing normalisation using standard methodology. Briefly, this involved stepwise stretching of the vessel and application of the Laplace relation to determine the internal circumference (L$_{100}$) of a vessel when relaxed and under an effective transmural pressure of 100 mm Hg (13.3 kPa).

The vessel was then set at an internal circumference of 0.9 L$_{100}$ at which many small arteries, including bovine and canine ocular arteries develop maximum or near maximum active tension. This setting has been used previously in functional investigations of bovine anterior ciliary arteries. The vessels were then left to equilibrate under their normalised tension for 30 minutes.

The functional integrity of the endothelium was assessed in each artery by the addition of bradykinin (BK; 10$^{-6}$ M) following contraction...
with 5-hydroxytryptamine (5-HT; $3 \times 10^{-7}$ M). The endothelium was considered intact if BK evoked a relaxation of greater than 60% of the 5-HT induced tone. In some arteries the endothelium was removed by gently rubbing the lumen of the vessel with a human hair. Histological studies have confirmed that this method successfully removes the endothelium and denudation was confirmed in the present study by the failure of the vessel to relax in response to BK ($10^{-6}$ M).

**INVFLUENCE OF THE ENDOTHELIUM ON VASOCONSTRICTOR RESPONSES**

The role played by the endothelium in modulating functional responses of bovine anterior ciliary arteries to vasoconstrictors was investigated using a variety of agonists. Cumulative concentration-response curves were obtained to the vasoconstrictors 5-HT ($10^{-6}$–$3 \times 10^{-3}$ M), noradrenaline (NA; $10^{-6}$–$3 \times 10^{-5}$ M), phenylephrine (PE; $10^{-6}$–$3 \times 10^{-5}$ M), prostaglandin (PG) $\tilde{F}_{2\alpha}$ ($10^{-6}$–$3 \times 10^{-5}$ M), endothelin-1 (ET-1; $10^{-11}$–$3 \times 10^{-7}$ M), and KCl (10–125 mM) in both endothelium intact and denuded vessels. Following each concentration-response curve the vessels were washed thoroughly with PSS and allowed to relax fully for at least 20 minutes before the next drug was tested.

The role of endothelium derived NO in the modulation of 5-HT induced contraction was assessed by constructing concentration-response curves in the absence of, and following incubation of arteries for 45 minutes with, the NO synthase inhibitor Nω-nitro-L-arginine (L-NNA; $10^{-4}$ M).

**STATISTICAL ANALYSIS**

Contractile responses of the vessels were expressed as active wall tension (mN/mm) and relaxation responses as a percentage of the induced contraction. For each concentration-response curve the molar concentration required to produce 50% of the maximum contraction ($EC_{50}$) was calculated by fitting the data to the Hill equation using the curve fitting program FIG P (Biosoft, UK). The sensitivity of the vessels to each constrictor agonist was assessed by constructing concentration-response curves in the absence of, and following incubation of arteries for 45 minutes with, the NO synthase inhibitor Nω-nitro-L-arginine (L-NNA; $10^{-4}$ M).

**Results**

Preliminary experiments demonstrated that vessels remained viable if stored in PSS at 4°C for 24 hours, with both vasoconstrictor and vasodilator function unaltered (data not shown). This extended the period during which experiments could be performed. The mean normalised lumen diameter of vessels used in the present study was 218 (6) µm (n=33). Vessels which had not had the endothelium removed relaxed by 79.8% (4.5%) (n=33) in response to BK, whereas in denuded arteries the relaxation was reduced to 51.3% (3.3%) (n=8) (Fig 1).

In quiescent arteries with an intact endothelium all six agonists tested, 5-HT, NA, PE, PGF$_{2\alpha}$, ET-1, and KCl, produced concentra-

<table>
<thead>
<tr>
<th>Maximum contraction ($E_{max}$) (mN/mm)</th>
<th>$pD_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact</strong></td>
<td><strong>Denuded</strong></td>
</tr>
<tr>
<td>5-HT</td>
<td>3.65 (0.25) (12)</td>
</tr>
<tr>
<td>NA</td>
<td>2.78 (0.28) (9)</td>
</tr>
<tr>
<td>PE</td>
<td>3.18 (0.35) (8)</td>
</tr>
<tr>
<td>PGF$_{2\alpha}$</td>
<td>2.81 (0.31) (8)</td>
</tr>
<tr>
<td>ET-1</td>
<td>4.21 (0.24) (9)</td>
</tr>
<tr>
<td>KCl</td>
<td>4.08 (0.31) (8)</td>
</tr>
</tbody>
</table>

Values are expressed as mean (SEM) (n). $p$ Values comparing maximum contractions and $pD_2$ values in endothelium intact versus denuded arteries.
function may alter both the basal vascular tone and the response of the vessel to circulating agonists and pharmacological agents and, consequently, is an important factor in both the development and treatment of disorders of the eye.

Functional responses of ocular vessels are heterogeneous, depending upon the anatomical origin of the vessel, its location within or outside the orbit, and also upon the part of the vessel selected (reviewed by Buckley et al 17). In the present investigation, vasoconstrictor agonists were used to provide information on the role of the endothelium in modulating the action of agonists derived from sympathetic nerves, aggregating platelets and damaged endothelial cells. KCl, a receptor independent vasoconstrictor, was included as a control and PGF2α was also included as many investigations have used this to precontract vessels for subsequent investigation of vasodilator activity. These agonists all produced sustained, concentration dependent responses that were not altered significantly by removal of the endothelium. The slight, but not significant, reduction in the maximum contraction following removal of the endothelium was probably due to slight damage to the vascular smooth muscle cells which is a recognised problem encountered when denuding small arteries. This was supported by the demonstration that inhibition of NO synthase using L-NNA did not result in a reduction of the magnitude of 5-HT induced contraction. Further experiments are required to investigate the effect of NO synthase inhibition on the responses to other constrictor agonists and also to determine the potential role of other vasoactive mediators such as PGI2 in regulating blood flow in this artery. These results suggest that basal release of NO, or any other vasoactive agent, is not a significant factor in bovine anterior ciliary arteries in an isometric system. This observation is supported by the absence of an increase in basal tone following the addition of the L-arginine analogue (L-NNA). In contrast, in vivo experiments using NO synthase inhibitors have demonstrated a significant reduction in blood flow to the anterior uvea in both rabbits and dogs indicating that basal NO release is involved in the regulation of uveal blood flow under resting conditions. Whether this difference reflects the different species used or the size of the vessels or is due to the different methodological approaches used requires further investigation. Investigations using porcine isolated ocular arteries have suggested that the influence of the endothelium on contractile responses is dependent upon the size and location of a particular vessel. Basal release of NO is more important in larger arteries, modulating vasoconstriction in porcine ophthalmic, but not ciliary, arteries. The results reported for the porcine ciliary artery are consistent with those obtained in the present study, whether the influence of the endothelium or endothelium derived NO on contractile response depends on vessel size in the bovine ophthalmic...
Role of the endothelium in modulating functional responses of isolated bovine anterior ciliary arteries to vasoconstrictor agonists

The use of NA and PE demonstrated that α adrenoceptors were present in the anterior ciliary artery. Sensitivity to the specific α1 adrenoceptor agonist, PE, was 100-fold lower than that to NA, suggesting that the latter may contract this vessel by stimulation of more than one receptor subtype. Indeed, bovine intraocular long posterior ciliary arteries have been shown to contract in response to both selective α1 and α2 adrenoceptor agonists. In contrast, α2 adrenoceptor agonists do not cause contraction in canine or human ciliary arteries with the contraction to NA in these vessels mediated solely by α1 adrenoceptors.  
NA can also relax some arteries by stimulating β adrenoceptors or by δ adrenoceptor mediated release of NO from the endothelium. As NA mediated vasoconstriction was unaffected by removal of the endothelium in the present study, α2 adrenoceptor mediated release of NO appears not to play a role in regulating tone in the bovine anterior ciliary artery.  
The inhibitory action of NO on platelet aggregation may become impaired in patients with endothelial cell dysfunction, allowing aggregating platelets to adhere to the vascular wall (releasing vasoactive agonists, including 5-HT), with consequent vasospasm. The 5-HT-induced vasoconstriction in the present investigation is most likely to be mediated by 5-HT1 receptors on the vascular smooth muscle cells as demonstrated in porcine ophthalmic and ciliary arteries.  
5-HT can also cause vessels to dilate by acting on 5-HT2 receptors on the endothelium.  
In conclusion, the results of the present study show that the endothelium does not modulate responses of isolated bovine anterior ciliary arteries to a variety of constrictor agonists. Furthermore, there does not appear to be a significant basal release of NO by this artery when studied in an in vitro system. This suggests that endothelial cell dysfunction associated with cardiovascular diseases is unlikely to significantly reduce blood flow to the ciliary body.
Role of the endothelium in modulating functional responses of isolated bovine anterior ciliary arteries to vasoconstrictor agonists
Christine H Buckley, Patrick W F Hadoke and Colm J O'Brien

Br J Ophthalmol 1998 82: 826-829
doi: 10.1136/bjo.82.7.826

Updated information and services can be found at:
http://bjo.bmj.com/content/82/7/826

These include:

References
This article cites 24 articles, 9 of which you can access for free at:
http://bjo.bmj.com/content/82/7/826#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/