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LABORATORY STUDY

Role of cyclooxygenase and haemoxygenase products in nitric oxide-independent vasodilatation in the porcine ciliary artery

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Abstract

Purpose **Vascular endothelial cell dysfunction has been noted in patients with normal pressure glaucoma. Although nitric oxide (NO) accounts for a large proportion of vasorelaxation in the posterior ciliary artery, considerable relaxation remains unexplained. We investigated the roles of haemoxygenase (HO) and cyclooxygenase (COX), which produce the vasodilators carbon monoxide (CO) and prostacyclin, respectively, in NO-independent endothelium-dependent vasodilatation in porcine posterior ciliary arteries.**

Methods **Isolated vascular rings were mounted in a Mulvaney-Halpern small vessel myograph for the measurement of isometric tension development. Vasodilator responses to bradykinin (BK) were elicited in each ring on three separate occasions following preconstriction with prostaglandin $F_{2\alpha}$: first in the absence of inhibitors, second in the presence of the NO synthase inhibitor *N*-nitro-L-arginine methyl ester (L-NAME, 10^{-3} M), and third in the presence of L-NAME and either a COX (indomethacin, 10^{-6} M) or an HO inhibitor (tin protoporphyrin-IX 10^{-5} M). Results were expressed as a percentage of the maximal relaxation in the presence of L-NAME alone.**

Results **Incubation with indomethacin ($n=6$), in the presence of L-NAME, significantly reduced ($P<0.01$) maximum BK-induced relaxation ($-103.5\pm8.8\%$) compared to paired rings in the presence of L-NAME alone ($-130.8\pm8.8\%$). HO inhibition did not reduce NO-independent, BK-induced relaxation when compared to paired control vessels.**

Conclusions **These data suggest that in the presence of L-NAME, a COX product accounts for a significant proportion of NO-independent vasodilatation. In contrast, endogenous CO production does not have a functionally significant role in the porcine ciliary artery.**

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Introduction

It has been suggested that an impaired blood supply to the optic nerve may be an important pathogenic mechanism contributing to the development of glaucoma.[1] There is an increased incidence of ocular and systemic vascular abnormalities in patients with glaucoma,[2–6] and abnormal function of the systemic endothelium has been noted in patients with normal pressure glaucoma.[7] Taken together, these observations suggest that vascular dysfunction or, more specifically, abnormalities of endothelial-derived vasodilator production may underlie the pathologic process.[8]

In most vascular beds, nitric oxide (NO) is an important vasodilator produced by the endothelium through the action of nitric oxide synthase (NOS). Prostacyclin (PGI_2) is another significant vasodilator, for example in the coronary circulation.[9] It is the predominant vasodilator product of cyclooxygenase (COX) and acts by increasing intracellular cAMP through G_s protein coupling to adenylate cyclase.[10] It is well recognised that endothelium-

derived vasodilators contribute to posterior ciliary artery vasodilatation in porcine and human ciliary arteries. Simultaneous blockade of both NO and PGI_2 production reduces endothelium-dependent vasodilatation by 30–60% in the porcine circulation.[11,12] Interestingly, the separate contributions of NO and PGI_2 have not been previously examined in ciliary arteries. Furthermore, the identity of the endothelium-dependent vasodilator responsible for the considerable residual dilatation remains unknown.

Carbon monoxide (CO) is a gas with physico-chemical properties similar to those of NO and there is evidence that CO may be an endogenous, endothelium-derived vasodilator.[13–16] NO and CO messenger systems may interact and influence each other synergistically or antagonistically.[17–19] Low concentrations of CO induce the release of NO and may mimic the vascular effects of NO, while high levels of CO inhibit NOS activity and NO generation.[20] CO is produced by the enzyme haemoxygenase (HO)[21,22] that has been localised to the vascular endothelium in the pig.[14] HO is expressed in the normal bovine eye[23] suggesting that CO may be an important vasodilator in the ophthalmic circulation. Thus, CO may account for some of the residual endothelium-dependent relaxation observed in the presence of NO and COX inhibition.

This study was undertaken to investigate the contribution of PGI_2 and the constitutive form of HO (HO-2) to NO-independent, endothelium-dependent vasodilatation in the porcine posterior ciliary circulation.

Materials and methods

Tissue preparation

Porcine eyes, removed immediately following slaughter, were obtained from a local abattoir. The enucleated eyes were transported to the laboratory in physiological salt solution (PSS, see Solutions and reagents) on ice, stored at 4°C, and used over 3 successive days. Storage had no effect on mean maximum prostaglandin F_2 alpha ($PGF_{2\alpha}$)-induced contraction and mean maximum endothelium-dependent relaxation in groups of eyes stored for 1, 2, and 3 days (unpublished data). Isolation of vessels was carried out on the morning of each experiment and the vessels cut into ring segments (length: 0.85–1.5 mm, internal diameter: 267–347 μm). Only one eye from each animal was used, with adjacent segments used in the paired test and control protocols. All experiments adhered to the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Experimental set-up

Isolated vessels were mounted for the measurement of isometric tension development as previously described.[24] In brief, the rings were mounted on a Mulvaney-Halpern small vessel dual myograph (Model 410A, Linton Instrumentation, Norfolk, England) using two 40 μm intraluminal wires.[25] Isometric tension was recorded as a function of time using an analogue to digital system (Biopac MP100 WS, Linton Instrumentation, Norfolk, England) connected to a desktop computer (Apple Macintosh Performa 6200). The rings were mounted in PSS that was continuously gassed with 5% CO_2 in air and maintained at 37°C. Neither pH nor pCO_2 were significantly altered in any of the conditions examined. Following a 1-h equilibration period, the rings were stretched to a resting optimum pretension corresponding to an intraluminal pressure of 100 mmHg (13.3 kPa) using a calculation based on the Laplace equation.

Experimental protocol

Following a 30 min equilibration period at optimum pretension, each ring was maximally contracted by three successive exposures to a high potassium solution (see Solutions and reagents) for 5 min and relaxed to pretension after each exposure by rinsing with PSS. $PGF_{2\alpha}$ was added in increasing concentrations (10^{-8}–$10^{-4.5}$ M) at 5 min intervals, yielding a cumulative concentration response curve (CCRC). Three agonists were examined as potential constricting agents for these experiments. The α-agonist phenylephrine produced very small contractions (mean maximum contraction 0.3 ($\pm$0.2) N/m or 5.3 ($\pm$3.0)% of 80 mM KCl-induced contraction), which did not allow an examination of endothelium-dependent relaxation. Serotonin produced a mean maximum contraction of 1.2 ($\pm$0.2) N/m (21.3$\pm$4.2% of 80 mM KCl-induced contraction); however, repeated exposure to this agent produced tachyphylaxis in the preparation, rendering it unsuitable. We chose $PGF_{2\alpha}$ as our vasoconstrictor; when used to induce submaximal contractions, it produced stable, repeatable contractions (mean maximum contraction 5.2 ($\pm$0.4) N/m or 87.7 ($\pm$6.6)% of 80 mM KCl-induced contraction). Relaxation was achieved by rinsing with PSS. A submaximal contraction (70% maximum) was elicited using $PGF_{2\alpha}$ and, when a stable tension was achieved, the response to cumulatively increasing concentration of BK ($10^{-10.5}$–10^{-6} M) was examined. Relaxation of 80% or greater of the submaximal contraction was taken to indicate the presence of a functional endothelium, and rings that relaxed less than this were discarded.[11,26] After washout, rings were incubated with N-nitro-L-arginine methyl ester (L-NAME

10^{-3} M) for 30 min, before eliciting a second submaximal $PGF_{2\alpha}$-induced contraction. The BK CCRC was repeated as before, completing the standard run-up protocol.

Following washout, the ring was incubated with an inhibitor of COX or HO (see below) for 30 min, while the adjacent ring segment underwent a control exposure (see below). The bath concentration of $PGF_{2\alpha}$ was increased in small increments until the submaximal tension obtained was similar to that elicited during the previous $PGF_{2\alpha}$-induced contractions, prior to the addition of the inhibitor. This was to ensure that the effect of subsequent addition of BK was always compared at the same submaximal tension. Once again the response to cumulatively increasing concentration of BK was examined. L-NAME (10^{-3} M) was continuously present in the bath solutions throughout all test and control protocols.

Three separate series of experiments were carried out. In Series 1, the effect of the COX inhibitor indomethacin (10^{-6} M) on endothelium-dependent relaxation was compared to that of its diluent (PSS). In Series 2, the effect of the HO inhibitor Tin protoporphyrin-IX (SnPP-IX, 10^{-5} M) on endothelium-dependent relaxation was compared to that of its diluent (PSS). Finally, the effect of SnPP-IX (10^{-5} M) was compared to that of a structurally similar compound, protoporphyrin-IX (PP-IX, 10^{-5} M), which does not inhibit HO (Series 3). In this last series of experiments, PP-IX was dissolved in pure ethanol and HCl (10^{-3} M). An identical volume of ethanol–HCl diluent was added to the ring that was exposed to SnPP-IX. All protocols in which protoporphyrins were used were carried out in a darkened room and the tissue bath was surrounded with aluminium foil to exclude light.[27]

A standard end procedure was carried out on completion of the experimental protocol. This comprised induction of maximum contraction by the addition of high potassium solution and relaxation by rinsing with PSS, repeated three times. If the maximal contraction of a ring following the protocol was not equal to, or greater than, 90% of the initial value, the data were excluded.

Solutions and reagents

PSS was composed of 119 mM NaCl, 4.7 mM KCl, 1.17 mM $MgSO_4$, 5.5 mM D-glucose, 25 mM $NaHCO_3$, 1.18 mM KH_2PO_4, 2.5 mM $CaCL_2$, and 0.026 mM K_2EDTA. The high potassium solution was composed of 48 mM NaCl, 80 mM KCl, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, 20 mM $NaHCO_3$, 0.9 mM Na_2HPO_4, and 2.4 mM $CaCl_2$. Syringe samples of the bath solution were taken at regular intervals throughout all experiments, for analysis of pH, PCO_2, and PO_2 by an automated blood–gas analyser (Ciba Corning Model 278, Medfield, MA, USA).

$PGF_{2\alpha}$ (10^{-2} M), BK (10^{-3} M), and SnPP-IX (10^{-2} M) were made up as stock solutions in distilled water and stored at -20°C until needed. A stock solution of indomethacin (10^{-2} M) was made up daily, in Na_2CO_3 (10^{-1} M), and further diluted in PSS to achieve a final bath concentration of 10^{-6} M. L-NAME (10^{-1} M) was made up in PSS and stored at -20°C until needed. PP-IX was dissolved in a solution of hydrochloric acid (10^{-3} M) in ethanol and was made up daily. All stock solutions underwent dilution in PSS to achieve the required bath concentrations.

All salts and chemicals were obtained from Sigma-Aldrich (Poole, Dorset, UK), except for SnPP-IX and PP-IX, which were obtained from Affiniti (Mamhead, Exeter, UK). Protoporphyrins were made up in a darkened room and all containers covered in aluminium foil. All concentrations are expressed as the final molar concentrations in the bath solution.

Data analysis

Contractile responses were calculated as the average of the digitised tension recordings over a 30 s period when the response had stabilised. The active wall tensions are expressed as force per unit length (N/m).

The purpose of Series 1 was to examine the contribution of COX products to NO-independent vasodilatation. Thus, the maximum BK-induced endothelium-dependent relaxation that remained following NOS inhibition (L-NAME) was taken as 100% and, following the addition of indomethacin, the residual endothelium-dependent relaxation was expressed as a percentage of this (100%) value. The concentration of BK that evoked 50% of the maximum relaxation (EC_{50} value) was calculated in the presence of each inhibitor and in the paired control ring. In the series of experiments looking at the role of HO products on residual endothelium-dependent relaxation (Series 2 and 3), relaxation was expressed in a similar manner, that is, as a percentage of the maximum BK-induced relaxation achieved in the presence of NOS inhibitor (L-NAME), in that ring, prior to HO inhibition. In the experiments examining the effect of continued exposure to L-NAME, relaxation was expressed as a percentage of the $PGF_{2\alpha}$-induced submaximal contraction in each ring. In this last series, the results were expressed in this way to allow comparison with the initial endothelium-dependent relaxation, in the absence of inhibitor.

All values shown are means $\pm$ standard error of the mean (SEM) and n equals the number of animals studied. To test for statistical differences between mean values, paired t-tests were used. For multiple comparisons of

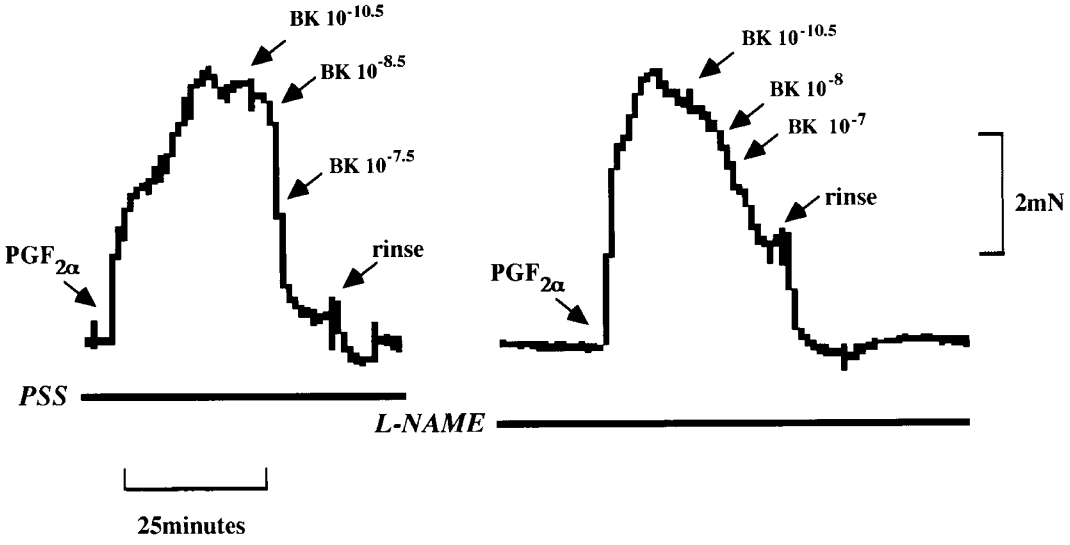

Figure 1 Reproduction of a typical original recording, showing induction of submaximal contraction on addition of $PGF_{2\alpha}$, followed by relaxation to cumulatively increasing concentrations of BK ($10^{-10.5}$–10^{-6} M) in control conditions and 30 min following the addition of L-NAME (10^{-3} M). The horizontal bars indicate the duration of exposure to each condition.

means across experimental groups, analysis of variance (ANOVA) was carried out and where a significant F-value was found, the Student–Newman–Keuls *post hoc* test (SNK) was used to assess the significance of the differences between specific means. A value of $P<0.05$ was accepted as statistically significant.

Results

The mean maximum wall tension developed in response to $PGF_{2\alpha}$ in the posterior ciliary artery preparation was 5.5 ($\pm$0.4) N/m. Mean maximum relaxation in response to BK (10^{-6} M) was −97.7 ($\pm$2.9)% of the submaximal, $PGF_{2\alpha}$-induced, tension.

Figure 1 shows a reproduction of an original experimental record, which illustrates that BK, in the absence of inhibitors, produced almost complete relaxation of a posterior ciliary artery, which has been preconstricted with $PGF_{2\alpha}$. It can be seen that in the presence of L-NAME (10^{-3} M), a substantial relaxation was still observed in response to BK, that is, NOS-independent, endothelium-dependent relaxation.

Series 1: In a group of rings ($n=6$), incubation with indomethacin significantly ($P<0.01$, ANOVA) attenuated NOS-independent, endothelium-dependent relaxation when compared to the paired control group (Figure 2 and Table 1). Addition of indomethacin did not alter mean baseline tension compared to that in the control group (data not shown).

Series 2: In the presence of SnPP-IX (10^{-5} M), BK caused a significantly ($P<0.01$, ANOVA) greater relaxation at

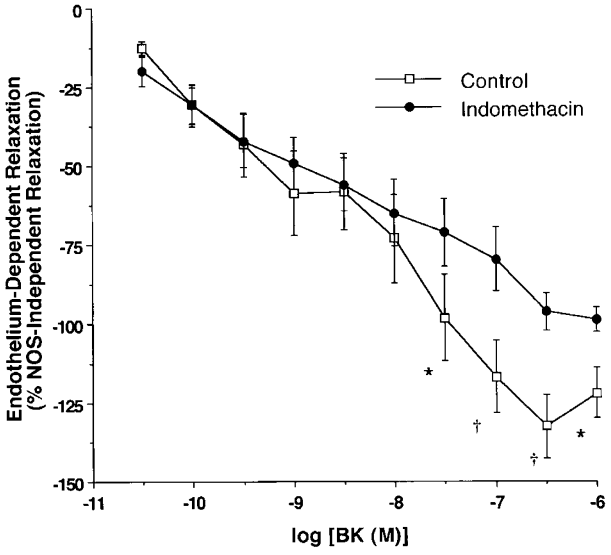

Figure 2 Mean ($\pm$SEM) residual relaxation in response to BK ($10^{-10.5}$–10^{-6} M) following NOS inhibition is shown, in the presence of indomethacin (10^{-6} M) and in paired control rings ($n=6$). Amplitude of relaxation is expressed as a percentage of maximum residual relaxation in the presence of L-NAME alone. *indicates significant difference from paired control curve ($P<0.05$, SNK). †indicates significant difference from paired control curve ($P<0.01$, SNK).

submaximal concentrations than in paired rings exposed to the PSS vehicle (Figure 3a and Table 1). However, maximal relaxation was similar in the two groups. No significant change in mean baseline tension was

Table 1 Effect of addition of SnPP-IX and indomethacin on endothelium-dependent relaxation to BK

Control/inhibitor	*Maximum relaxation*		*EC_{50} BK*	
	Control	*Inhibitor*	*Control*	*Inhibitor*
PSS/SnPP-IX (n=9)	−116.2±15.1	−122.9±11.6	−7.9±0.2	−8.7±0.2*
PP-IX/SnPP-IX (n=8)	−149.1±18.0	−107.5±16.7	−8.2±0.2	−8.8±0.4
Na_2CO_3/indomethacin (n=7)	−130.8±8.8	−103.5±8.8*	−8.3±0.3	−8.5±0.4

Values are mean±SEM. The maximum relaxations during CCRCs to BK, on submaximal $PGF_{2\alpha}$-induced contraction, on addition of the inhibitor SnPP-IX (10^{-5} M) compared to PSS or PP-IX (10^{-5} M), or indomethacin compared to Na_2CO_3 are shown. Maximum relaxation is expressed as a percentage of maximum relaxation in the presence of L-NAME alone. The EC_{50} values are expressed as logarithm BK bath concentration in moles. *indicates significant difference from control value ($P<0.05$, paired t-test).

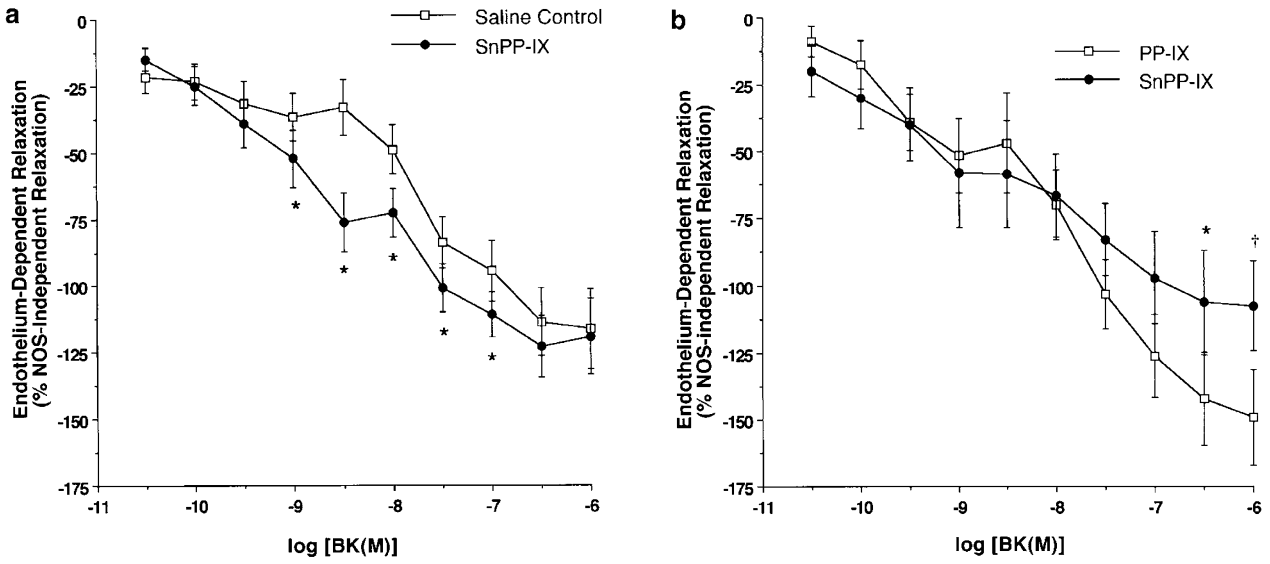

Figure 3 (a) Mean (±SEM) residual relaxation in response to BK ($10^{-10.5}$–10^{-6} M) following NOS inhibition, in the presence of SnPP-IX (10^{-5} M) and in paired control rings exposed to saline vehicle ($n=8$). Amplitude of relaxation is expressed as a percentage of maximum residual relaxation in the presence of L-NAME alone. *indicates significant difference from paired control curve ($P<0.01$, SNK). (b) Mean (±SEM) residual relaxation in response to BK ($10^{-10.5}$–10^{-6} M) following NOS inhibition, in the presence of SnPP-IX (10^{-5} M) and in paired control rings exposed to PP-IX (10^{-5} M). Amplitude of relaxation is expressed as a percentage of maximum residual relaxation in the presence of L-NAME alone ($n=9$). *indicates significant difference from paired control curve ($P<0.05$, SNK). †indicates significant difference from paired control curve ($P<0.01$, SNK).

observed following the addition of SnPP-IX when compared to PSS (data not shown). It is interesting to note that, in the control group, NOS-independent relaxation examined on the second occasion was larger than when it was first tested earlier in the experimental protocol (see Effect of continued exposure to L-NAME).

Series 3: In the presence of SnPP-IX (10^{-5} M), BK caused a significantly ($P<0.01$, SNK) lesser relaxation at the higher concentrations tested than in paired rings exposed to PP-IX (Figure 3b and Table 1). No significant change in mean baseline tension was observed following the addition of SnPP-IX when compared to the addition of PP-IX (data not shown).

Effect of continued exposure to L-NAME

We noted that in the PSS control group of Series 2, NOS-independent, endothelium-dependent relaxation appeared to increase as the period of NOS inhibition continued. To allow a quantitative examination of this, we expressed BK-induced relaxation as a percentage of the $PGF_{2\alpha}$-induced submaximal tension (per cent submaximal tension) during each of the four exposures of the control group to BK. Figure 4 shows that 30 min after the initial addition of L-NAME (10^{-3} M), BK-induced relaxation in response to BK was significantly ($P<0.01$, ANOVA) reduced. However, when tested again after approximately a further 60 and 120 min of exposure

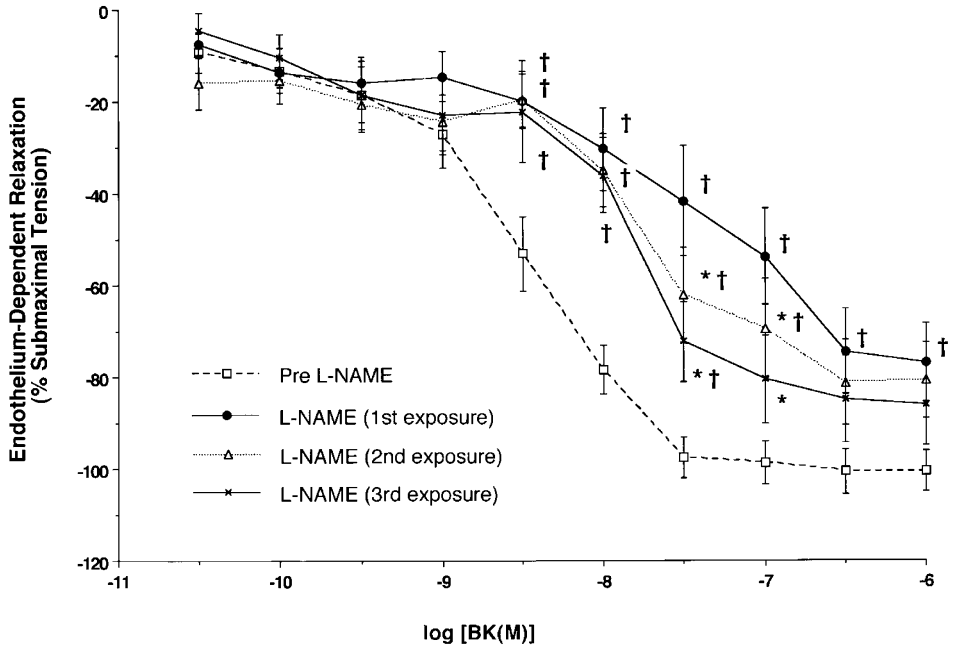

Figure 4 Mean (±SEM) relaxation in response to repeated exposures to BK ($10^{-10.5}$–10^{-6} M) over a period of 2 h of continuous exposure to L-NAME (10^{-3} M). Amplitude of relaxation is expressed as a percentage of submaximal $PGF_{2\alpha}$-induced contraction ($n=8$). *indicates significant difference from first curve in the presence of L-NAME ($P<0.01$, SNK). †indicates significant difference from pre-L-NAME (control) curve ($P<0.01$, SNK).

to L-NAME, relaxation induced by BK was significantly ($P<0.01$, ANOVA) greater than on initial exposure to L-NAME. On all occasions tested, relaxation in the presence of the NOS inhibitor was significantly less than in its absence ($P<0.01$, ANOVA). The mean EC_{50} concentration, expressed as $\log_{10}$[BK(M)], under pre-L-NAME (control) conditions was −8.62 (±0.15), while those following the first, second, and third exposures to BK in the presence of L-NAME were −7.93 (0.34), −7.85 (0.12), and −8.25 (0.22), respectively. Although the mean EC_{50} on the occasion of the second exposure remained significantly different from that during the control exposure ($P<0.05$), it was not significantly different during the third exposure ($P=0.10$).

Discussion

Our finding, that inhibition of NOS-reduced relaxation in response to BK by approximately one-third, agrees with the results of Haefliger *et al*[12] and supports the idea that, while NO is a major endothelium-derived vasodilator, it does not account for all the endothelium-dependent relaxation. Zhu *et al*[28] showed that removal of the endothelium abolishes the relaxing effect of BK, demonstrating that the residual relaxation in question is endothelium dependent.

Exogenously applied $PGF_{2\alpha}$ evokes contractions in the ciliary circulation in many species (eg porcine, bovine, canine, lupine vessels[29–31]). Its ability to produce stable, repeatable contractions on repeated exposure makes it useful in the investigation of vascular reactivity. Like many mediators, $PGF_{2\alpha}$ may influence vessel tone through a number of different pathways. It has been shown to cause contraction by stimulating TP prostanoid receptors in bovine long posterior ciliary arteries.[29] It can also cause relaxation by stimulating EP_2 prostanoid receptors in smooth muscle cells, stimulating FP prostanoid receptors in the endothelium causing release of NO[32,33] and causing the release of endogenous prostaglandins, such as PGE_2 in smooth muscle cells.[34] Thus, the degree of vasoconstriction produced is the result of a balance of vasoconstrictor and vasodilator actions. In the presence of inhibitors of one of these specific pathways, for example NOS or COX, the effect of a given concentration of a $PGF_{2\alpha}$ is altered. Use of a constant preconstricting concentration of $PGF_{2\alpha}$ in the present experiments would have led to differing submaximal tensions after the addition of NOS and COX inhibitors. Thus, any apparent effect on the response to an endothelium-dependent vasodilator might then be owing to altered initial tension and not simply to an effect on the endothelium. To avoid this problem, we did not induce the contractions with a standard concentration of $PGF_{2\alpha}$, but rather titrated the

concentration of $PGF_{2\alpha}$ to elicit a similar submaximal tension on all occasions.

An obvious candidate mediator that might account for NO-independent vasodilatation is PGI_2. Previous research in the ophthalmic and posterior ciliary circulations has been carried out on a background of COX inhibition,[11,12] and therefore the contribution of PGI_2 has not been directly determined. We demonstrate, for the first time, that incubation with indomethacin, following inhibition of NOS, significantly reduced endothelium-dependent relaxation in the posterior ciliary artery, indicating an important role for PGI_2, similar to that seen in other circulations.[35] Indeed, the present results may underestimate the contribution of PGI_2 to vascular control in the ciliary circulation. It has been suggested that NO-induced retinal and choroidal vasorelaxation is only partly mediated by the action of cGMP in smooth muscle and that NO acts largely by stimulating PGI_2 formation in the endothelium.[36] Thus, the importance of PGI_2 may be underestimated in the presence of NOS inhibition and, conversely, the magnitude of NO-induced relaxation may be distorted by background inhibition of PGI_2.

The potential role of HO-derived CO as an endothelium-dependent vasodilator has not been examined previously in the posterior ciliary circulation. SnPP-IX is a synthetic haem analogue that is a powerful competitive inhibitor of HO.[37] To assess the role of HO in endothelium-dependent relaxation, we compared its effect on BK-induced relaxation to that of two separate controls, saline vehicle and the structurally similar compound PP-IX. In the absence of a metal ion, protoporphyrin does not inhibit HO activity.[14] We found that SnPP-IX did not reduce relaxation compared to saline vehicle control, demonstrating that HO does not have a significant role in mediating endothelium-dependent relaxation in porcine ciliary arteries. This is in contrast to other vascular beds, where HO-derived CO has been shown to have an important role.[13–16] The lesser endothelium-dependent relaxation observed in the presence of SnPP-IX, when compared to PP-IX, is similar to the findings previously reported in the pulmonary circulation.[14] However, since SnPP-IX did not inhibit NO-independent relaxation (Series 2), this latter finding suggests that PP-IX enhanced BK-induced relaxation. In support of this, it can be seen that the maximum endothelium-dependent relaxation in the presence of PP-IX in the third series
of experiments was greater than that in the saline control group in the second series of experiments (comparing Figures 3a and b). A potential explanation for enhanced relaxation in the presence of PP-IX is that this agent can activate guanylyl cyclase[38] and may have caused activation of this pathway in the absence of endogenous NO.

The importance of NO-independent vasodilatation is further emphasised by the partial recovery in vasodilatation with time following NOS inhibition observed in the present study. Repeated BK CCRCs in the presence of L-NAME had not, to our knowledge, been previously examined. The increase in relaxation seen consistently in the second and third BK CCRCs, relative to the response seen in the initial relaxation curve, demonstrates that NO-independent mechanisms may become upregulated in the presence of NOS inhibition *in vitro*. However, recovery is not complete as indicated by the persistent difference from control values following the third exposure to L-NAME. McCulloch *et al*[39] have previously reported that, in isolated rat superior mesenteric arteries, NO may reduce endothelium-derived hyperpolarizing factor (EDHF) activity through a cyclic GMP-dependent pathway and suggested that, in disease conditions, loss of basal NO production may cause the EDHF component of endothelium-dependent relaxation to become functionally greater. EDHF was first identified because of the residual relaxation seen in the presence of NOS and COX inhibition.[40] This, as yet, unidentified factor is neither a NOS nor COX product, but is a major mediator of endothelium-dependent relaxation in many vascular beds. Recent evidence suggests that EDHF mediates its effect by either, directly of indirectly, opening K^+ channels on vascular smooth muscle cells, or by facilitating electric coupling through myoendothelial gap junctions.[41,42] Metabolites of arachidonic acid produced by cytochrome P450 are postulated to be the elusive EDHF.[43] Taken together with the results presented here, those previous reports suggest that in disease conditions which impair NOS expression or activity, other endothelium-dependent relaxation mechanisms including EDHF may become upregulated and act as a partial substitute.

Recognition of the increase in endothelium-dependent relaxation with time has important implications for experimental design. Firstly, time-based controls are imperative; comparison of relaxation curves before and after addition of inhibitors would produce an erroneous conclusion. Secondly, it accounts for the relaxation in both control and test rings being greater than 100% of the first NO-independent relaxation (Figures 2, 3a and b and Table 1).

In conclusion, we have shown that vasodilator products of the COX pathway account for a significant proportion of NO-independent endothelium-dependent relaxation in porcine posterior ciliary arteries. We have demonstrated that CO produced by HO cannot account for the significant residual relaxation following

NOS inhibition. Previous work to elucidate the contribution of endothelium-dependent relaxation to the pathogenesis of glaucoma has predominantly focused on NO; the results of the present study show that abnormalities of the COX pathway and other unidentified vasodilator pathways may also contribute and warrant further study.

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