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RESEARCH PAPER

Differential effects of nitric oxide synthase inhibitors on endothelium-dependent and nitrergic nerve-mediated vasodilatation in the bovine ciliary artery

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Background and purpose: We have previously demonstrated that L-NMMA (N^G-monomethyl-L-arginine) selectively inhibits vasodilatation produced by endothelium-derived nitric oxide but not nitrergic nerves in the bovine penile artery. The present study investigated whether L-NMMA had a similar selective action in the bovine ciliary artery. We also investigated whether two recently introduced inhibitors of neuronal nitric oxide synthase (nNOS), AAAN (N-(4S)-4-amino-5-[aminoethyl]aminopentyl-N′-nitroguanidine) and L-NPA (N^G-propyl-L-arginine), produced selective blockade of vasodilatation induced by nitrergic nerves but not endothelium-derived nitric oxide.
Experimental approach: Rings of bovine ciliary artery were suspended in a wire myograph for tension recording. Neurogenic (nitrergic) vasodilatation was elicited by electrical field stimulation, and endothelium-dependent, nitric oxide-mediated dilatation was evoked using bradykinin.
Key results: L-NMMA inhibited vasodilatation induced by endothelium-derived nitric oxide but not the nitrergic nerves. In fact, L-NMMA, acted like L-arginine in protecting nitrergic vasodilatation against inhibition by L-NAME (N^G-nitro-L-arginine methyl ester). AAAN had no effect on vasodilatation induced by either nitrergic nerves or endothelium-derived nitric oxide, but L-NPA inhibited both with equal potency.
Conclusions and implications: In the bovine ciliary artery, L-NMMA acts as a selective inhibitor of the vasodilatation induced via endothelial NOS, without affecting that operating via nNOS. Furthermore, the putative nNOS inhibitors, AAAN and L-NPA failed to produce the expected selective inhibition of nitrergic vasodilatation in this artery.
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Keywords: artery; autonomic nerves; EDHF; endothelium; endothelium-derived hyperpolarizing factor; nitrergic nerves; nitric oxide; nitric oxide synthase; vasodilatation

Abbreviations: AAAN, *N*-(4S)-4-amino-5-[aminoethyl]aminopentyl-*N*′-nitroguanidine; EFS, electrical field stimulation; ChTx, charybdotoxin; EDHF, endothelium-derived hyperpolarizing factor; eNOS, endothelial nitric oxide synthase; L-NAME, N^G-nitro-L-arginine methyl ester; L-NOARG, N^G-nitro-L-arginine; L-NPA, N^G-propyl-L-arginine; L-NMMA, N^G-monomethyl-L-arginine; NOS, nitric oxide synthase; nNOS, neuronal nitric oxide synthase

Introduction

Nitrergic vasodilator nerves running in the parasympathetic outflow innervate the retinal and ciliary vascular beds in a wide range of mammalian species, including humans (for reviews see Koss, 1999; Toda and Okamura, 2003). In the bovine intraocular long posterior ciliary artery, electrical field stimulation (EFS) evokes biphasic vasodilatation, consisting of an initial component peaking at ~10 s, which decays rapidly, but is followed by a slower component peaking at 50 s. The initial rapid component is abolished by the nitric oxide synthase (NOS) inhibitors, N^G-nitro-L-arginine (L-NOARG) and N^G-nitro-L-arginine methyl ester (L-NAME), and by the inhibitor of soluble guanylate cyclase, ODQ, and is clearly nitrergic, but the identity of the neurotransmitter mediating the slower component is the subject of debate (Wiencke *et al*., 1994; Overend *et al*., 2005).

Previous work has revealed that NOS inhibitors that are N^G-substituted analogues of L-arginine do not all uniformly

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block nitrergic neurotransmission in bovine tissues. For example, in the bovine retractor penis muscle, nitrergic transmission is blocked by L-NOARG and L-NAME, but not by L-NMMA (Liu *et al*., 1991; Martin *et al*., 1993). Indeed, in this tissue, N^G-monomethyl-L-arginine (L-NMMA) appears to act as an alternative substrate for nitric oxide (NO) production as, like the endogenous substrate, L-arginine, it can both prevent the onset of and reverse already established blockade of nitrergic transmission induced by L-NOARG or L-NAME. Interestingly, in the bovine penile artery, L-NMMA also fails to inhibit nitrergic transmission, but shares the ability of L-NOARG to block endothelium-dependent, NO-mediated vasodilatation (Liu *et al*., 1991). Thus, in the bovine penile artery, L-NMMA selectively inhibits vasodilatation mediated by endothelial nitric oxide synthase (eNOS), while acting as an alternative substrate for dilatation occurring through neuronal nitric oxide synthase (nNOS).

The aim of this study was to determine if L-NMMA has the ability to selectively block vasodilatation induced by endothelium-derived NO but not nitrergic nerves in the bovine ciliary artery, as it does in the penile artery. Furthermore, the selectivity of two more recently introduced putative inhibitors of nNOS, *N*-(4S)-4-amino-5-(aminoethyl) aminopentyl-*N*′-nitroguanidine (AAAN) and N^G-propyl-L-arginine (L-NPA) (Zhang *et al*., 1997b; Hah *et al*., 2001) was investigated by comparing their actions on vasodilatation produced by endothelium-derived NO and nitrergic nerves in the bovine ciliary artery.

Methods

Preparation of bovine ciliary artery rings for tension measurement
Bovine eyes were obtained from a local abattoir within 90 min of killing. At the laboratory, the intraocular long posterior ciliary artery (~400 μm internal diameter (i.d.)) was dissected into 2 mm long ring segments and mounted in wire myographs (Multi Myograph model 610; Danish Myo Technology). The vessels were maintained at 37°C in Krebs solution containing (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11.5; and gassed with O_2 containing 5% CO_2. Isometric tension was recorded and displayed using a PowerLab 4/20 and Chart v5 (both ADInstruments, Hastings, UK). Tension was applied to the vessels to give a transmural pressure equivalent to ~100 mm Hg (actual equivalents 103.2±0.4 mm Hg in vessels of 458±6 μm i.d., $n=125$). Tissues were allowed to equilibrate for 30 min before experiments were carried out.

Experimental protocols involving electrical field stimulation
Ciliary artery rings were mounted on myographs jaws containing a pair of parallel platinum electrodes (Danish Myo Technology) for EFS. EFS, consisting of square wave pulses (10–15 V, 0.3 ms pulse width, 10 s train length, 0.5–32 Hz), was delivered using a S88 stimulator (Grass, Quincy, USA). Stimulation parameters were designed to evoke maximal neurogenic (tetrodotoxin-sensitive; Overend *et al*., 2005) vasodilatation, while avoiding direct muscle stimulation. All experiments were carried out in the presence of ~70% of maximal tone, induced by using the thromboxane A_2 mimetic, U46619 (0.1–1 μM), and after adrenergic neurone blockade using guanethidine (30 μM). Under these conditions, EFS evokes a biphasic vasodilatation, comprising an initial nitrergic component peaking at 10 s, followed by a slower component peaking at 50 s, mediated by an unknown transmitter (Overend *et al*., 2005). In keeping with previous findings, NOS inhibitors had no effect on the second component of neurogenic vasodilatation (data not shown), and only the effects on the nitrergic component are presented in the results.

Control frequency–response curves to EFS (0.5–32 Hz, 10 s trains) were obtained for each vessel before a second curve was generated in the presence of the NOS inhibitors, L-NAME, L-NMMA, AAAN or L-NPA (all 100 μM, 1 h). Preliminary experiments showed that control frequency–response curves were reproducible over a period of up to 4 h (data not shown). In some experiments, the concentration dependence of the ability of NOS inhibitors to inhibit neurogenic vasodilatation was examined. In these experiments, EFS was delivered at a single frequency (16 Hz, 10 s) at 15 min intervals. When reproducible neurogenic vasodilatation had been obtained, the effects of increasing concentrations of L-NAME (0.1–100 μM), L-NMMA (10 μM–1 mM) or L-NPA (0.1–100 μM) were examined. Other experiments were conducted to determine if L-arginine or L-NMMA could inhibit the blockade of neurogenic vasodilatation induced by L-NAME. In these experiments, reproducible neurogenic vasodilatation (16 Hz, 10 s) was first obtained in the presence of either L-arginine or L-NMMA (both 1 mM, 1 h) before the effects of increasing concentrations of L-NAME (0.1 μM–1 mM) were examined.

Experimental protocols involving endothelium-dependent, NO-mediated dilatation to bradykinin
Following constriction of ciliary artery rings with U46619, endothelium-dependent dilatation was evoked using bradykinin (1 μM). As will be seen in the Results, this dilatation was not solely mediated by NO. The NO-mediated component was, however, isolated by treating tissues with apamin and charybdotoxin (both 100 nM) to block endothelium-derived hyperpolarizing factor (EDHF) (Waldron and Garland, 1994; Zygmunt and Högestätt, 1996), together with indomethacin (10 μM) to block cyclooxygenase. Under these conditions, the effects of the NOS inhibitors, L-NAME, L-NMMA, AAAN or L-NPA, were then examined on the NO-mediated component of dilatation induced by bradykinin.

At the end of all experiments, papaverine (500 μM) was added to determine the level of tone present, and all dilatations were expressed as a percentage of this U46619-induced tone.

Drugs and chemicals
AAAN, apamin, L-arginine hydrochloride, bradykinin triacetate, guanethidine sulfate, indomethacin, L-NAME, L-NMMA, papaverine hydrochloride and U46619 (9,11-dideoxy-11α,9α-epoxy-methanoprostaglandin$F_{2\alpha}$) were all obtained from Sigma (Poole, UK). Charybdotoxin was

obtained from Latoxan (Valence, France). L-NPA was obtained from Alexis Biochemicals (Nottingham, UK). All drugs were dissolved and diluted in 0.9% saline, with the following exceptions: L-NPA (1 mM stock) in 100% ethanol; indomethacin (0.01 M stock) in Na_2HCO_3 (0.04 M) and U46619 (1 mM stock) in 50% ethanol.

Statistical analysis

Results are expressed as the mean ± s.e.m. of *n* observations, each from a separate vessel from a different eye. Statistical comparisons were made using one-way analysis of variance (ANOVA) and the Bonferroni post-test, with the aid of a computer program, Prism (GraphPad, San Diego, USA). A probability (*P*) less than or equal to 0.05 was considered significant.

Results

Neurogenic dilatation of the bovine ciliary artery

In the presence of submaximal U46619 (0.1–1 μM)-induced tone and the adrenergic neurone blocker, guanethidine (30 μM), EFS (10–15 V, 0.3 ms pulse width, 10 s train length) of bovine ciliary artery rings evoked frequency (0.5–32 Hz)-dependent dilatation, optimal at 32 Hz. As found previously (Overend *et al*., 2005), this dilatation was biphasic, comprising an initial rapid component peaking at 10 s, followed by a slower component peaking at 50 s. Figure 1 shows frequency–response curves for the first component of dilatation.

Effects of L-NAME, L-NMMA and L-arginine on neurogenic dilatation

The first component of neurogenic dilatation was abolished at all frequencies by the NOS inhibitor, L-NAME (100 μM, Figure 1a). Furthermore, when stimulated at a single frequency (16 Hz, 10 s), L-NAME produced concentration-dependent inhibition over the range 0.1–100 μM, with a pIC_{50} of 5.74 ± 0.16 (Figure 2). In contrast, L-NMMA (10 μM–1 mM) failed to inhibit neurogenic dilatation at any frequency (Figures 1a and 2). Pretreatment with L-arginine or L-NMMA (both 1 mM, 1 h) protected against subsequent inhibition of neurogenic dilatation (16 Hz, 10 s) by L-NAME, shifting its apparent pIC_{50} to 4.07 ± 0.11 and 3.50 ± 0.26, respectively ($P<0.001$ for both, Figure 2). The potencies of L-arginine and L-NMMA in protecting against inhibition of neurogenic dilatation by L-NAME were not significantly different.

Effects of nNOS inhibitors on neurogenic dilatation

The effects of two putative nNOS inhibitors, AAAN (Hah *et al*., 2001) and L-NPA (Zhang *et al*., 1997b), were examined on the first component of neurogenic dilatation. AAAN (100 μM) had no effect, whereas L-NPA abolished dilatation at all frequencies (Figure 1b). Furthermore, when stimulated at a single frequency (16 Hz, 10 s), L-NPA produced concentration-dependent inhibition over the range 0.1–100 μM, with a pIC_{50} of 4.95 ± 0.42 (Figure 3).

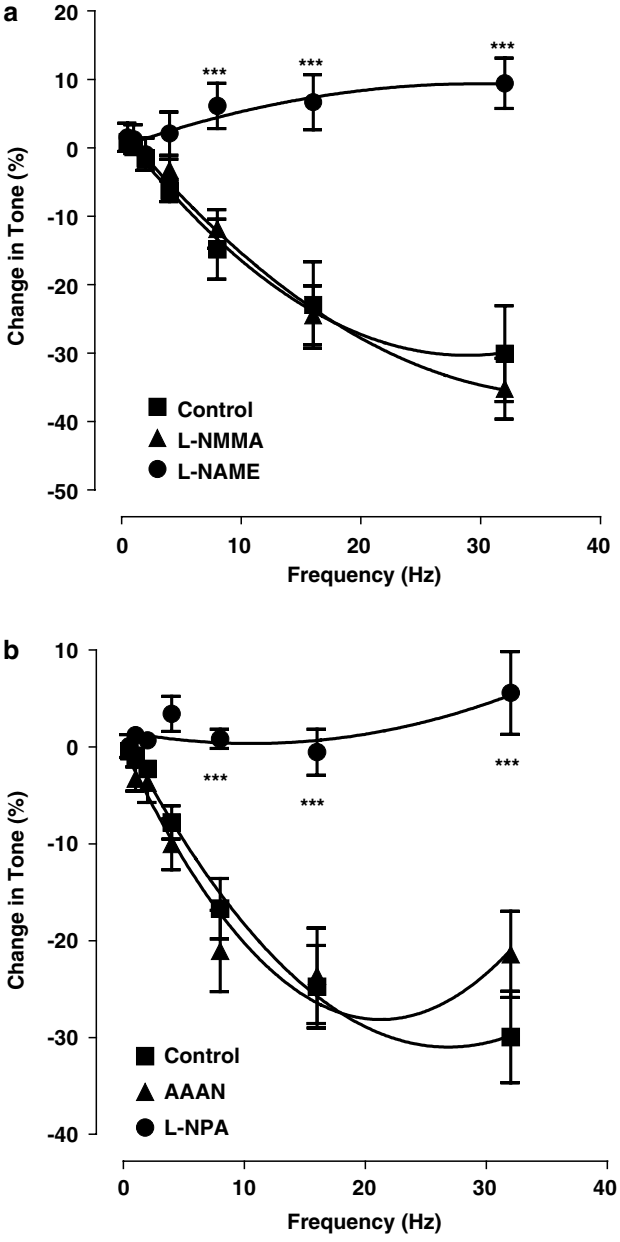

Figure 1 Frequency–response curves showing the first component of neurogenic dilatation elicited by EFS (0.5–32 Hz, 10 s trains) in control bovine ciliary artery rings, and the blockade of this dilatation by the NOS inhibitors (**a**) L-NAME, but not L-NMMA and (**b**) L-NPA, but not AAAN (all at 100 μM). Data are mean ± s.e.m. (vertical lines) of 8–12 observations. ***$P<0.001$, indicates a significant difference from control.

Effects of NOS inhibitors on endothelium-dependent, NO-mediated dilatation

In the presence of submaximal U46619 (0.1–1 μM)-induced tone, bradykinin (10 nM–1 μM) elicited concentration-dependent dilatation (maximum of 58 ± 4%, Figure 4a). L-NAME (100 μM) had no significant effect by itself on this dilatation. However, when the NO-mediated component of bradykinin-induced dilatation was isolated in the presence of inhibitors of EDHF (apamin and charybdotoxin, both 100 nM) and cyclooxygenase (indomethacin, 10 μM), L-NAME (100 μM) significantly inhibited this response.

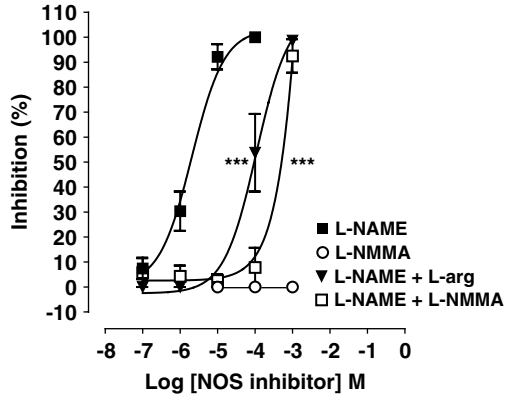

Figure 2 Graphs showing that neurogenic dilatation of bovine ciliary artery rings elicited by EFS (16 Hz, 10 s) is inhibited in a concentration-dependent manner by L-NAME, but unaffected by L-NMMA. In addition, pretreatment with L-arginine or L-NMMA (both 1 mM for 1 h) protected neurogenic dilatation against subsequent blockade by L-NAME. Data are mean ± s.e.m. (vertical lines) of 5–8 observations. ***$P<0.001$ indicates a significant difference from L-NAME alone.

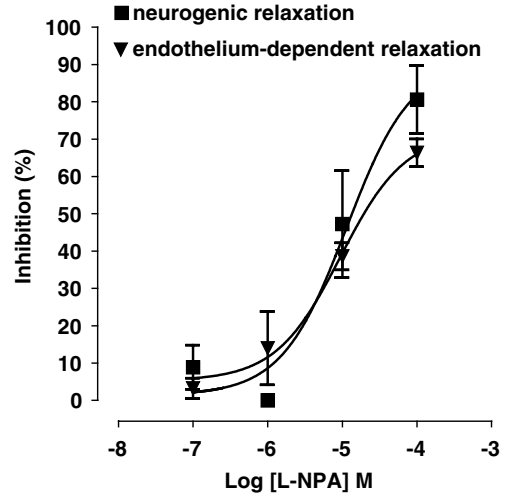

Figure 3 Graphs showing that both neurogenic (16 Hz, 10 s) and bradykinin (1 μM)-induced, NO-mediated dilatation of bovine ciliary artery rings are inhibited in a concentration-dependent manner by L-NPA. Data are mean ± s.e.m. (vertical lines) of 4–9 observations.

L-NMMA (100 μM) itself inhibited bradykinin (1 μM)-induced dilatation to a small degree, but as with L-NAME, it produced a marked inhibition when the NO-mediated component was isolated following inhibition of EDHF and cyclooxygenase (Figure 4b).

Furthermore, the NO-mediated component of bradykinin (1 μM)-induced dilatation was unaffected by AAAN (100 μM, Figure 4c), but was markedly inhibited by L-NPA (100 μM, Figure 4d). Indeed, the latter compound produced concentration-dependent inhibition over the range 0.1–100 μM, with a pIC_{50} of 5.03 ± 0.14 (Figure 3).

Discussion

The major new observations made in this study are that the NOS inhibitor, L-NMMA, does not block nitrergic vasodilatation in the bovine ciliary artery, but acts like the endogenous substrate, L-arginine, to prevent the inhibition induced by L-NAME. This occurs despite the ability of L-NMMA to inhibit endothelium-dependent, NO-mediated dilatation in the same tissue. Furthermore, two putative nNOS-selective inhibitors, AAAN and L-NPA (Zhang *et al*., 1997b; Hah *et al*., 2001) did not behave as expected in the bovine ciliary artery: AAAN failed to inhibit vasodilatation mediated by either the nitrergic nerves or endothelium-derived nitric oxide (EDNO) and L-NPA inhibited both, but with equal potency.

A large number of N^G-substituted analogues of L-arginine have been introduced as inhibitors of NOS (Hibbs *et al*., 1987; Rees *et al*., 1989, 1990; Moore *et al*., 1990). The most commonly used agents, L-NOARG and L-NAME, are not isoform selective and reliably inhibit responses mediated by the L-arginine-NO system in a wide range of species. Although L-NMMA is also commonly used as a non-isoform-selective NOS inhibitor, many studies demonstrate anomalous findings with this agent. For example, L-NMMA does not block nitrergic transmission in the bovine retractor penis muscle, but acts like the endogenous substrate, L-arginine, to inhibit blockade by L-NOARG or L-NAME (Liu *et al*., 1991; Martin *et al*., 1993). Furthermore, in the bovine penile artery, L-NMMA also fails to block nitrergic transmission, despite being able to inhibit the endothelium-dependent, NO-mediated dilatation induced by acetylcholine (Liu *et al*., 1991). In addition, in the rat aorta and pulmonary artery, L-NMMA enhances rather than inhibits the production of NO, assessed by chemiluminescence detection, and inhibits the basal but not agonist-stimulated, endothelium-dependent dilatation produced by NO (Archer and Hampl, 1992; Frew *et al*., 1993).

The above anomalous results with L-NMMA have been found despite the general observation that it inhibits all three isoforms of NOS in standard enzyme assays (Moore *et al*., 1996). Nevertheless, more detailed biochemical analysis of murine macrophage inducible nitric oxide synthase (iNOS) has revealed that L-NMMA does not behave as a simple competitive inhibitor, but as an alternative substrate and mechanism-based inhibitor of the enzyme (Olken and Marletta, 1993). According to this scheme, L-NMMA is initially metabolized to N^G-hydroxy-N^G-methyl-L-arginine, and finally to NO and L-citrulline, but with the intermediate production of a 'suicide inhibitor' that slowly and irreversibly blocks the enzyme. These findings may, therefore, account for the seemingly anomalous ability of L-NMMA to augment NO production at some sites while blocking it at others. However, why L-NMMA fails to block nitrergic transmission at sites like the bovine ciliary artery is not entirely clear. One possible explanation is that at these sites the rate of formation of the suicide inhibitor is exceedingly slow. This seems unlikely because, although most of our experiments ran for 1–2 h, we failed to see any inhibitory effects even after prolonged treatment for up to 6 h (data not shown). It is, therefore, possible that at these sites, the particular isoforms of NOS either do not produce the suicide inhibitor or are insensitive to it. Further work is required to clarify this point.

According to present findings, L-NMMA fails to block nitrergic transmission in the bovine ciliary artery, but acts

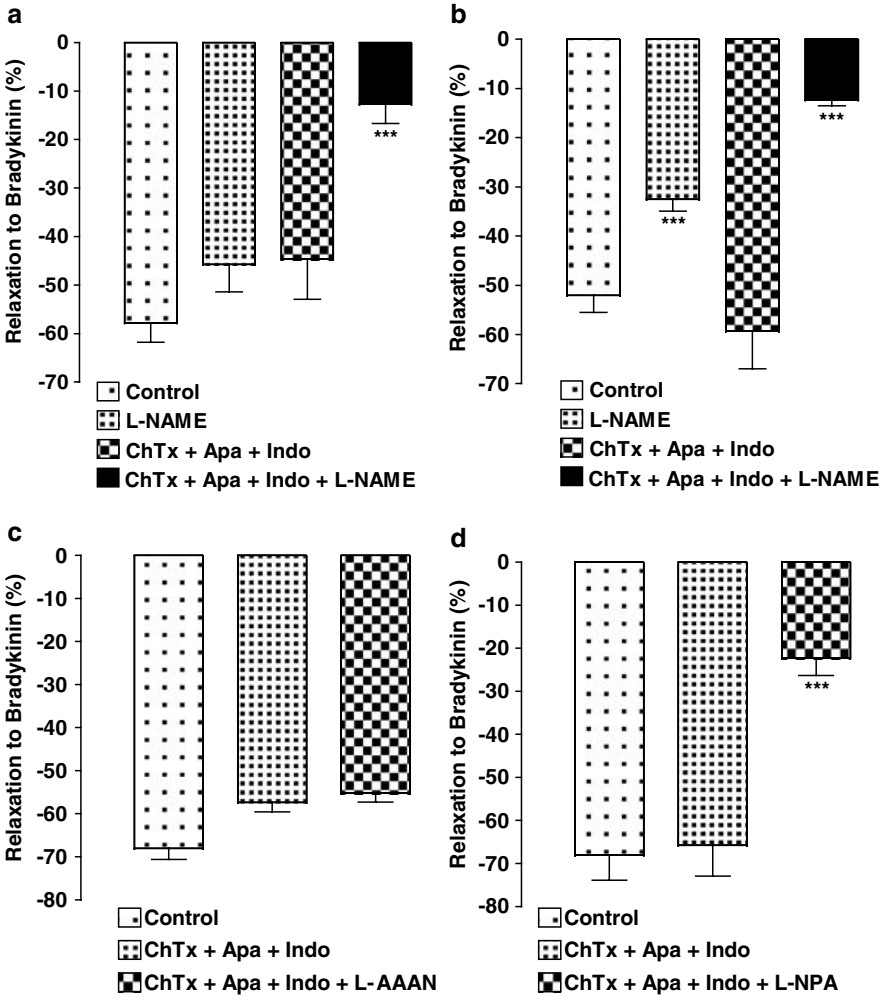

Figure 4 Graphs showing bradykinin (1 μM)-induced, endothelium-dependent dilatation in control bovine ciliary artery rings, and the component of dilatation mediated solely by NO observed in rings treated with the EDHF and cyclooxygenase inhibitors, apamin (Apa, 100 nM), charybdotoxin (ChTx, 100 nM) and indomethacin (Indo, 10 μM). Also shown are the effects of the nNOS inhibitors, (**a**) L-NAME, (**b**) L-NMMA, (**c**) AAAN and (**d**) L-NPA (all at 100 μM), on the NO-mediated component of dilatation, following inhibition of EDHF and cyclooxygenase. Data are mean$\pm$s.e.m. (vertical lines) of 6–11 observations. ***$P<0.001$ indicates a difference from control.

like L-arginine in inhibiting blockade by L-NAME, which are therefore in keeping with previous results on other nitrergically-innervated tissues (retractor penis muscle and penile artery) from this species (Liu *et al*., 1991; Martin *et al*., 1993). The inability of L-NMMA to block nitrergic transmission may, therefore, be a general feature of bovine tissues.

Our examination of the actions of L-NMMA on endothelium-dependent, NO-mediated dilatation in the bovine ciliary artery was initially confounded because of the simultaneous activation of other endothelium-derived factors. At sites where EDHF and NO are both active, it is, however, well established that blockade of either component alone may produce no effect or only a small reduction in the magnitude of the vasodilatation observed (Mügge *et al*., 1991; Tare *et al*., 2000; McNeish *et al*., 2003). The remaining vasodilatation will then be mediated solely by the other component and therefore be sensitive to inhibitors of that pathway. In keeping with this, we found that, following blockade of EDHF with apamin and charybdotoxin (Waldron and Garland, 1994; Zygmunt and Högestätt, 1996) and cyclooxygenase with indomethacin, vasodilator responses mediated solely by endothelium-derived NO could be elicited with bradykinin. These dilator responses, unlike those produced by the nitrergic nerves in the ciliary artery, were inhibited by L-NMMA. They were also inhibited by L-NAME. Thus, our findings in the bovine ciliary artery that L-NMMA inhibits endothelium-dependent, NO-mediated dilatation but not that produced by its nitrergic nerves parallels earlier findings on the bovine penile artery (Liu *et al*., 1991) and suggests that this may be a general property in this species.

Since the discovery of NO, great effort has been expended in developing isoform-selective inhibitors of NOS, both as investigational tools and as potential therapeutic agents (Fukuto and Chaudhuri, 1995; Hobbs *et al*., 1999; Li and Poulos, 2005). We, therefore, compared the effects of L-NMMA and L-NAME on bovine ciliary artery with those of some more recently introduced nNOS-specific inhibitors. The two agents we examined were AAAN, which has a $\sim$2500-fold greater selectivity for rat nNOS over bovine

eNOS (Hah *et al*., 2001) and L-NPA, which is ~150-fold more selective for bovine nNOS than eNOS (Zhang *et al*., 1997a, b). We found that in the bovine ciliary artery, AAAN at concentrations up to 100 μM failed to affect vasodilatation induced either by the nitrergic nerves or endothelium-derived NO. A lack of effect on endothelium-dependent vasodilatation might have been expected, given its poor K_i for bovine eNOS (314 μM, Hah *et al*., 2001). Nevertheless, the failure to block nitrergic transmission was surprising, in view of its K_i of 0.12 μM for rat nNOS. Whether the failure of AAAN to block nitrergic transmission in the bovine ciliary artery results from poor tissue penetration, a major difference in K_i values for bovine and rat nNOS, or some other factor, remains to be established. Equally disappointing were the actions of L-NPA (Zhang *et al*., 1997a, b) on the bovine ciliary artery. Although this agent did inhibit nitrergic vasodilatation, it failed to exhibit the ~150-fold selectivity for nNOS over eNOS seen in enzyme assays (Zhang *et al*., 1997b). Indeed, L-NPA blocked endothelium-dependent, NO-mediated vasodilatation over the same concentration range as for blockade of nitrergic transmission. Our findings with AAAN and L-NPA therefore serve as a reminder that it is not always possible to extrapolate findings in biochemical assays to functional responses in intact tissues.

In conclusion, in the bovine ciliary artery, L-NMMA acts as a selective blocker of endothelium-dependent, NO-mediated vasodilatation, but has no inhibitory effect on dilatation produced by nitrergic nerves. In fact, L-NMMA behaves similarly to L-arginine in protecting nitrergic vasodilatation against blockade by L-NAME. No agent was identified that selectively inhibits vasodilatation mediated by the nitrergic nerves. Of the two putative nNOS inhibitors tested, AAAN failed to inhibit vasodilatation induced either by nitrergic nerves or endothelium-derived NO, and L-NPA inhibited both, but with no evidence of selectivity.

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

References

Archer SL, Hampl V (1992). N^G-monomethyl-L-arginine causes nitric oxide synthesis in isolated arterial rings: trouble in paradise. *Biochem Biophys Res Commun* **188**: 590–596.

Frew JD, Paisley K, Martin W (1993). Selective inhibition of basal but not agonist-stimulated activity of nitric oxide in rat aorta by N^G-monomethyl-L-arginine. *Br J Pharmacol* **110**: 1003–1008.

Fukuto JM, Chaudhuri G (1995). Inhibition of constitutive and inducible nitric-oxide synthase: potential selective inhibition. *Annu Rev Pharmacol Toxicol* **35**: 165–194.

Hah JM, Roman LJ, Martasek P, Silverman RB (2001). Reduced amide bond peptidomimetics. (4S)-*N*-(4-amino-5-[aminoalkyl] aminopentyl)-*N*′-nitroguanidines, potent and highly selective inhibitors of neuronal nitric oxide synthase. *J Med Chem* **44**: 2667–2670.

Hibbs JB, Taintor RR, Vavrin Z (1987). Macrophage cytotoxicity–role for L-arginine deiminase and imino-nitrogen oxidation to nitrite. *Science* **235**: 473–476.

Hobbs AJ, Higgs A, Moncada S (1999). Inhibition of nitric oxide synthase as a potential therapeutic target. *Annu Rev Pharmacol Toxicol* **39**: 191–220.

Koss MC (1999). Functional role of nitric oxide in regulation of ocular blood flow. *Eur J Pharmacol* **374**: 161–174.

Li HY, Poulos TL (2005). Structure–function studies on nitric oxide synthases. *J Inorganic Biochem* **99**: 293–305.

Liu X, Gillespie JS, Gibson IF, Martin W (1991). Effects of N^G-substituted analogues of L-arginine on NANC relaxation of the rat anococcygeus and bovine retractor penis muscles and the bovine penile artery. *Br J Pharmacol* **104**: 53–58.

Martin W, Gillespie JS, Gibson IF (1993). Actions and interactions of N^G-substituted analogues of L-arginine on NANC neurotransmission in the bovine retractor penis and rat anococcygeus muscles. *Br J Pharmacol* **108**: 242–247.

McNeish AJ, Nelli S, Wilson WS, Dowell FJ, Martin W (2003). Differential effects of ascorbate on endothelium-derived hyperpolarizing factor (EDHF)-mediated vasodilatation in the bovine ciliary vascular bed and coronary artery. *Br J Pharmacol* **138**: 1172–1180.

Moore PK, al-Swayeh OA, Chong NWS, Evans RA, Gibson A (1990). -N^G-nitro-arginine (L-NOARG), a novel, L-arginine-reversible inhibitor of endothelium-dependent relaxation *in vitro*. *Br J Pharmacol* **99**: 408–412.

Moore WM, Keith Webber R, Fok KF, Jerome GM, Kornmeier CM, Tjoeng FS *et al*. (1996). Inhibitors of human nitric oxide synthase isoforms with the carbamidine moiety as a common structural element. *Bioorg Med Chem* **4**: 1559–1564.

Mügge A, Antonio J, Lopez G, Piegors DJ, Breese KR, Heistad DD (1991). Acetylcholine-induced vasodilatation in rabbit hindlimb *in vivo* is not inhibited by analogues of arginine. *Am J Physiol* **260**: H242–H247.

Olken NM, Marletta MA (1993). N^G-monomethyl-L-arginine functions as an alternative substrate and mechanism-based inhibitor of nitric oxide synthase. *Biochemistry* **32**: 9677–9685.

Overend J, Wilson WS, Martin W (2005). Biphasic neurogenic vasodilatation in the bovine intraocular long posterior ciliary artery: involvement of nitric oxide and an additional unidentified neurotransmitter. *Br J Pharmacol* **145**: 1001–1008.

Rees DD, Palmer RMJ, Hodson HF, Moncada S (1989). A specific inhibitor of nitric oxide formation form L-arginine attenuates endothelium-dependent relaxations. *Br J Pharmacol* **96**: 418–424.

Rees DD, Palmer RMJ, Schulz R, Hodson HF, Moncada S (1990). Characterisation of three inhibitors of endothelial nitric oxide synthase *in vitro* and *in vivo*. *Br J Pharmacol* **101**: 746–752.

Tare M, Parkington HC, Coleman HA (2000). EDHF, NO and a prostanoid: hyperpolarization-dependent and -independent relaxation in guinea pig arteries. *Br J Pharmacol* **130**: 605–618.

Toda N, Okamura T (2003). The pharmacology of nitric oxide in the peripheral nervous system of blood vessels. *Pharmacol Rev* **55**: 271–324.

Waldron GJ, Garland CJ (1994). Effect of potassium channel blockers on L-NAME insensitive relaxations in rat small mesenteric artery. *Can J Physiol Pharmacol* **72**: 11.

Wiencke AK, Nilsson H, Nielsen PJ, Nyborg NCB (1994). Nonadrenergic noncholinergic vasodilatation in bovine ciliary artery involves CGRP and neurogenic nitric oxide. *Invest Opthalmol Vis Sci* **35**: 3268–3277.

Zhang HQ, Dixon RP, Marletta MA, Nikolic D, Van Breemen R, Silverman RB (1997a). Mechanism of inactivation of neuronal nitric oxide synthase by N^{ω}-allyl-L-arginine. *J Am Chem Soc* **119**: 10888–10902.

Zhang HQ, Fast W, Marletta MA, Martasek P, Silverman RB (1997b). Potent and selective inhibition of neuronal nitric oxide synthase by N^{ω}-propyl-L-arginine. *J Med Chem* **40**: 3869–3870.

Zygmunt PM, Högestätt ED (1996). Role of potassium channels in endothelium-dependent relaxation resistant to nitroarginine in the rat hepatic artery. *Br J Pharmacol* **117**: 1600–1606.