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Endothelium-dependent sensory NANC vasodilatation: involvement of ATP, CGRP and a possible NO store

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1 Non-adrenergic non-cholinergic (NANC) vasodilator nerves regulate tone in certain vascular beds. We have investigated the mechanisms of the NANC dilator response in the isolated small mesenteric artery of the rabbit by use of the tension myograph.

2 Small second or third order (150–300 μm in diameter) arteries of the rabbit mesenteric bed were mounted in a Mulvany tension myograph. Responses to electrical field stimulation (EFS) and exogenous vasodilators were investigated.

3 EFS (0.5–16 Hz, 10 V, 0.3 ms for 5 s), in the presence of guanethidine (5 μM) and atropine (1 μM) produced frequency-dependent relaxation of small arteries. Pretreatment with tetrodotoxin (1 μM) abolished the relaxation and desensitization with capsaicin (10 μM) strongly inhibited the relaxation.

4 Pretreatment with a P2Y-purinoceptor antagonist, basilen blue (3 μM) or a human calcitonin gene-related peptide (hCGRP) receptor antagonist, $hCGRP_{8-37}$ (1 μM) suppressed the NANC relaxation by approximately 40–60 % in each case and combined pretreatment almost abolished the relaxation.

5 The EFS-induced relaxation was suppressed by endothelium-removal, pretreatment with the soluble guanylyl cyclase inhibitor ODQ (1 μM) and the NO scavenger oxyhaemoglobin (OxyHb; 20 μM) but not by NO synthase inhibitors N^G-nitro-L-arginine methyl ester (L-NAME; 300 μM) or N^G-nitro-L-arginine (L-NOARG; 300 μM). Combined pretreatment with ODQ and $CGRP_{8-37}$ almost abolished the relaxation.

6 A P2Y-purinoceptor agonist, 2-methylthio ATP, produced endothelium-dependent relaxation which was inhibited by L-NAME and ODQ (1 μM), whilst hCGRP produced endothelium-independent and ODQ-insensitive relaxation.

7 Ultraviolet light (320 nm, 5 shots over 20 s) produced relaxation that was blocked by both OxyHb and ODQ but not by N^G-monomethyl-L-arginine (L-NMMA, 300 μM).

8 The present study suggests that EFS-induced NANC relaxation of the mesenteric small artery of the rabbit is mediated mainly by capsaicin-sensitive sensory C-fibres and that both ATP and CGRP are involved. The action of ATP released by EFS appears to be endothelium-dependent and involve activation of soluble guanylyl cyclase, but is resistant to inhibitors of NO synthase. The response to CGRP is endothelium-independent. These results show that ATP and CGRP account fully for the NANC relaxation of this vessel type and that the endothelium is involved in NANC-induced relaxation. The endothelium-dependent part of the response is consistent with the release of NO, either from NO synthase, incompletely inhibited by the NO synthase inhibitors, or by some preformed stores.

Keywords: Capsaicin; nitric oxide; soluble guanylyl cyclase; ATP; calcitonin gene-related peptide (CGRP)

Introduction

Neuronal control of vascular tone is provided by reflex responses of the central nervous system and by local peripheral neuronal activation. It is well established that the sympathetic and, to a lesser extent, the parasympathetic nervous systems regulate vascular tone but the vasculature is also innervated by non-adrenergic non-cholinergic (NANC) nerves. Dilator NANC nerves in the vasculature are thought to oppose the constrictor sympathetic nervous system. In accordance with such a hypothesis, studies have shown that NANC nerves modulate responses to sympathetic stimulation *in vitro* (Ahluwalia & Vallance, 1996) and *in vivo*. Putative NANC mediators include the peptides calcitonin gene-related peptide (CGRP) and substance P (SP), and non-peptide mediators including nitric oxide (NO) and adenosine 5′-triphosphate (ATP) (Lundberg, 1996). These mediators, either singly or as co-transmitters, contribute to NANC dilatation in a range of vascular beds.

In 1988, Kawasaki and co-workers were the first to demonstrate that CGRP is a mediator of electrical field stimulation (EFS)-induced NANC relaxation (Kawasaki *et al*., 1988; 1990). Since this initial observation CGRP has been identified as a NANC transmitter in many vascular beds (see Bell & McDermott, 1996). However, the cerebral vasculature possesses dense nitrergic innervation, i.e. nerves that release NO on stimulation (Toda, 1995), and in the rabbit portal vein NO acts as a co-transmitter with ATP to produce neurogenic NANC vasodilatation (Brizzolara *et al*., 1993). Thus the mediators released by NANC nerves may differ between species and between beds.

The nerve type(s) mediating NANC responses have not been clearly identified. Sensory C-fibres release peptide and non-peptide mediators peripherally and these nerves may contribute to NANC responses (for review Lundberg, 1996). Capsaicin is a selective neurotoxin for sensory C-fibres (Kawasaki *et al*., 1988) and in the rat mesenteric bed NANC-induced relaxation is blocked in tissues pretreated with caspaicin. Capsaicin-sensitive sensory C-fibres (CSPANs) have been implicated as the nerves mediating NANC

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vasorelaxation in pig coronary arteries (Franco-Cereceda & Rudehill, 1989), rat gastric submucosal arterioles (Chen & Guth, 1995) and bovine intraocular anterior ciliary arteries (Wiencke *et al*., 1994). In this study we explored the NANC responses in isolated mesenteric small arteries of the rabbit, determined whether CSPANs contribute to these responses and identified the transmitters involved.

Methods

Male New Zealand white rabbits (3–3.5 kg) were killed by injecting a lethal dose (50 mg kg^{-1}) of sodium pentobarbitone. The jejunal portion of the mesentery was removed and placed in Krebs solution of the following composition (mM): NaCl 118, KC1 4.69, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.18, $NaHCO_3$ 25, glucose 11 and disodium EDTA 0.027. Third order jejunal branches of the superior mesenteric artery were carefully dissected. Ring segments (3 mm in length; 150–300 μm in internal diameter) were mounted horizontally between 2 stainless steel wires (40 μm in diameter) in an automated tension myograph (JP Trading, Aarhus, Denmark) as described previously (Mulvany & Halpern, 1977). Vessels were maintained at 37°C in Krebs solution bubbled with 5% CO_2 and 95 % O_2 (pH 7.4). After an equilibration period of 45 min, vessels were stretched in a stepwise manner to determine the relationship between the passive tension and internal circumference according to Laplace's equation. From this relationship, the internal diameter was determined. Vessels were then stretched to 90% of the diameter achieved when under a transmural pressure of 100 mmHg. In some experiments, the endothelium was removed by passing a human hair through the lumen of the vessel.

Following normalization, each vessel was contracted repeatedly with phenylephrine (PE EC_{max}, 10–30 μM) until the response was constant. The integrity of the endothelium was assessed by measuring the response to acetylcholine (1 μM) in vessels submaximally precontracted (approximate EC_{75}) with PE (3–10 μM). The endothelium was considered intact if acetylcholine produced greater than 70% relaxation of PE-contracted vessels and denuded if less than 10% relaxation was produced.

Electrical field stimulation

All frequency-dependent responses to EFS (0.5–16 Hz, 10 V, 0.3 ms for 5 s) were investigated in vessels pretreated with guanethidine (5 μM) and atropine (1 μM) for 30 min (to block sympathetic and cholinergic neurotransmission, respectively) and then submaximally precontracted with PE (3–10 μM). To confirm that the responses to EFS were neuronal in origin, some preparations were pretreated with tetrodotoxin (1 μM). To determine whether the EFS responses were sensory in origin, sensory neurones were desensitized by pretreatment with capsaicin (10 μM) for 20 min, followed by 30 min washing.

To investigate the involvement of endogenously-released dilators in the responses to EFS, frequency-response curves were constructed in the presence and absence of the following antagonists and enzyme inhibitors: (i) to determine whether P2Y-receptors are involved, vessels were pretreated for 15 min with the P2Y-receptor antagonist basilen blue (3 μM) (Olsson & Pearson, 1990). (ii) To determine whether the sensory neuropeptides CGRP or SP are involved, vessels were pretreated for 15 min with human calcitonin gene-related peptide$_{8\text{-}37}$ (hCGRP$_{8\text{-}37}$, 1 μM) or (3a**R**, 7a**R**)-7, 7-diphenyl-2-[1-immuno-2-(2-methoxyphenyl)ethyl] po-hydroisoindol-4-one (RP 67,580, 1 μM) to block CGRP and NK_1 receptors, respectively. (iii) To determine whether the NO-guanylyl cyclase pathway is involved, vessels were pretreated for 30 min with N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) or N^G-nitro-L-arginine (L-NOARG, 300 μM), or 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (ODQ, 1 μM) to inhibit NO synthase and soluble guanylyl cyclase, respectively (Garthwaite *et al*., 1995; Moro *et al*., 1996). The effect of oxyhaemoglobin (OxyHb), an NO scavenger (Martin *et al*., 1986) was also investigated. Vessels were pretreated with OxyHb (10 μM) for 30 min before the application of PE and then again at the time of PE application to give a total concentration of 20 μM. (iv) To determine whether prostanoid generation is involved, vessels were pretreated with the cyclo-oxygenase inhibitor indomethacin (5 μM) for 30 min.

The frequency-response curves in drug treated vessels were compared to those produced in control untreated vessels dissected from the same segment of blood vessel. Responses to EFS in endothelium-denuded vessels were compared to the responses in paired endothelium-intact vessels. For these experiments the concentration of PE used for the precontraction was titrated to give an equivalent level of precontraction in all experiments, which was 75% of the maximum response to PE obtained before drug application.

Exogenous vasodilators

Responses to exogenous vasodilators were assessed in vessels precontracted with PE (3–10 μM). Relaxation concentration-response curves to 2-methylthio ATP (0.01–100 nM), a P2Y-receptor selective agonist, were constructed in the presence or absence of basilen blue (3 μM). The effects of endothelial removal or pretreatment with either L-NAME (300 μM) or ODQ (1 μM) on the concentration-response curve to 2-methylthio ATP were also determined. Since repeated application of 2-methylthio ATP resulted in rapid desensitization of responses, it was not possible to construct repeat concentration-response curves in each tissue. The concentration-response curves in endothelial denuded preparations or following drug treatment were compared to those responses achieved in intact or control untreated preparations taken from the same vessel.

Relaxation concentration-response curves to hCGRP (0.01–100 nM) were constructed in the absence or presence of hCGRP$_{8\text{-}37}$ (1 μM) or ODQ (1 μM). The effect of removal of the endothelium on hCGRP-induced relaxation was also determined.

Photorelaxation

Following equilibration, vessels were precontracted with a submaximally effective concentration of PE. Once tone was stable, vessels were subjected to u.v. light: 5 brief exposures over 20 s at a wavelength of 320 nm (Hi-Tech Scientific u.v.-light source). This was repeated at 15 min intervals. To explore the role of the NO-guanylyl cyclase pathway in u.v. light-induced relaxation, vessels were pretreated with N^G-monomethyl-L-arginine (L-NMMA, 300 μM), OxyHb (20 μM) or ODQ (1 μM). L-NAME or L-NOARG were not used in this study since these compounds possess a nitro group in their structures which on exposure to u.v. light releases NO (Bauer & Fung, 1994).

Drugs

CGRP and hCGRP$_{8-37}$ (Bachem), ODQ (1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one; synthesized in The Cruciform

Project) and sodium salt of 2-methylthio ATP (ICN Biomedicals Inc.). RP 67580 ((3a**R**, 7a**R**)-7, 7-diphenyl-2-[1-immuno-2-(2-methoxyphenyl)ethyl] po-hydroisoindol-4-one) was a generous gift from Dr C. Garret (Rhone Poulenc, Vitry, France). All other drugs used were purchased from Sigma (Poole, U.K.).

Analysis of data

In all experiments, relaxation responses are expressed as a percentage decrease of maximal relaxation. Data are expressed as mean ± s.e.mean. Data were compared by unpaired *t* test or analysis of variance followed by Fisher's LSD. $P<0.05$ was considered significant.

Results

The mean diameter of the vessels used in this study was 206.4 ± 6.2 μm ($n=168$).

EFS-induced NANC relaxation

In vessels contracted with PE and pretreated with guanethidine (5 μM) and atropine (1 μM), EFS (0.5–16 Hz) produced frequency-dependent relaxation (Figure 1a). This relaxation was abolished by tetrodotoxin (1 μM; $n=4$, $P<0.01$; Figure 1b) and strongly inhibited by desensitization with capsaicin (10 μM; $n=6$, $P<0.01$; Figure 1c), which produced 75–90 % inhibition of the response at each frequency (Figure 1d).

Role of ATP, CGRP and substance P in EFS-induced relaxation

Pretreatment with basilen blue (3 μM) or $hCGRP_{8\text{-}37}$ (1 μM) significantly attenuated the EFS-induced relaxation ($n=6-8$, $P<0.05$ and $P<0.01$, respectively, see Figure 2). In contrast, the SP antagonist, RP 67,580 (1 μM), at a concentration which shifted the concentration-response curve to exogenous SP (1–1000 nM) to the right 74 fold (SP $EC_{50}=1.9\pm0.8$ nM, SP + RP 67,580 $EC_{50}=139.7+88.5$ nM), had no significant effect on EFS-induced relaxation ($n=4$, data not shown). Combined pretreatment with basilen blue (3 μM) and $hCGRP_{8\text{-}37}$ (1 μM) produced greater inhibition than when either agent was given alone ($P<0.05$; Figure 2). The combination completely abolished the relaxation in 3 of 5 vessels studied.

Role of the endothelium and NO in EFS-induced relaxation

Removal of the endothelium attenuated the EFS-induced relaxation ($n=7$, $P<0.05$; Figure 3a). Similarly OxyHb (20 μM; $n=6$, $P<0.05$; Figure 3b) or ODQ (1 μM; $n=6$, $P<0.05$; Figure 3c) attenuated the EFS relaxation. Combined treatment with ODQ (1 μM) and $CGRP_{8-37}$ (1 μM) almost abolished EFS-induced relaxation ($n=8$, $P<0.01$; Figure 3c). In contrast, pretreatment with either L-NAME (300 μM; $n=9$; Figure 3a), or L-NOARG (300 μM; $n=4$; data not shown) had no significant effects. Indomethacin (5 μM, $n=3$) also had no significant effect on the response to EFS (data not shown).

Mechanisms of 2-methylthio ATP-induced relaxation

2-Methylthio ATP (0.01–100 nM) caused concentration-dependent relaxation ($EC_{50}=0.62\pm0.18$ nM) of precontracted vessels. Basilen blue (3 μM) shifted the concentration-response curve to 2-methylthio ATP (0.01–100 nM) to the right ($P<0.01$, $EC_{50}=8.3\pm4.0$ nM; Figure 4a). L-NAME (300 μM), ODQ (1 μM) or removal of the endothelium ($P<0.01$ and $P<0.05$) also caused significant rightward shift of the relaxation concentration-response curve to 2-methylthio ATP (EC_{50} values of 3.1 ± 0.8 nM, 2.2 ± 0.3 nM and 1.4 ± 0.2 nM, respectively; Figure 4b).

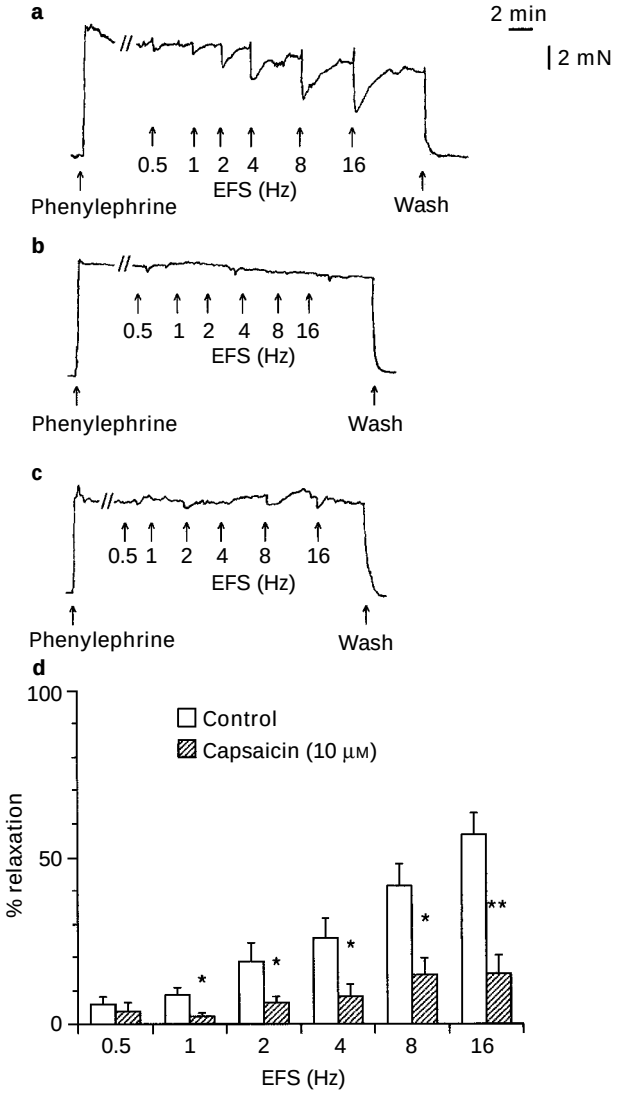

Figure 1 NANC relaxation responses to electrical field stimulation (EFS; 10 V, 0.3 ms duration, 0.5–16 Hz for 5 s). In all cases vessels were pretreated with guanethidine (5 μM) and atropine (1 μM) and precontracted with phenylephrine (6–10 μM). Typical frequency-dependent relaxation of rabbit mesenteric small arteries are shown in (a) control vessel, (b) in a vessel pretreated with tetrodotoxin (1 μM) and (c) in a vessel pretreated with capsaicin (10 μM). The effect of pretreatment with capsaicin (10 μM, $n=6$) on the responses to EFS ($n=5$) are shown in (d). Values shown are mean with s.e.mean shown by vertical lines; $*P<0.05$ and $**P<0.01$.

Mechanisms of CGRP-induced relaxation

$hCGRP_{8-37}$ (1 μM) shifted the concentration-response curve to hCGRP (0.01–100 nM) by approximately 10 fold without suppressing the maximum response ($n=4$, data not shown). hCGRP-induced relaxation ($EC_{50}=2.9\pm0.9$ nM) was unaffected by removal of the endothelium or pretreatment with ODQ (1 μM) (EC_{50} values of 3.4 ± 1.1 nM and 2.5 ± 0.6 nM, respectively; Figure 5).

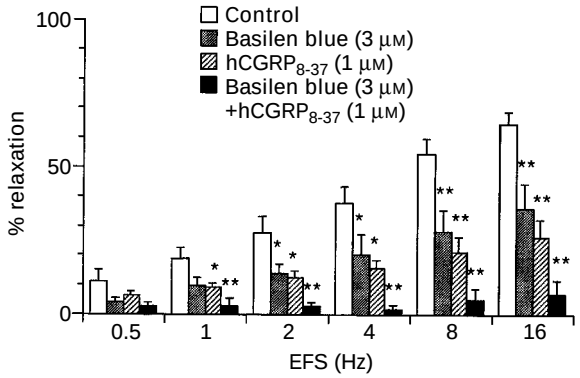

Figure 2 Effects of CGRP receptor and P2Y-receptor blockade on electrical field stimulation (EFS)-induced relaxation of rabbit mesenteric small arteries. Responses to EFS (10 V, 0.3 ms duration, 0.5–16 Hz for 5 s) in control vessels ($n=13$), in vessels pretreated with the P2Y-receptor antagonist, basilen blue (3 μM, $n=6$), in vessels pretreated with human calcitonin gene-related peptide (hCGRP) receptor antagonist, $hCGRP_{8-37}$ (1 μM, $n=8$) and in vessels pretreated with both $hCGRP_{8-37}$ and basilen blue ($n=5$). Values shown are mean $\pm$ s.e.mean. $^*P<0.05$ and $^{**}P<0.01$.

Photorelaxation

Brief 20 s exposure of precontracted vessels to u.v. light caused transient relaxation as shown in Figure 6 (23.3 $\pm$ 3.8 %, n = 8). Responses to u.v. light were completely abolished by pretreatment with either OxyHb ($n=6$, $P<0.001$) or ODQ ($n=6$, $P<0.001$), but were unaffected by pretreatment with L-NMMA ($n=7$).

Discussion

Mechanisms of EFS-induced relaxation were investigated in isolated small resistance arteries of the rabbit mesenteric bed. Our results show that the EFS-induced NANC relaxant response in these vessels is due to activation of capsaicin-sensitive fibres and mediated by CGRP and ATP. The response is partially endothelium-dependent and involves activation of guanylyl cyclase, but is not attenuated by the substrate-based NO synthase inhibitors L-NAME and L-NOARG.

EFS of vessels pretreated with guanethidine and atropine caused frequency-dependent relaxation that was blocked by the Na^+ channel blocker, tetrodotoxin, confirming that the relaxant response was neuronally-mediated. Pretreatment with capsaicin, a neurotoxin that shows selectivity for sensory C-fibres, significantly inhibited the NANC relaxation by approximately 80%, suggesting that the EFS-induced NANC relaxation was mediated predominantly by CSPANs. CSPANs are a heterogeneous population and the content of their nerve-endings varies between vascular beds (Maggi, 1995). Blockade of CGRP receptors with the CGRP receptor antagonist $hCGRP_{8-37}$ showed that the response to EFS was in part mediated by the release of CGRP. This is in agreement with other studies demonstrating that CGRP is an important NANC vasodilator in the mesenteric vascular bed (Kawasaki *et al.*, 1988; 1990). However, the NK_1 receptor blocker RP 67,580 (1 μM), at a concentration shown to block dilator responses to exogenous SP, had no effect on the EFS responses, indicating that SP is not involved in the dilator component of the NANC response.

Recently it has become clear that other non-peptide substances may be released from sensory C-fibres, including

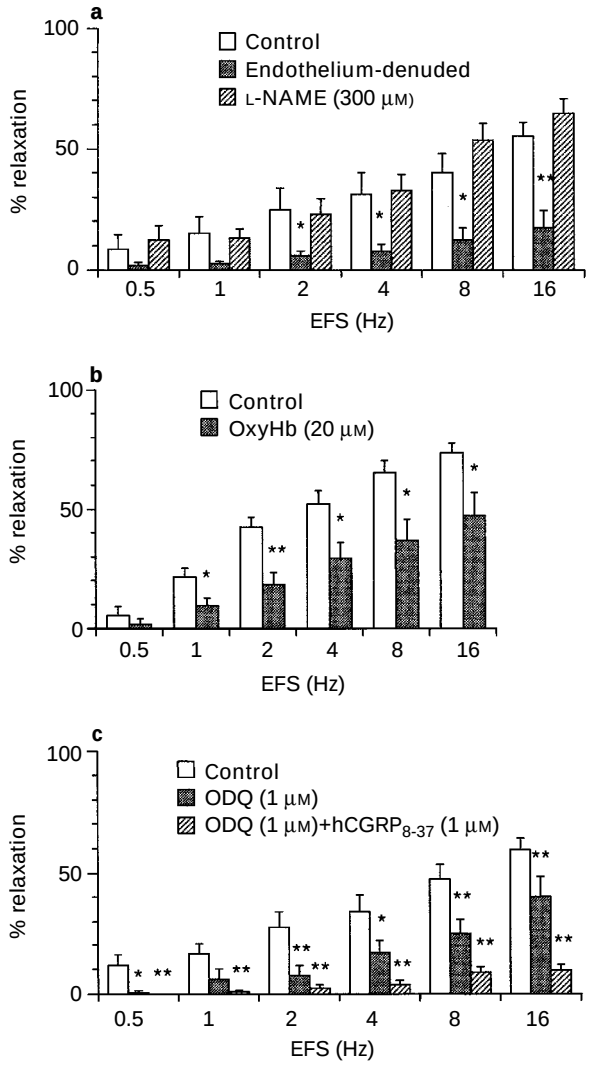

Figure 3 Effect of modulation of the NO-guanylyl cyclase pathway on electrical field stimulation (EFS)-induced relaxation of the rabbit mesenteric small arteries. Responses to EFS (10 V, 0.3. ms duration, 0.5–16 Hz for 5 s) in (a) control vessels ($n=7$), vessels denuded of endothelium ($n=7$) and in endothelium-intact vessels pretreated with the NO synthase inhibitor L-NAME (300 μM, $n=9$), (b) control vessels ($n=9$), vessels pretreated with OxyHb (20 μM, $n=6$) and (c) control vessels ($n=9$), vessels pretreated with ODQ (1 μM, $n=6$) and vessels pretreated with both ODQ (1 μM) and $hCGRP_{8-37}$ (1 μM, $n=8$). Values shown are mean $\pm$ s.e.mean. $^*P<0.05$ and $^{**}P<0.01$.

ATP and NO (for review see Ahluwalia & Cellek, 1997). ATP acts as a vasoconstrictor via interaction with P2X-receptors located on the vascular smooth muscle (Chapal & Loubatieres-Mariani, 1983; Kennedy & Burnstock, 1985; Kennedy *et al.*, 1985) and causes vascular relaxation by activation of P2Y-receptors located on the endothelium (Demay & Vanhoutte, 1981). In the present study the selective P2Y-receptor agonist, 2-methylthio ATP, caused concentration-dependent relaxation of precontracted mesenteric arteries that was shifted to the right by the selective P2Y-receptor antagonist, basilen blue (Chaudhry *et al.*, 1993). Pretreatment with basilen blue also significantly attenuated the relaxation responses to EFS by approximately 40%, indicating that EFS-induced relaxation is, at least in part, mediated by P2Y receptor activation. The combination of $CGRP_{8-37}$ with basilen blue produced a substantially greater inhibitory effect than when either antagonist was given alone. Indeed, in 3 out of 5 experiments the response to EFS was abolished by this combined

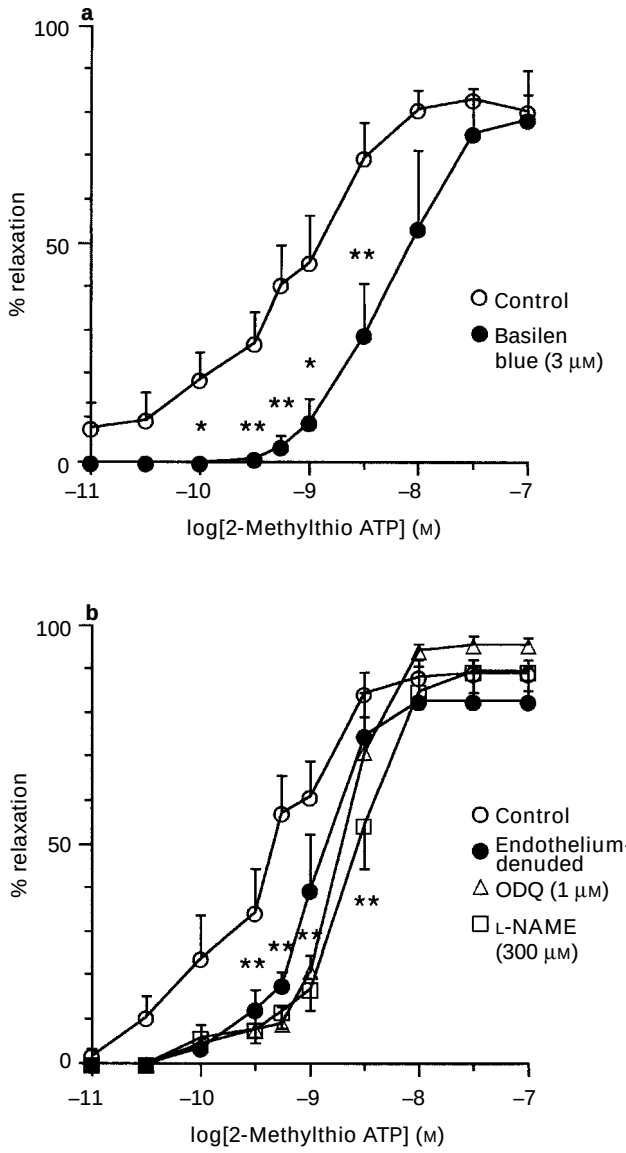

Figure 4 The mechanisms of 2-methylthio ATP-induced relaxation of rabbit mesenteric small arteries. (a) Relaxation concentration-response curves to the selective P2Y-receptor agonist 2-methylthio ATP in the absence ($n=6$) and presence of the P2Y-receptor antagonist, basilen blue (3 μM, $n=6$), (b) Relaxation concentration-response curves to 2-methylthio ATP in control vessels ($n=9$), endothelium-denuded vessels ($n=5$) and endothelium-intact vessels treated with, ODQ (1 μM, $n=4$) or L-NAME (300 μM, $n=5$). Values shown are mean$\pm$s.e.mean (vertical lines).*$P<0.05$ and **$P<0.01$.

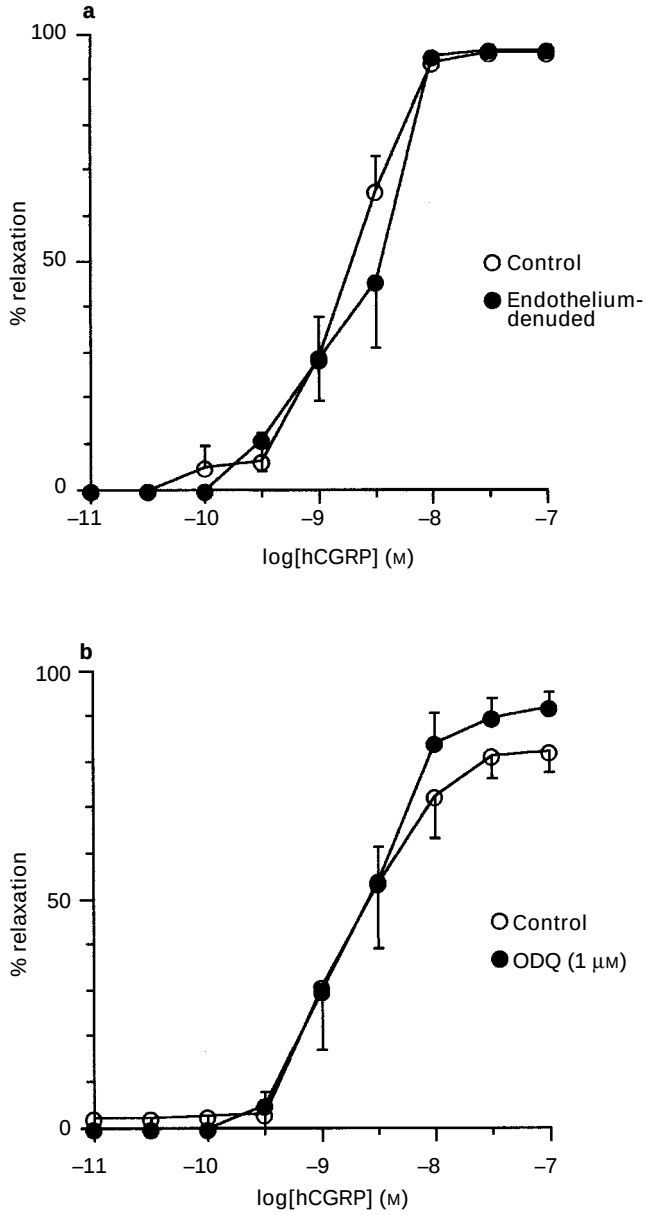

Figure 5 Mechanisms of CGRP-induced relaxation of rabbit mesenteric small arteries. (a) Relaxation concentration-response curves to hCGRP in endothelium intact ($n=5$) and endothelium denuded ($n=5$) vessels. (b) Concentration-response curves to hCGRP in control vessels ($n=6$) and vessels pretreated with ODQ (1 μM, $n=4$). All values are means$\pm$s.e.means (vertical lines).

antagonist pretreatment. Together these results suggest that both CGRP and ATP mediate the NANC relaxation of rabbit mesenteric small arteries.

The endothelium appears to play an important role in the NANC relaxant response since removal of the endothelium resulted in approximately 50% inhibition of EFS-induced relaxation. Prostanoid generation was not involved in this endothelium-dependent response since indomethacin had no effect on the EFS response. In contrast EFS-induced relaxation was attenuated by pretreatment with the NO scavenger, OxyHb, and by the specific inhibitor of guanylyl cyclase, ODQ. These results are consistent with EFS stimulating the release of an NO-like substance from the endothelium. Since the combination of basilen blue and $CGRP_{8-37}$ produced almost complete blockade of NANC relaxation, it is likely that NO acts as a second messenger of either the ATP or CGRP component of the response. The present studies with exogenously applied CGRP and the ATP analogue, 2-methylthio ATP, showed that NO is involved in the mediation of responses following P2Y-receptor activation and not CGRP receptor activation. The response to low concentrations of 2-methylthio ATP is endothelium-dependent and attenuated by ODQ whereas the response to CGRP was independent of the endothelium and unchanged in the presence of ODQ. Combination of ODQ and $CGRP_{8-37}$ produced almost complete block of EFS-induced relaxation, an effect similar to that achieved by blockade of both CGRP and ATP receptors. The simplest explanation of these findings is that ATP released by EFS mediates relaxation through activation of P2Y-receptors located on the endothelium, resulting in the release of NO.

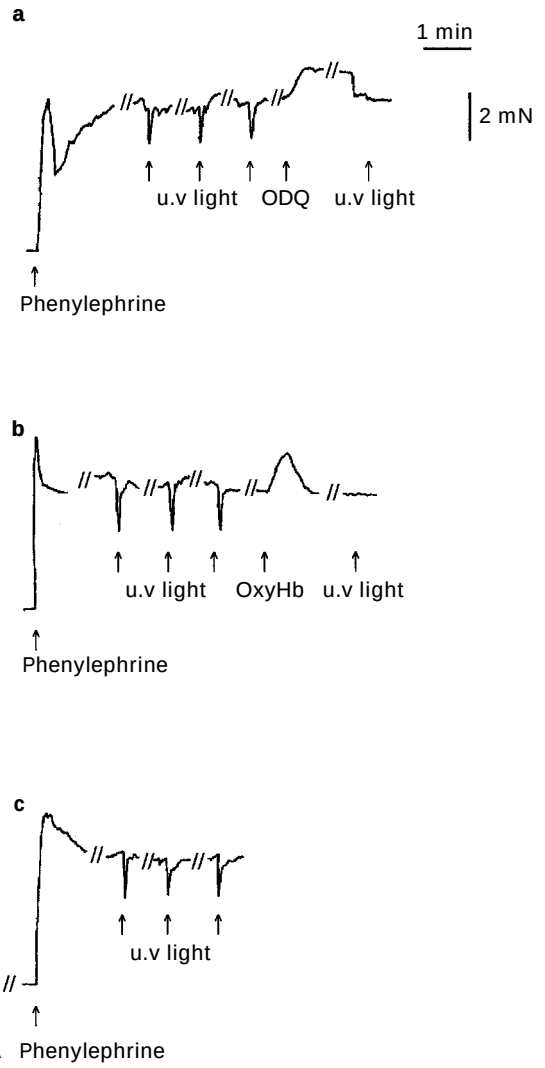

Figure 6 Typical relaxation responses to brief exposure (5 brief exposures over 20 s of u.v. light at a wavelength of 320 nm). Pretreatment with ODQ (1 μM) or the NO scavenger OxyHb (20 μM) abolished the response.

Despite the apparent involvement of endothelium-derived NO, EFS-induced relaxation was not attenuated by the NO synthase inhibitors L-NAME and L-NOARG, at concentrations known to inhibit endothelium-dependent relaxation (Rees *et al*., 1990). These results are consistent with previous findings indicating a failure of these NO synthase inhibitors, in certain instances, to suppress relaxation that appears to be mediated by NO (Kemp & Cocks, 1997). One possible explanation is that the NO synthase inhibitors cannot fully inhibit NO generation, allowing some residual NO production. Alternatively, it has been suggested that in some situations NO, attached to a carrier, may be stored and released. The most striking example of this is in the salivary glands of the *Rhodnius Prolixis* where NO is stored bound to a haem-protein containing ferric iron (Ribeiro *et al*., 1993; Champagne *et al*., 1995). Stores of NO have also been postulated to be present in both the endothelium and smooth muscle (see Ignarro, 1990; Venturini *et al*., 1993). In order to explore the possibility that stores of NO exist in rabbit mesenteric small arteries, we used u.v. light to induce relaxation. It is thought that u.v. light causes the release of NO from stores (Furchgott, 1955; Furchgott *et al*., 1985; Chaudhry *et al*., 1993) and thereby causes relaxation. u.v. light elicited relaxation of mesenteric arteries that was blocked by ODQ and OxyHb and not altered by L-NMMA. This profile is similar to that produced by EFS and our results are consistent with the possibility that EFS might induce the release of a preformed store of NO. Further experiments will be required to test this hypothesis directly and to explore the chemical nature of any store that might exist. It is unclear why the NO synthase inhibitors effectively attenuated the response to exogenous 2-methylthio ATP, but were ineffective at blocking the response to the presumed release of ATP in response to EFS.

In conclusion, the present study suggests that EFS-induced NANC relaxation of the mesenteric small artery of the rabbit involves activation of CSPANs and that release of ATP and CGRP account for the responses seen. It is not known whether these mediators are co-released or whether the activation of CSPANs stimulates the release of ATP from other nerves or indeed from the endothelium itself. However, there is evidence to suggest that ATP may be released directly from capsaicin-sensitive nerves (Holton, 1959; Sweeney *et al*., 1989). Our study clearly shows that EFS induces endothelium-dependent and endothelium-independent relaxation; the former is due to ATP and stimulation of NO/cyclicGMP and the latter is mediated by CGRP.

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