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Innervation and nitric oxide modulation of mesenteric arteries of the Golden hamster

Bethan Hill, Vera Ralevic *, Rahima Crowe, Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

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Abstract

Immunohistochemical and pharmacological techniques were used to examine perivascular nerves, endothelium and the effects of inhibition of nitric oxide synthesis on responses in mesenteric arteries/perfused mesenteric arterial beds of the Golden hamster. Frequency-dependent vasoconstrictions to electrical field stimulation and dose-dependent vasoconstrictions to noradrenaline were significantly augmented by N^G-nitro-L-arginine methyl ester (10^{-5} M), an inhibitor of nitric oxide synthase. In preparations with tone raised with methoxamine (10 μM) dose-dependent relaxations to ATP, but not to acetylcholine, were blocked by N^G-nitro-L-arginine methyl ester. In the presence of guanethidine (5 μM) to block sympathetic neurotransmission there was no neurogenic relaxation to electrical field stimulation. Furthermore, the sensory neurotoxin capsaicin (0.05–5 nmol) did not elicit relaxation. Immunohistochemical studies demonstrated dense plexuses of fibres immunoreactive for tyrosine hydroxylase and neuropeptide Y, a plexus of moderate density for calcitonin gene-related peptide and an absence of fibres immunoreactive for substance P and vasoactive intestinal polypeptide. Of particular interest is the finding that whereas sympathetic perivascular nerves and nitric oxide regulate the function of hamster mesenteric arteries, there is no apparent motor function of calcitonin gene-related peptide-containing sensory nerves.

Keywords: Endothelium; (Golden hamster); Mesenteric arterial bed; Nitric oxide (NO); Perivascular nerve; Sensory nerve

1. Introduction

Sympathetic perivascular nerves comprise the most significant contribution to the control of vasoconstrictor tone; the most common combination of neurotransmitters found in these nerves is noradrenaline, adenosine 5′-triphosphate (ATP) and neuropeptide Y (Burnstock, 1990). Relatively few vessels receive innervation from the parasympathetic division of the autonomic nervous system; alongside the classical parasympathetic transmitter acetylcholine many neurones have been found to contain and release vasoactive intestinal polypeptide. Primary sensory neurones have been shown to possess dual function: in addition to their afferent capacity whereby they relay information from the periphery to the central nervous system, these nerves may also have an efferent (motor) function on the target tissues which they innervate and as a consequence have been termed sensory-motor nerves (Burnstock, 1985). Substance P and calcitonin gene-related peptide are contained within and released from sensory nerves and thus are regarded as sensory neurotransmitters. Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), an extract of chilli peppers, causes selective degeneration of Aδ and C fibres in neonates and in the adult animal effects depletion of the neurotransmitter content of sensory axons and terminals. Thus, capsaicin has been used extensively as a marker for sensory nerves (Nagy, 1982; Fitzgerald, 1983; Maggi and Meli, 1988).

A role for the endothelium in the control of vasodilatation was first described in 1980 (Furchgott and Zawadzki, 1980) and it has since been confirmed that endothelial cells play a crucial role in the adjustment of vascular blood flow (Furchgott et al., 1984; Angus and Cocks, 1989; Ryan and Rubanyi, 1992). Endogenous nitric oxide, released basally and after receptor-mediated stimulation of endothelial cells, is an important modulator of vasodilatation and may additionally modulate neurotransmission and the effects of vasoconstrictors.

In the present study we investigate the effects of perivascular nerves and endothelium in the control of tone of mesenteric arteries of the Golden hamster. Specifically, we examine immunohistochemically the innervation of the

* Corresponding author. Tel.: (44-171) 387-7050; Fax: (44-171) 419-3873.

vascular bed by subsets of perivascular nerves and characterize pharmacologically vascular responses to electrical field stimulation of perivascular nerves and to exogenous application of agonists. Modulation of responses by endogenous nitric oxide was also examined using an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester.

2. Materials and methods

3-Month-old male Golden hamsters (*Mesocricetus auratus*) were used in the study. The hamsters were placed in a closed chamber and killed by asphyxiation with carbon dioxide. The mesenteric arterial beds were then isolated and set up for perfusion and pharmacological analysis, or dissected out and processed for immunohistochemical examination.

2.1. Immunohistochemistry of the isolated mesenteric arterial bed

The mesenteric arterial beds were isolated and placed in Krebs solution. They were then pinned out onto pieces of Sylgard silicone resin and excess adipose and connective tissue removed. The tissue was fixed in 4% paraformaldehyde in phosphate buffered saline for 2 h, washed three times in phosphate buffered saline, subsequently washed eight times in 80% alcohol in order to permeabilize cell membranes and then washed a further three times in phosphate buffered saline. The tissues were stored in phosphate buffered saline containing 0.01% sodium azide (BDH, Poole, UK) at 4°C until processed.

An indirect immunohistochemical fluorescence technique was used in order to investigate the presence of nerves immunoreactive for protein gene product (a general neuronal marker), calcitonin gene-related peptide, substance P, vasoactive intestinal polypeptide, neuropeptide Y and tyrosine hydroxylase (an enzymatic marker for noradrenaline). The whole mount stretch preparations were placed in a humid chamber and incubated for 18 h at room temperature with rabbit polyclonal antibodies at a dilution of 1:1000 for each of the antibodies. The whole mounts were then washed three times in phosphate-buffered saline/Triton X-100 before being incubated for 1 h at room temperature with a biotinylated goat anti-rabbit immunoglobulin G at a dilution of 1:250. Subsequently the tissues were washed again three times in phosphate-buffered saline/Triton X-100 and then incubated with streptavidin conjugated fluorescein isothiocyanate at room temperature for 1 h to expose the antigen-antibody binding sites. This was followed with a further three washes in phosphate-buffered saline/Triton X-100 and the vessels were then counterstained with pontamine sky blue to reduce background fluorescence. This completed, the preparations were unpinned and transferred to glass slides upon which they were stretched out and dried, before being mounted with phosphate-buffered saline/glycerol-Citifluor and coverslipped.

2.2. Photomicroscopy

The slides were viewed under a Zeiss fluorescence microscope with the appropriate FITC filter and a KP 360 filter to counteract the red light seen due to the pontamine sky blue stain. Selected fields were photographed using TMAX P3200 film.

2.3. Pharmacology of the isolated perfused mesenteric arterial bed

To isolate the tissue for pharmacological investigation the abdomen of the hamster was opened by a midline incision and the superior mesenteric artery revealed. The artery was then cannulated with a Krebs-filled hypodermic needle (0.8 mm bore). The mesenteric bed was dissected away from the gut by cutting close to the intestinal border (as originally described for the rat mesenteric arterial preparation, McGregor, 1965) and modified for the hamster (Ralevic and Burnstock, 1996). Each bed was placed on a wire mesh (5×7 cm) in a humid chamber (custom-made at University College London), attached to a peristaltic pump (model 7554-30, Cole-Parmer Instrument, Chicago, IL, USA) and perfused at a constant flow rate of 3 ml min^{-1} with oxygenated Krebs solution at 37°C. The composition of the Krebs solution was as follows: (mM) NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2/5% CO_2. Because flow through the mesenteric arterial bed was kept constant, changes in perfusion pressure (mmHg) were indicative of changes in mesenteric arterial resistance. Responses (mmHg) were measured as changes in perfusion pressure via a pressure transducer (model 79D, Grass Instrument, Quincy, MA, USA). Preparations were allowed to equilibrate for 30 min before experimentation.

2.4. Vasoconstriction at basal tone

Transmural stimulation of perivascular nerves was achieved by passing a current (model 79D stimulator, Grass) between two electrodes, one being the needle cannulating the artery and the other the wire grid upon which the preparation rested. Electrical field stimulation was at pulses of 90 V, with a pulse width of 1 ms for a range of frequencies (4–64 Hz) for 30 s. Vasoconstriction was abolished by guanethidine (5 μM) confirming its sympathetic nature. The response was also blocked by tetrodotoxin (1 μM) qualifying its neuronal nature.

Vasoconstrictor responses to increasing doses of noradrenaline (0.005–1500 nmol) and adenosine 5′-triphosphate (ATP, 5–5000 nmol) were recorded at basal tone. Doses were administered via a neoprene rubber injection

port as 50 μl boluses and the tone was allowed to return to baseline before a subsequent injection was given.

After a further 15 min equilibration period, vasodilator responses at raised tone were determined.

2.5. Endothelium-dependent and -independent vasodilatation

Methoxamine, an α_1-adrenoceptor agonist, was added at a concentration of approximately 16 μM to the perfusate in order to raise the tone of the preparations. When a stable increase in tone above baseline of 50–100 mmHg had been achieved vasodilator responses to two endothelium-dependent vasodilators, acetylcholine (0.005–500 nmol) and ATP (0.005–500 nmol) were examined. Responses to sodium nitroprusside (0.005–500 nmol) were also examined.

2.6. Nitric oxide inhibition

The above protocol was repeated in separate preparations in the presence of a nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (30 μM). Dose-response curves to electrical field stimulation, vasoconstrictor responses to noradrenaline and ATP and vasodilator responses to acetylcholine, ATP and sodium nitroprusside were obtained in the same manner as before. The sensitivities of the preparations to methoxamine were increased in the presence of N^G-nitro-L-arginine methyl ester, therefore the concentration of methoxamine used was reduced in order to achieve a similar increase in tone as in the controls.

2.7. Peptide-mediated vasoconstriction and vasodilatation

Single, relatively high doses (0.5 nmol) of calcitonin gene-related peptide, substance P, vasoactive intestinal polypeptide and neuropeptide Y were administered to the preparations at resting tone. In the presence of methoxamine to raise the tone of the preparation, vasodilator responses to calcitonin gene-related peptide, substance P, vasoactive intestinal polypeptide and neuropeptide Y (0.0005–0.5 nmol) were examined.

2.8. Electrical field stimulation and capsaicin at raised tone

In methoxamine raised-tone preparations and in the presence of guanethidine (5 μM) to block sympathetic neurotransmission, perivascular nerves were initially stimulated at 60 V, 0.1 ms, 1–12 Hz, for 30 s, parameters which are known to excite capsaicin-sensitive primary afferents in the rat isolated perfused mesenteric arterial bed (Kawasaki et al., 1988; Ralevic et al., 1993). Infusion of tetrodotoxin (3 μM) and capsaicin (1 μM) were used to assess the neuronal contribution to the response. In the absence of evidence for a neuronal origin for the slight vasodilator response, the parameters were reduced (e.g., 40 V, 0.05 ms) to try to determine a tetrodotoxin-sensitive relaxation.

2.9. Statistical analysis

Frequency-response curves to electrical field stimulation and dose-response curves to noradrenaline and ATP were plotted as the mean (±S.E.M.) increases in perfusion pressure above baseline (mmHg). Dose-response curves to acetylcholine, ATP and sodium nitroprusside were plotted as the mean (±S.E.M.) percentage decrease in perfusion pressure. pD_2 values were determined as the negative log of the dose (nmol) required to produce a response that was 50% of maximum. When response curves did not reach a maximum pD_{30} values were calculated, where 30 represents 30 mmHg for constrictor responses at basal tone. Statistical difference between two groups was determined by Student's *t*-test; $P < 0.05$ was taken as significant.

2.10. Drugs

Acetylcholine (chloride), adenosine 5′-triphosphate (sodium salt), N^G-nitro-L-arginine methyl ester (hydrochloride), methoxamine (hydrochloride), 8-methyl-*N*-vanillyl-6-nonenamide (capsaicin), noradrenaline (bitartrate) and sodium nitroprusside were obtained from Sigma (Poole, UK). Ismelin (guanethidine) was obtained from Ciba-Geigy (Horsham, UK). Calcitonin gene-related peptide, neuropeptide Y, substance P and vasoactive intestinal polypeptide were from Cambridge Research Biochemicals (Northwick, UK). Drugs were made up in distilled water, apart from noradrenaline, which was made up as a stock solution of 30 mM in 0.1 M ascorbic acid, and capsaicin, which was made up as a stock solution of 10 mM in 100% ethanol.

Antibodies to calcitonin gene-related peptide and tyrosine hydroxylase were obtained from Affiniti (Exeter, UK), antibodies to vasoactive intestinal polypeptide came from Incstar (Wokingham, UK), to substance P from Genosys (Cambridge, UK), protein gene product from Ultraclone (Cambridge, UK) and neuropeptide Y from UCB Bioproducts (Belgium). Biotinylated goat anti-rabbit IgG and streptavidin-conjugated isothiocyanate were obtained from Amersham. Citifluor came from City University and Sylgard was from ICI.

3. Results

3.1. Characterization of the innervation of the mesenteric arterial bed

To investigate the innervation of the hamster mesenteric arterial vascular bed studies were performed on whole mount stretch preparations from control animals using

antisera against protein gene product, tyrosine hydroxylase, neuropeptide Y, substance P, calcitonin gene-related peptide and vasoactive intestinal polypeptide.

Incubation with protein gene product revealed a dense perivascular plexus which formed a close network on the vessel wall. Numerous nerve bundles were seen to run parallel with and across the vessel. Small diameter arterioles were supplied by a dense plexus of fibres compared with a relatively sparse plexus of fibres on large diameter arteries (Fig. 1). Staining of fibres for tyrosine hydroxylase and peptides similarly showed that small diameter arteries were more densely innervated than large diameter fibres.

Fibres immunoreactive for tyrosine hydroxylase formed a dense plexus on the vessel wall (Fig. 2A). There was a similar pattern of innervation, but slightly less dense plexus of fibres immunoreactive for neuropeptide Y (Fig. 2B).

Calcitonin gene-related peptide fibres formed a plexus of moderate density, much less dense than those formed by tyrosine hydroxylase-immunoreactive and neuropeptide Y-immunoreactive fibres (Fig. 2C).

There was an absence of fibres immunoreactive for substance P and vasoactive intestinal polypeptide.

3.2. Electrical field stimulation of sympathetic nerves at basal tone

Electrical field stimulation (4–64 Hz) elicited frequency-dependent vasoconstrictor responses (Fig. 3a). The contractile responses were short-lived and were completely blocked with guanethidine (5 μM) confirming their sympathetic nature.

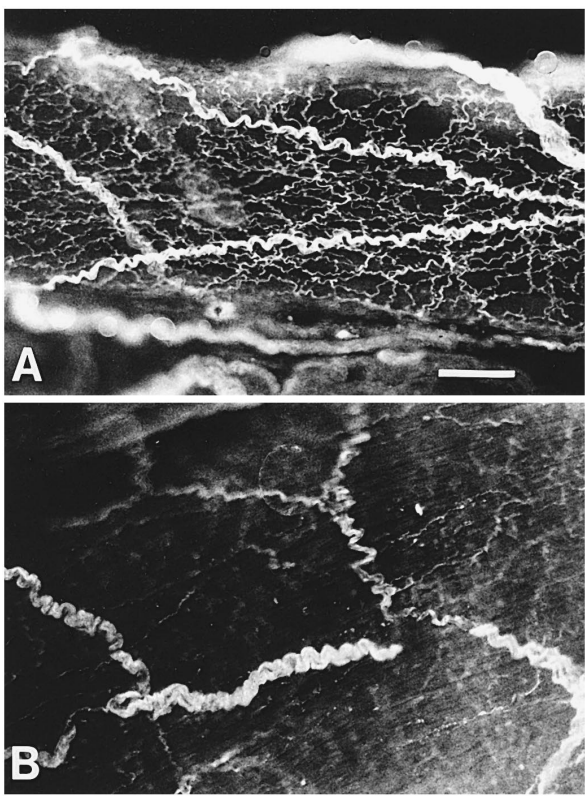

Fig. 1. PGP-immunohistochemical analysis of mesenteric arteries of the hamster. (A) small branch of the mesenteric arterial bed; (B) superior mesenteric artery. Bar = 68 μm.

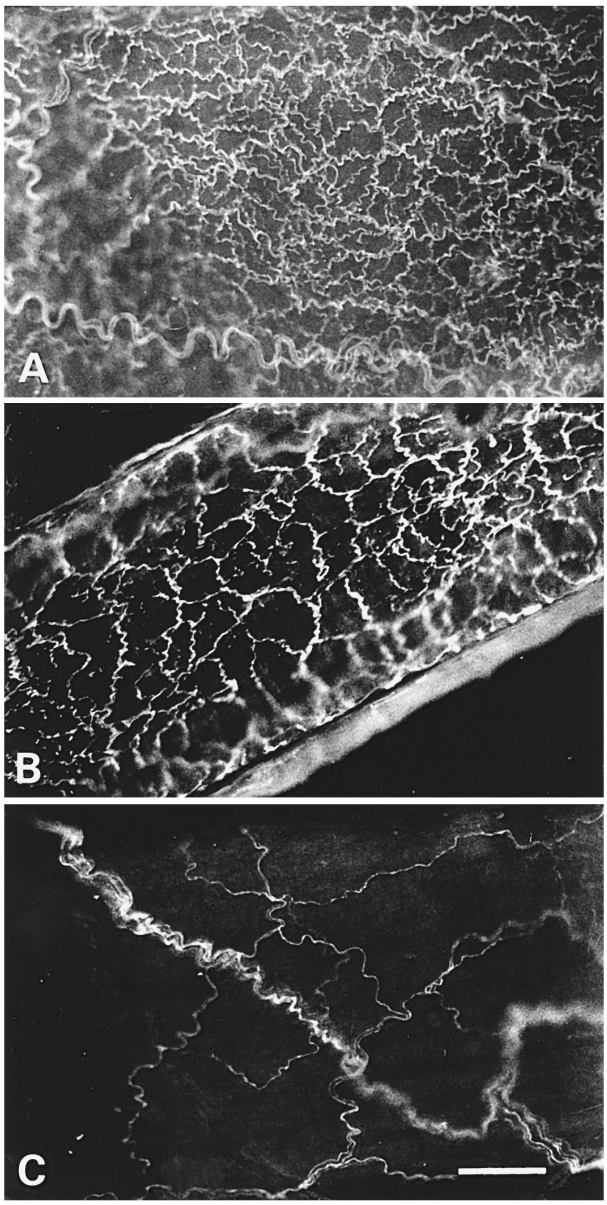

Fig. 2. Immunoreactivity of mesenteric arteries of the Golden hamster. (A) immunoreactivity for tyrosine-hydroxylase; (B) immunoreactivity for neuropeptide Y; (C) immunoreactivity for calcitonin gene-related peptide. Bar = 68 μm.

3.3. Vasoconstrictor responses to noradrenaline and ATP

Exogenous noradrenaline (0.005–1500 nmol) produced dose-dependent vasoconstriction (Fig. 3b). Low doses elicited transient contractions and responses became longer-lasting as the doses increased. ATP (5–500 nmol) elicited dose-dependent transient vasoconstriction of the mesenteric arterial preparations (Fig. 3c). pD_2 values and maximum heights of constriction are given in Table 1.

Table 1
Maximal response and pD_2 or pD_{30} values for vasoconstrictors and vasodilators of hamster mesenteric arterial beds in the absence and presence of L-NAME

		Control	L-NAME (3×10^{-5} M)
Stimulation frequency	F_{50} (Hz) [a]	18.72 ± 0.71	18.15 ± 1.19
	Max. height (mmHg)	119.27 ± 17.51	234.17 ± 27.00 *
NA	pD_2	7.81 ± 0.16	8.81 ± 0.14 * *
	Max. height (mmHg)	189.15 ± 18.93	355.71 ± 16.74 * *
ATP	pD_{30}	6.17 ± 0.15	6.52 ± 0.24
	Max. height (mmHg)	–	–
ACh	pD_2	10.08 ± 0.18	9.85 ± 0.20
	Max. relaxation (% of tone)	63.11 ± 6.26	57.68 ± 10.44
ATP	pD_{30}	9.88 ± 0.29	8.12 ± 0.32 *
	Max. relaxation (% of tone)	–	–
SNP	pD_2	9.01 ± 0.16	9.37 ± 0.12
	Max. relaxation (% of tone)	64.43 ± 6.93	62.89 ± 11.04

Significant difference from control * $P < 0.01$; * * $P < 0.001$. [a] F_{50} denotes the frequency (Hz) which is required to elicit 50% of the maximum response. Dashed line indicates that the maximum responses were not achieved. Values are the means of 4–17 experiments. L-NAME: N^G-nitro-L-arginine methyl ester.

3.4. Effect of N^G-nitro-L-arginine methyl ester on vasoconstrictor responses

N^G-nitro-L-arginine methyl ester (30 μM) had no significant effect on basal perfusion pressure of the preparations. Perfusion pressure of the preparations in the absence of N^G-nitro-L-arginine methyl ester was 35.1 ± 4.3 mmHg ($n = 17$) and in the presence of N^G-nitro-L-arginine methyl ester was 36.7 ± 7.9 mmHg ($n = 6$).

In the presence of N^G-nitro-L-arginine methyl ester the maximum height of the constrictor response to electrical field stimulation was significantly enhanced (Fig. 3a). Both the maximal height and the sensitivity of responses to noradrenaline were enhanced in the presence of N^G-nitro-L-arginine methyl ester, causing a leftward shift in the dose-response curve (Fig. 3b). Responses to ATP were not significantly affected by N^G-nitro-L-arginine methyl ester (Fig. 3c). pD_2 values and maximum heights in the pres-

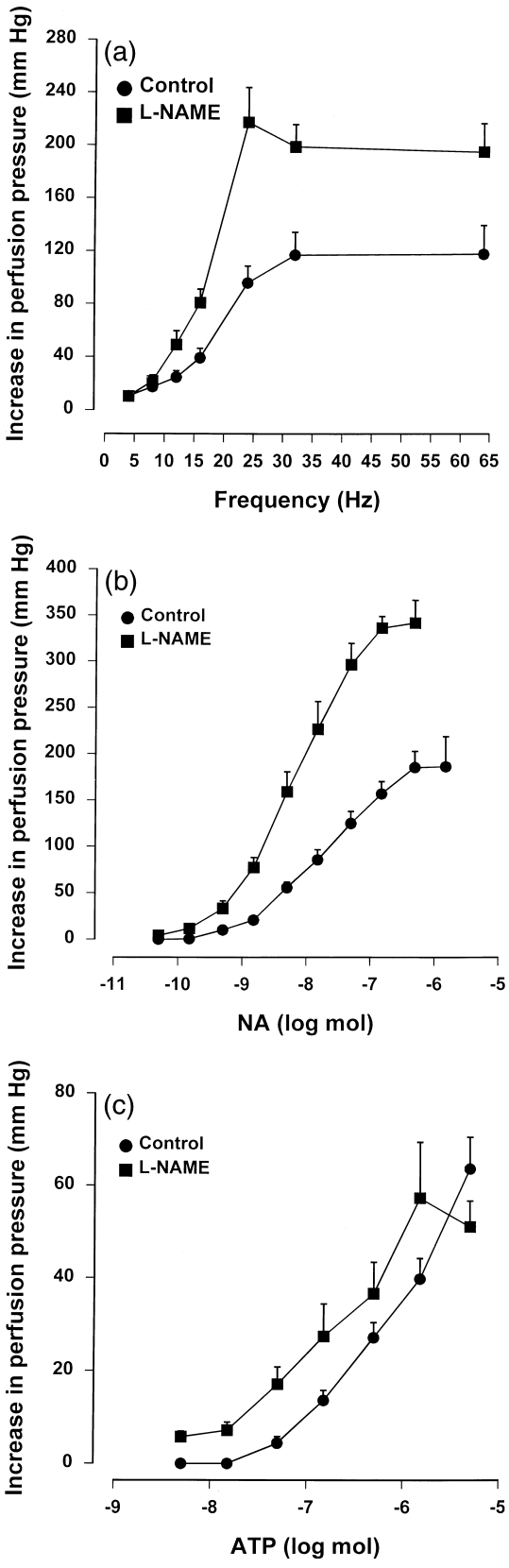

Fig. 3. (a) Frequency-dependent constrictor responses (increase in perfusion pressure, mmHg) of the isolated mesenteric arterial bed of the Golden hamster to electrical field stimulation (4–64 Hz, 1 ms, 90 V, 30 s) in the absence (●, $n = 15$) and presence (■, $n = 6$) of N^G-nitro-L-arginine methyl ester (30 μM). (b) Dose-dependent contractile responses (increase in perfusion pressure, mmHg) of the isolated mesenteric arterial bed of the Golden hamster to noradrenaline (NA; 0.005–1500 nmol) in the absence (●, $n = 17$) and presence (■, $n = 7$) of N^G-nitro-L-arginine methyl ester (30 μM). (c) Dose-dependent contractile responses (increase in perfusion pressure, mmHg) of the isolated mesenteric arterial bed of the Golden hamster to adenosine 5′-triphosphate (ATP; 5–500 nmol) in the absence (●, $n = 14$) and presence (■, $n = 7$) of N^G-nitro-L-arginine methyl ester (30 μM).

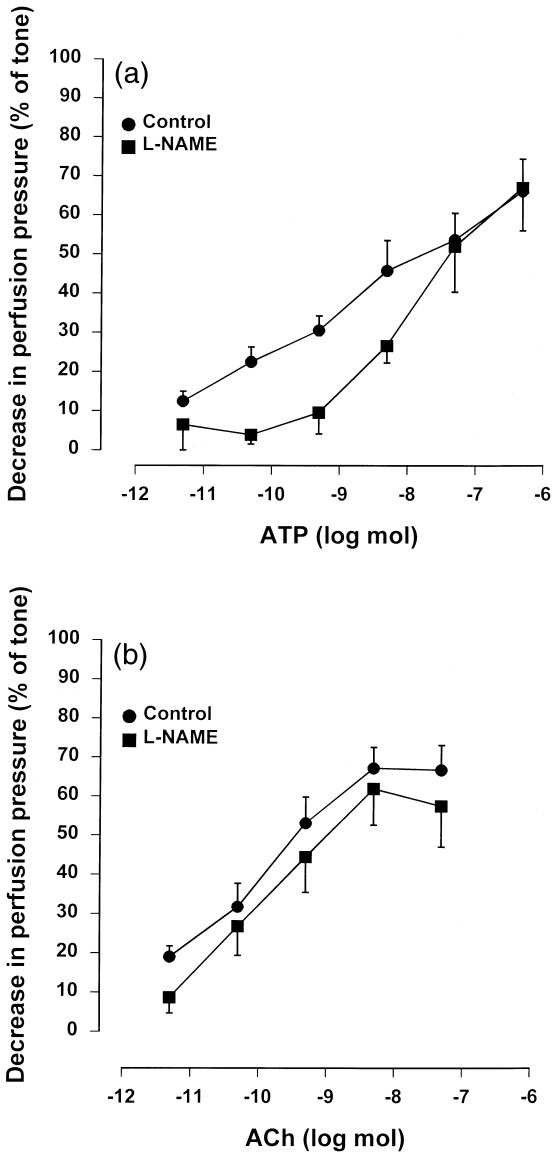

Fig. 4. Dose-dependent relaxation (decrease in perfusion pressure, % of tone) of the isolated mesenteric arterial bed of the Golden hamster to (a) adenosine 5′-triphosphate (ATP; 0.005–500 nmol) ($n = 7$), (b) acetylcholine (ACh; 0.05–500 nmol) ($n = 12$), in the absence (•) and presence (■) of N^G-nitro-L-arginine methyl ester (30 μM) ($n = 4$–7).

ence of N^G-nitro-L-arginine methyl ester are given in Table 1.

3.5. Vasodilator responses to acetylcholine, ATP and sodium nitroprusside

Methoxamine, added at a concentration of 16.3 ± 2.2 μM raised the tone of the preparations above baseline by 57.9 ± 3.8 mmHg ($n = 12$). Acetylcholine, ATP and sodium nitroprusside (0.005–500 nmol) mediated dose-dependent relaxations at raised tone (Fig. 4). For each of the agents used pD_2/pD_{30} values and maximum relaxations are given in Table 1.

3.6. Effects of N^G-nitro-L-arginine methyl ester on vasodilator responses to acetylcholine, ATP and sodium nitroprusside

In order to achieve a similar level of tone as in the control preparations a lower concentration of methoxamine was required to raise the tone in the presence of N^G-nitro-L-arginine methyl ester (30 μM): 4.5 ± 1.2 μM methoxamine was used to achieve an increase in tone above baseline of 58.3 ± 3.4 mmHg ($n = 7$).

Responses to ATP were significantly attenuated by N^G-nitro-L-arginine methyl ester (Fig. 4a). There was an increase in the pD_{30} value, but no reduction of the maximum relaxation (Table 1). Responses to acetylcholine were not affected by N^G-nitro-L-arginine methyl ester (Fig. 4b, Table 1). Responses to sodium nitroprusside were unaffected by N^G-nitro-L-arginine methyl ester (Table 1).

3.7. Electrical field stimulation at raised tone

In the methoxamine raised-tone preparation in the presence of guanethidine (5 μM) to block sympathetic neurotransmission, electrical field stimulation (1–12 Hz, 0.1 ms, 60 V, 30 s) at parameters known to excite sensory-motor nerves in rat mesenteric arteries, elicited small relaxations; however, these were not blocked by tetrodotoxin (1 μM) ($n = 4$) indicating direct smooth muscle relaxation. Reducing the parameters of stimulation (see methods) also did not produce tetrodotoxin-sensitive responses.

Capsaicin at 0.05 nmol, a dose known to elicit marked relaxation of the rat mesenteric arterial bed (Ralevic et al., 1994), did not have any relaxant effect in the hamster mesenteric bed. Application of higher doses of capsaicin (0.5–5 nmol) elicited only constrictor responses ($n = 6$).

3.8. Effects of neuropeptides on the mesenteric arterial bed

At basal tone, substance P, vasoactive intestinal polypeptide, neuropeptide Y and calcitonin gene-related peptide (all at 0.5 nmol) had no effect on the mesenteric arterial bed preparations ($n = 4$). At raised tone calcitonin gene-related peptide was a potent vasodilator, active in the range 0.0005–0.5 nmol). A dose of 0.5 nmol produced a relaxation of $42.21 \pm 3.97\%$ ($n = 7$). Vasoactive intestinal polypeptide (0.005–0.5 nmol) had slightly less potent vasodilator actions; a dose of 0.5 nmol produced a relaxation of $34.28 \pm 4.19\%$ ($n = 7$). Substance P (0.5 nmol) had no effect.

4. Discussion

Perivascular nerves innervating the hamster mesenteric arterial bed were characterized pharmacologically and immunohistochemically in the present study. In addition, the effect of nitric oxide as a modulator of mesenteric arterial

sympathetic neurotransmission and of vasoconstriction, and vasodilatation function was examined.

Immunohistochemical examination of the hamster mesenteric arterial bed using antisera to protein gene product (a general neuronal marker) revealed that the small diameter second and third order branches of the bed were much more densely innervated than the larger superior mesenteric artery. This pattern of innervation is similar to that observed in many other vascular beds (Burnstock, 1975) and is consistent with the fact that the smaller vessels have a more important role in the control of vascular resistance. Sympathetic perivascular nerves were the most abundant nerve type, as indicated by the highest levels of fibres immunoreactive for tyrosine hydroxylase. A functional correlate for these nerves in hamster mesenteric arteries was provided by showing vasoconstriction to electrical field stimulation which was abolished by guanethidine. Although this response can also be blocked by the α_1-adrenoceptor antagonist prazosin (Ralevic and Burnstock, 1996), this does not exclude the possibility of sympathetic cotransmission of ATP and/or neuropeptide Y. In the rat mesenteric arterial bed, vasoconstriction to electrical field stimulation is also abolished by prazosin (Longhurst et al., 1986); however, in rat small mesenteric arteries a prazosin-resistant purinergic component has been shown (Sjöblom-Widfeldt et al., 1990).

Tyrosine hydroxylase-containing nerves were found to form a dense plexus on the wall of the smaller mesenteric vessels. There was a similar pattern of innervation but slightly less dense innervation by fibres immunoreactive for neuropeptide Y which is consistent with the fact that most neuropeptide Y is contained within and released from sympathetic nerves as a neurotransmitter/neuromodulator (Burnstock, 1990). However, it is possible that some of these neuropeptide Y-immunoreactive fibres were non-sympathetic as has been shown in rat mesenteric arteries (Aberdeen et al., 1991). The lack of functional responses to neuropeptide Y in hamster mesenteric arteries at either basal or raised tone suggests that the role of neuropeptide Y is that of a neuromodulator, as shown in many other vessels including mesenteric arteries of the rat (Westfall et al., 1987, 1990; Gustafsson and Nilsson, 1990; Kawasaki et al., 1991).

Sympathetic constrictor responses to electrical field stimulation were significantly augmented in the presence of an inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester, suggesting a role for nitric oxide as a modulator of sympathetic neurotransmission. Since constrictor responses to noradrenaline were also augmented by N^G-nitro-L-arginine methyl ester this effect appears to be post- rather than pre-junctional. There was a trend for contractile responses to ATP, which are mediated via P_{2X} purinoceptors (Ralevic and Burnstock, 1996), to be greater in the presence of N^G-nitro-L-arginine methyl ester, although this did not reach statistical significance. Augmentation of contractile responses of vessels by inhibitors of nitric oxide synthase and following removal of the endothelium has been reported previously (Ralevic and Burnstock, 1988; Li and Duckles, 1992; Adeagbo et al., 1994). The increase in responses may be due to the removal of an opposing vasodilatation mediated via actions of the agonist at endothelial receptors as well as to inhibition of basal release of nitric oxide from endothelial cells. Thus, nitric oxide contributes significantly to the control of constrictor tone in the hamster mesenteric arterial bed under normal conditions, but does not appear to equally modulate responses mediated by noradrenaline receptors and P_{2X} purinoceptors.

At raised tone N^G-nitro-L-arginine methyl ester significantly attenuated vasodilator responses to ATP, but not those to acetylcholine. Both ATP and acetylcholine elicit endothelium-dependent vasodilatation of hamster mesenteric arteries at raised tone (Ralevic and Burnstock, 1996). However, ATP, but not acetylcholine can also act at vasoconstriction-mediating receptors on the smooth muscle. Thus, the combination of vasoconstriction together with N^G-nitro-L-arginine methyl ester-mediated inhibition of vasodilatation might explain why there was significant attenuation of vasodilator responses to ATP but not those to acetylcholine. It is also possible that the N^G-nitro-L-arginine methyl ester-resistant component of the vasodilator response is mediated by endothelium-derived hyperpolarizing factor (Adeagbo and Triggle, 1993; Parsons et al., 1994). Responses to the nitric oxide-donor sodium nitroprusside were unaffected by N^G-nitro-L-arginine methyl ester, in contrast to the augmented vasodilatation to sodium nitroprusside which has been reported in the rat mesenteric arterial bed (Ralevic et al., 1991).

Calcitonin gene-related peptide-immunoreactive fibres were found to form a plexus of moderate density compared to tyrosine hydroxylase containing nerves in hamster mesenteric arteries. These are likely to be sensory fibres since calcitonin gene-related peptide is a recognized transmitter in these nerves, where it typically coexists with substance P. Thus, it was interesting that we found no substance P-immunoreactive fibres in the hamster mesenteric arterial bed, suggesting that calcitonin gene-related peptide does not coexist with substance P. Similarly, the density of substance P-fibres has been reported to be sparse in rat mesenteric arteries, whereas innervation by calcitonin gene-related peptide-immunoreactive fibres is relatively dense (Kawasaki et al., 1988).

In the hamster mesenteric arterial bed electrical field stimulation at raised tone and in the presence of guanethidine to block sympathetic neurotransmission did not elicit a neurogenic functional response. This is in contrast to the rat isolated mesenteric arterial bed where electrical field stimulation at similar parameters elicits frequency-dependent vasodilatation due to activation of sensory-motor nerves (Kawasaki et al., 1988; Ralevic et al., 1993, 1994). This efferent function of sensory-motor nerves in rat mesenteric arteries is due to the release of the potent

vasodilator calcitonin gene-related peptide (Kawasaki et al., 1988; Han et al., 1990). In both hamster (present study) and rat (Kawasaki et al., 1988) mesenteric arterial beds substance P is a weak vasodilator. It is possible that, in contrast to sensory nerves in rat mesenteric arteries, sensory nerves in hamster mesenteric arteries have only an afferent function. This raises questions about the role of calcitonin gene-related peptide contained within nerves in hamster mesenteric arteries and for the postjunctional receptors for this peptide, since exogenously applied calcitonin gene-related peptide was a potent vasodilator.

The sensory neurotoxin capsaicin, a potent activator of a subpopulation of sensory nerves and mediator of vasodilatation of rat mesenteric arteries (Kawasaki et al., 1988; Ralevic et al., 1994), was ineffective as a vasodilator of hamster mesenteric arteries. Indeed, at the highest doses used this agent elicited vasoconstriction, which may be due to a direct effect on the smooth muscle as has been shown in some other vessels (Toda et al., 1972; Duckles, 1986; Edvinsson et al., 1990). It is possible that sensory nerves in hamster mesenteric arteries are capsaicin-insensitive. However, the results showing a lack of responsiveness to electrical field stimulation might be taken as further evidence for their possessing only afferent function.

The potent vasodilator actions of exogenous vasoactive intestinal polypeptide, but absence of nerves immunoreactive for this peptide, raises questions about its physiological source and role in the control of vascular tone in hamster mesenteric arteries.

In conclusion, we have characterized pharmacologically and immunohistochemically the innervation and function of perivascular nerves in mesenteric arteries of the hamster. Sympathetic perivascular nerves are important in vasoconstriction, but there is a lack of evident motor effects of calcitonin gene-related peptide-containing nerves, despite the presence of postjunctional receptors for this peptide. Endogenous nitric oxide is a modulator of sympathetic neurotransmission, but has only a small role as a mediator of endothelium-dependent vasodilatation in hamster mesenteric arteries.

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