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Endothelium-dependent Sensory Non-adrenergic Non-cholinergic Vasodilatation in Rat Thoracic Aorta: Involvement of ATP and a Role for NO

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Abstract

The involvement of non-adrenergic non-cholinergic (NANC) transmitters, such as adenosine 5′-triphosphate (ATP) and nitric oxide (NO), in the neurogenic relaxation of rat thoracic aorta was investigated in vessel segments suspended for isometric tension recording by polygraph.

Responses to electrical field stimulation (EFS) and exogenous vasodilator were investigated in vessels precontracted with 5-hydroxytryptamine. EFS (100 V, 2–16 Hz, for 10 s at 3-min intervals), in the presence of guanethidine (10 μM) and atropine (10 μM) produced frequency-dependent relaxations. Pretreatment with tetrodotoxin (1 μM) markedly reduced the relaxation and desensitization with capsaicin (10 μM) significantly inhibited the relaxation. Exogenously added ATP caused concentration-dependent relaxations. Mechanical removal of the endothelium significantly inhibited EFS- and ATP-induced relaxation by $30 \pm 3\%$ and $37 \pm 2\%$, respectively. Pretreatment with a P_1-purinoceptor antagonist, 8-phenyltheophylline (10 μM) or P_{2X}-purinoceptor antagonist, Evans blue (10 μM) did not influence the relaxations to EFS and exogenously added ATP. In contrast, the P_{2Y}-purinoceptor antagonist, basilen blue (100 μM) markedly reduced the relaxations to EFS by $52 \pm 4\%$ in the endothelium-intact preparations. However, in the endothelium-denuded preparations and capsaicin-pretreated preparations, basilen blue did not change relaxations elicited by EFS. The NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) also significantly inhibited the relaxations to EFS and ATP by $40 \pm 6\%$ and $30 \pm 2\%$, respectively, in the endothelium-intact preparations but had no effect on the relaxations in the endothelium-denuded preparations or capsaicin-pretreated preparations. In addition, the EFS-induced relaxations were also inhibited $43 \pm 7\%$ by pretreatment with 1*H*-[1,2,4]-oxadiazolo[4,3-α]quinoxalin-1-one (ODQ; 1 μM), soluble guanylate cyclase inhibitor.

This study suggests that the NANC nerve system is present in the thoracic aorta of rat, mediating vasodilatation by sensory nerves. ATP, as a neurotransmitter released from sensory nerves, activates P_{2Y}-purinoceptors located on the endothelium and stimulates the NO/cyclic GMP pathway, resulting in vasodilatation.

It is well known that sympathetic, parasympathetic and non-adrenergic, non-cholinergic (NANC) nerves regulate vascular tone. Putative NANC mediators include peptide mediators such as calcitonin gene-related peptide (CGRP) and substance P, and non-peptide mediators such as nitric oxide (NO) and adenosine 5′-triphosphate (ATP) (Lundberg 1996). The nerve type mediating NANC responses has not been clearly identified.

Peripheral sensory neurons release peptide and non-peptide mediators and these nerves may contribute to NANC responses (Lundberg 1996). Capsaicin is a selective neurotoxin for sensory nerves (Kawasaki et al 1988). Capsaicin-sensitive sensory nerves have been implicated as the nerves mediating NANC vasorelaxation in various vas-

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cular beds (Chen & Guth 1995; Kakuyama et al 1998).

It has been proposed that NANC nerves containing NO or ATP regulate the vascular tone in certain vascular beds (Burnett et al 1992). NO was first identified as endothelium-derived relaxing factor (EDRF) and later proposed as a putative NANC neurotransmitter in both central and peripheral neurons, including those involved in the control of cardiovascular function (Bredt et al 1990; Bredt & Snyder 1992). Nitrergic perivascular nerves have been demonstrated by immunohistochemical localization of NO synthase (NOS) (Bredt et al 1990; Burnett et al 1992) and blockers of NOS have been shown to reduce inhibitory neurotransmission (Ahlmer et al 1991; Toda & Okamura 1992), thus providing evidence for functional nitrergic innervation of certain vascular beds.

ATP is now considered to be a neurotransmitter in both neural and vascular preparations (Edwards et al 1993; Evans et al 1993). It acts as a co-transmitter, which may occur in either sympathetic neurons co-stored with noradrenaline or in parasympathetic neurons together with acetylcholine. The possibility of ATP being released from sensory nerves has also been suggested (Ahluwalia & Cellek 1997). Both neuronally released and exogenously applied ATP induces vasodilation through P_{2Y}-purinoceptors in the portal vein and pulmonary arteries (Liu et al 1992; Brizzolara et al 1993).

Recently, it was suggested that NANC neurogenic relaxation of lamb coronary small arteries is mediated by ATP, relaxed by P_{2Y}-purinoceptors (Simonsen et al 1997), this is released from capsaicin-sensitive sensory neurons, and mediated endothelium-dependent NANC relaxation in small mesenteric artery of the rabbit (Kakuyama et al 1998).

In this study, we explored NANC neurotransmission in rat isolated thoracic aorta to determine whether sensory nerves contribute to these responses. We also investigated the involvement of ATP as a transmitter and determined a role for NO in NANC neurotransmission.

Methods

Tissue preparation

Male Sprague–Dawley rats (200–250 g) were used and thoracic aorta were carefully excised to remove connective tissue and placed in Krebs solution of the following composition (mM): NaCl 118, KCl 4·8, $CaCl_2$ 2·5, KH_2PO_4 1·2, $MgSO_4$ 1·2, $NaHCO_3$ 24 and glucose 11. Ring segments (3–5 mm in length) were mounted vertically between 2 stainless steel wires in organ baths filled with Krebs solution. One wire was fixed and the other attached to a force transducer (FT03 Grass Instruments Co., Quincy, MA). Changes in isometric force were recorded on a polygraph (Grass model 79).

Vessels were maintained at 37°C in Krebs solution bubbled with 5% CO_2 and 95% O_2 (pH $7{\cdot}45 \pm 0{\cdot}05$). Rings were allowed to equilibrate at resting tension (1 g) in the bath for at least 60 min and washed with fresh Krebs solution every 15 min during the equilibration periods.

In the endothelial denudation study, endothelium was removed by gently rubbing the intraluminal surface with a roughened wire. The functional denudation of the endothelium was confirmed by loss of the relaxant responses to acetylcholine (1–10 μM) in segments that had been pre-contracted with 5-HT before the experiment in all vessel rings.

Electrical field stimulation

Electrical field stimulation (EFS) (100 V, 2–16 Hz, for 10 s at 3-min intervals) was conducted with two parallel platinum electrodes positioned at each end of the vessel ring and connected to a stimulator (Grass S48).

For studying relaxation to EFS, vessel rings were pretreated with guanethidine (10 μM) and atropine (10 μM) for 20 min to block sympathetic and cholinergic neurotransmission and then pre-contracted with 100 μM 5-hydroxytryptamine (5-HT). After a stable contraction was obtained, frequency–response relationships (2–16 Hz) were constructed and the rings were washed three times and allowed to equilibrate for 30 min after EFS to permit the rings to recover completely from the relaxant responses to EFS.

In our present study, all frequency-dependent responses to EFS were investigated. To determine whether the EFS responses were neuronal or sensory in origin, vessels were pretreated by tetrodotoxin (1 μM) and capsaicin (10 μM) for 20 min, followed by several washings. Vessels were re-contracted 30 min later with 5-HT and EFS-induced responses were re-established.

Exogenous vasodilators

The vessel rings were pre-contracted with 100 μM 5-HT and concentration–response curves to exogenous ATP in the absence and presence of 10 μM 8-phenyltheophylline, 10 μM Evans blue and 100 μM basilen blue were obtained. The effects of endothelial removal or pretreatment with N^G-nitro-

L-arginine methyl ester (L-NAME) (100 μM) on the concentration–response curve to exogenous ATP were also determined. The concentration–response curves in endothelium-denuded preparations or drug-treated preparations were compared with those responses achieved with intact or control untreated preparations taken from the same vessel.

Drugs

Atropine sulphate/guanethidine monosulphate (1 : 1), tetrodotoxin, L-NAME, ATP, 5-HT oxalate salt, acetylcholine chloride, indomethacin and 1*H*-[1,2,4] oxadiazolo[4,3-α]quinoxalin-1-one (ODQ) were purchased from Sigma Chemical Co. (St Louis, MO). (E)-capsaicin, basilen blue and Evans blue tetrasodium salt were purchased from Tocris Cookson Ltd (Langford, UK). 8-Phenyltheophylline was purchased from RBI (Natrick, MA). The solutions were prepared on the day of the experiment and administered in volumes not exceeding 0·5% of the bath volume.

Analysis of data

In all experiments, relaxation responses are presented as a percentage of the 5-HT-induced contraction. Vasorelaxant responses to EFS in the presence of various inhibitors or their vehicles were compared with EFS responses before adding these inhibitors or vehicles and expressed as percentage inhibition. Values are presented as mean ± s.e.m.; n indicates the number of ring segments used. Statistical analysis of results was performed by use of paired and unpaired Student's *t*-test or analysis of the variance. $P < 0{\cdot}05$ was considered to be significant.

Results

EFS-induced NANC relaxation

5-HT (100 μM) induced sustained contractions and guanethidine/atropine suppressed adrenergic and cholinergic response to EFS (data not shown). In vessels contracted with 5-HT and pretreated with guanethidine/atropine (all 10 μM) to block the adrenergic and cholinergic responses, EFS (2–16 Hz) produced frequency-dependent relaxation (Table 1).

Characteristics of EFS-induced NANC relaxation

To confirm that the response to EFS were neuronal in origin, some preparations were pretreated with tetrodotoxin (1 μM). EFS induced frequency-dependent relaxations, which were completely suppressed by tetrodotoxin (1 μM, n = 4, $P < 0{\cdot}01$, Table 1). To determine whether the EFS responses were sensory in origin, vessels were desensitized by pretreatment with capsaicin (10 μM) for 20 min, followed by several washes. Thirty minutes later vessels were re-contracted with 5-HT and EFS-induced responses were re-established. Desensitization with capsaicin (10 μM) significantly inhibited the responses (26–34% inhibition, n = 5, $P < 0{\cdot}05$; Table 2).

To determine whether the nitrergic nerves were involved, endothelium-denuded vessel rings were pretreated with L-NAME (100 μM, NOS inhibitor) for 10 min. In the endothelium-denuded preparation, L-NAME (100 μM) had no significant effects (Table 3). EFS-induced NANC relaxation was significantly depressed (30 ± 3%, at each frequency) by endothelium-removal (n = 4, $P < 0{\cdot}05$ and $P < 0{\cdot}01$; Table 3).

Table 1. Effect of tetrodotoxin on response to EFS in rat isolated thoracic aorta.

	Relaxation (% of 5-HT contraction)	
EFS (Hz)	Control	Tetrodotoxin
2	23 ± 4·2	5 ± 1·8**
4	40 ± 4·2	4 ± 1·6**
8	44 ± 5·5	3 ± 1·0**
16	50 ± 6·3	2 ± 1·2**

In vessels contracted with 5-HT and pretreated with guanethidine (10 μM)/atropine (10 μM), EFS (2–16 Hz) produced frequency-dependent relaxation. However EFS-induced relaxation was completely suppressed by tetrodotoxin (1 μM), indicating that the response was neuronally mediated. Values are mean ± s.e.m., n = 4. **$P < 0{\cdot}01$ vs control by Student's *t*-test.

Table 2. Effect of desensitization with capsaicin on the response to EFS in rat isolated thoracic aorta.

EFS (Hz)	Relaxation (% of 5-HT contraction)	
	Control	Capsaicin
2	28·5 ± 2·7	18·8 ± 3·0*
4	27·9 ± 2·8	20·9 ± 3·3*
8	34·4 ± 2·3	23·1 ± 3·3*
16	40·3 ± 2·0	30·1 ± 3·7*

Desensitization with capsaicin (10 μM), sensory neurotoxin, significantly inhibited EFS-induced relaxation, which suggested that the responses are mediated by capsaicin-sensitive sensory nerves. Values are mean ± s.e.m., n = 5. *$P < 0{\cdot}05$ vs control values by Student's *t*-test.

Table 3. Effect of endothelium removal and L-NAME on response to EFS in rat isolated thoracic aorta.

EFS (Hz)	Relaxation (% of 5-HT contraction)		
	Endothelium (+)	Endothelium (−)	Endothelium (−) + L-NAME
2	28·3 ± 2·7	19·8 ± 2·2*	17·7 ± 1·3
4	30·7 ± 2·8	21·3 ± 0·4*	22·9 ± 1·9
8	34·8 ± 2·3	25·5 ± 1·2**	27·0 ± 1·5
16	40·0 ± 2·0	27·0 ± 1·2	31·2 ± 1·2

Endothelium removal significantly suppressed the response to EFS. In the endothelium-denuded preparation, L-NAME (100 μM, NOS inhibitor) did not change the response to EFS. This suggests that the endothelium-dependent pathway is involved in EFS-induced relaxation and that NO released neuronally can be excluded. Values are mean ± s.e.m., n = 4. *$P < 0.05$, **$P < 0.01$ vs endothelium intact preparation by analysis of variance.

Table 4. Effects of purinoceptor antagonists on response to exogenously added ATP in rat isolated thoracic aorta.

−log[ATP] (M)	Relaxation (% of 5-HT contraction)			
	Control	+ 8-Phenyltheophylline	+ Evans blue	+ Basilen blue
8	0	0	2	0
7	0	1	8·0 ± 4·5	0
6	35·0 ± 4·3	31·0 ± 2·8	47·0 ± 4·9	5·0 ± 4·8
5	82·0 ± 3·2	66·0 ± 5·2	81 ± 4·9	58·0 ± 4·3
4	84·0 ± 5·3	82·0 ± 2·0	91 ± 5·0	74·0 ± 1·5
pD_2	5·76 ± 0·16	5·40 ± 0·10	5·85 ± 0·10	5·17 ± 0·08*

ATP caused concentration-dependent relaxation in 5-HT-induced contraction that was shifted to the right by the selective P_{2Y}-purinoceptor antagonist, basilen blue (100 μM). The P_1-purinoceptor antagonist, 8-phenyltheophylline (10 μM) and the P_{2X}-purinoceptor antagonist, Evans blue (10 μM) had no effect on the responses to exogenously added ATP. pD_2 indicates the negative logarithm of half-maximal relaxation; 5-HT induced maximal contraction. Values are mean ± s.e.m., n = 4. *$P < 0.05$ vs control by Student's *t*-test.

Effects of exogenous ATP and purinoceptor antagonists

ATP (10^{-7}–10^{-4} M) induced reproducible concentration-dependent relaxations of rat thoracic aorta (negative logarithm of half-maximal relaxation (pD_2) = 5·76 ± 0·16, n = 4) and maximum relaxations of 84·0 ± 5·29% (n = 3) of the 5-HT-induced contraction (Table 4). Transient contractile responses to ATP were observed only at the high concentration (10^{-4}–10^{-3} M).

The P_1-purinoceptor antagonist 8-phenyltheophylline (8-PT, 10 μM) did not influence the relaxation elicited by exogenously added ATP, with pD_2 = 5·40 ± 0·10 (n = 4) and maximum relaxations of 82 ± 2% (n = 4; Table 4).

The P_{2X}-purinoceptor antagonist Evans blue (10 μM) also failed to affect the concentration-response curve of exogenous ATP, with pD_2 = 5·85 ± 0·10 and maximum relaxations of 91 ± 5% (n = 4; Table 4).

Table 5. Effect of endothelium-removal and L-NAME on response to exogenously added ATP in rat isolated thoracic aorta.

−log[ATP] (M)	Relaxation (% of 5-HT contraction)		
	Endothelium (+)	Endothelium (−)	L-NAME
8	0	0	0
7	0	0	0
6	35·0 ± 4·3	0***	2·0 ± 0·7***
5	82·0 ± 3·2	4·0 ± 4·1***	5·0 ± 0·4***
4	84·0 ± 5·3	47·0 ± 3·2**	54·0 ± 0·8*
pD_2	5·76 ± 0·16	3·90 ± 0·14**	3·93 ± 0·04**

ATP caused concentration-dependent relaxation in 5-HT-induced contraction that was shifted to the right by endothelium-removal and NOS inhibitor, L-NAME (100 μM); maximal relaxation was significantly reduced. pD_2 indicates the negative logarithm of half-maximal relaxation; 5-HT induced maximal contraction. Values are mean ± s.e.m., n = 4. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs endothelium intact preparation by Student's *t*-test

Table 6. Effects of purinoceptor antagonists on response to EFS in rat isolated thoracic aorta.

EFS (Hz)	Relaxation (% of 5-HT contraction)				
	Control	8-Phenyltheophylline	Evans blue	Basilen blue	Indomethacin
2	21·9 ± 1·8	24·6 ± 2·9	20·8 ± 3·7	12·4 ± 1·4**	21·1 ± 0·7
4	25·4 ± 1·8	27·5 ± 2·6	24·8 ± 3·9	16·2 ± 1·6**	25·1 ± 1·2
8	29·7 ± 1·9	29·2 ± 1·7	31·3 ± 4·2	18·1 ± 2·8*	27·8 ± 1·2
16	33·5 ± 2·0	29·2 ± 1·0	35·6 ± 2·0	20·0 ± 4·4*	32·9 ± 1·0

Pretreatment with basilen blue (100 μM) significantly attenuated the relaxation to EFS in endothelium-intact preparations, indicating that EFS-induced relaxation is, at least in part, mediated by activation of P_{2Y}-purinoceptor. Indomethacin (10 μM) had no effect on the EFS-induced relaxation, suggesting that prostanoid generation from endothelium is not involved in EFS-induced relaxation. Values are mean ± s.e.m.; n = 4. *$P < 0.05$, **$P < 0.01$ by Student's *t*-test.

In contrast, the P_{2Y}-purinoceptor antagonist basilen blue (100 μM) significantly reduced the response to exogenously added ATP with $pD_2 = 5.17 \pm 0.08$ (n = 4, $P < 0.05$) but did not affect the maximum relaxation, which was 84 ± 5·29% before and 74 ± 1·53% after treatment (n = 4; Table 4).

Effects of endothelial cell removal and L-NAME on relaxation to ATP

To determine whether ATP-elicited relaxations are endothelium dependent, the effects of mechanical endothelial cell removal and NOS blockade were examined on ATP-elicited relaxation. In endothelium-denuded preparations, ATP-elicited relaxations were significantly reduced from 84·0 ± 5·3% to 47·0 ± 3·2% (n = 4, $P < 0.01$, paired *t*-test; Table 5) and pD_2-values for ATP were 5·76 ± 0·16 and 3·90 ± 0·14 in endothelium-intact and -denuded preparations, respectively (n = 4, $P < 0.01$, paired *t*-test; Table 5).

Pretreatment with L-NAME (100 μM) significantly reduced maximum relaxation to 54·0 ± 0·8% with a pD_2-value of 3·93 ± 0·04 (n = 4, $P < 0.05$ and $P < 0.01$; Table 5).

Effects of purinoceptor antagonists on EFS-induced relaxation

Pretreatment with 8-phenyltheophylline (8-PT, 10 μM) or Evans blue (10 μM) did not influence the relaxation elicited by EFS (n = 4). In contrast, pretreatment with basilen blue (100 μM) caused a 40 ± 3% inhibition at each frequency (n = 4, $P < 0.05$ and $P < 0.01$; Table 6). Indomethacin (10 μM , cyclooxygenase inhibitor) did not influence the EFS-induced relaxation (n = 4; Table 6).

Effect of basilen blue on responses to EFS

In the endothelium-intact preparations, pretreatment with basilen blue (100 μM) significantly inhibited the responses to EFS (52 ± 4% inhibition

Table 7. Effect of basilen blue on response to EFS in rat isolated thoracic aorta.

EFS (Hz)		Relaxation (% of 5-HT contraction)		
		Endothelium (+)	Endothelium (−)	Capsaicin
2	Control	28·0 ± 2·7	19·9 ± 1·8	18·7 ± 3·0
	Basilen blue	12·0 ± 1·4**	21·8 ± 2·9	19·1 ± 2·0
	L-NAME	15·0 ± 2·7**	17·5 ± 1·3	19·9 ± 2·5
4	Control	31·0 ± 2·8	21·3 ± 0·4	20·8 ± 3·3
	Basilen blue	16·0 ± 1·6**	25·7 ± 2·8	20·8 ± 2·9
	L-NAME	17·0 ± 0·6**	22·9 ± 1·9	20·0 ± 2·6
8	Control	35·0 ± 2·3	24·7 ± 1·0	27·2 ± 3·3
	Basilen blue	18·0 ± 2·7**	30·7 ± 2·8	22·5 ± 4·0
	L-NAME	22·1 ± 2·0**	27·1 ± 1·5	22·0 ± 3·0
16	Control	40·0 ± 2·0	28·3 ± 2·0	29·9 ± 3·7
	Basilen blue	20·0 ± 4·4*	32·1 ± 2·4	24·0 ± 4·4
	L-NAME	27·1 ± 3·3*	31·3 ± 1·2	24·8 ± 2·4

In the endothelium-intact preparation, basilen blue (100 μM) and L-NAME (100 μM) significantly reduced the response to EFS, but did not change the response in the endothelium-denuded preparation. Basilen blue and L-NAME had no effect on the response in the capsaicin-pretreated preparations. These results suggest that NO is released from the endothelium and provide evidence that ATP released from sensory nerves only stimulates NO generation through P_{2Y}-purinoceptor located on the endothelium. Values are mean ± s.e.m.; n = 5. *$P < 0.05$, **$P < 0.01$ vs control values by analysis of variance.

at each frequency; n = 6, $P < 0.01$ and $P < 0.05$; Table 7), but in endothelium-denuded preparations it had no significant effect (Table 7). In addition, basilen blue had no effects on the EFS-induced relaxation in the capsaicin-pretreated preparations (Table 7).

Effect of L-NAME on responses to EFS

In the endothelium-intact preparations, pretreatment with L-NAME (100 μM) caused a 40 ± 6% inhibition at each frequency (n = 5, $P < 0.05$ and $P < 0.01$; Table 7), but had no significant effect in endothelium-denuded preparations (Table 7). L-NAME had no effects on the EFS-induced relaxation in the capsaicin-pretreated preparations (Table 7).

Effect of ODQ on the responses to EFS

ODQ (1 μM, soluble guanylate cyclase inhibitor) significantly suppressed the relaxation to EFS (n = 5, $P < 0.05$ and $P < 0.01$; Table 8) with 36–50% inhibition being observed (maximum at 16 Hz).

Discussion

The mechanism of EFS-induced relaxation was investigated in isolated thoracic aorta of the rat. Our results show that the EFS-induced NANC relaxant response in these vessels is partially due to activation of capsaicin-sensitive neurons and partially mediated by ATP. The response is endothelium-dependent and involves activation of guanylate cyclase by endothelium-derived NO.

EFS of vessels pretreated with guanethidine and atropine caused frequency-dependent relaxation that was reduced by the Na^+-channel blocker, tetrodotoxin, indicating the presence of an inhibitory NANC neurogenic component in rat thoracic aorta. The small reduction of EFS-induced relaxations produced by atropine and a combination of atropine and guanethidine suggest that both cholinergic and adrenergic responses contribute to the neurogenic relaxation of the thoracic aorta, but the main component of the EFS-induced relaxation was inhibitory NANC in nature.

Table 8. Effect of ODQ on response to EFS in rat isolated thoracic aorta.

EFS (Hz)	Relaxation (% of 5-HT contraction)	
	Control	ODQ
2	15·3 ± 2·4	15·3 ± 1·0
4	23·0 ± 3·1	16·5 ± 0·6
8	29·1 ± 2·6	18·8 ± 1·9*
16	34·8 ± 1·9	17·6 ± 1·1**

The soluble guanylyl cyclase inhibitor, ODQ (1 μM) significantly suppressed the relaxation to EFS, indicating that the NO/cyclic GMP pathway is involved in the response to EFS. Values are mean ± s.e.m.; n = 5. *$P < 0.05$ and **$P < 0.01$ vs control values by Student's *t*-test.

Pretreatment with capsaicin, a neurotoxin that shows selectivity for sensory nerves, significantly inhibited the NANC relaxation, suggesting that the EFS-induced NANC relaxation is mediated by sensory nerves. Capsaicin depletes sensory peptides, including CGRP and substance P, when administered systemically at high doses (Buck & Burks 1986; Wharton et al 1986) and desensitizes sensory nerves when given locally (Maggi et al 1987, 1990). Capsaicin-sensitive sensory nerves are a heterogeneous population and the content of their nerve endings varies between vascular beds (Maggi 1995).

Recently, it has become clear that other non-peptide substances may be released from sensory nerves, including ATP and NO (Ahluwalia & Cellek 1997). ATP acts as a vasoconstrictor via interaction with P_{2X}-purinoceptors located on vascular smooth muscle (Chapal & Loubatieres-Mariani 1983; Kennedy & Burnstock 1985; Kennedy et al 1985) and causes vascular relaxation by activation of P_{2Y}-purinoceptors located in the endothelium (DeMey & Vanhoutte 1981). ATP has been previously shown to mediate NANC relaxation to EFS in pulmonary arteries (Liu et al 1992), portal vein (Brizzolara et al 1993) and lamb coronary small arteries (Simonsen et al 1997).

In our study, to investigate the involvement of endogenously released dilators in the responses to EFS, frequency–response curves were constructed in the presence and absence of antagonists and enzyme inhibitors. To determine whether sensory nerve-released ATP is involved, vessels were pretreated for 10 min with the P_1-purinoceptor antagonist 8-phenyltheophylline (10 μM), the P_{2X}-purinoceptor antagonist Evans blue (10 μM) or the P_{2Y}-purinoceptor antagonist basilen blue (100 μM). To determine whether the NO-guanylate cyclase pathway is involved, endothelium-intact vessel rings were pretreated for 10 min with L-NAME (100 μM) or ODQ (1 μM) to inhibit NOS or soluble guanylate cyclase, respectively. To determine whether prostanoid generation is involved, vessel rings were pretreated with the cyclooxygenase inhibitor indomethacin (10 μM) for 10 min. The frequency–response curves in drug-treated vessels were compared with those produced in control

untreated vessels. Responses to EFS in endothelium-denuded vessels were compared with the responses in endothelium-intact vessels.

We found that ATP caused a concentration-dependent relaxation of pre-contracted vessels that was shifted to the right by the selective P_{2Y}-purinoceptor antagonist basilen blue. Pretreatment with basilen blue also significantly attenuated the relaxation to EFS in endothelium-intact preparations, indicating that EFS-induced relaxation is, in part, mediated by activation of P_{2Y}-purinoceptors. In endothelium-denuded preparations, basilen blue did not affect the responses to EFS or exogenously added ATP. Neuronally released ATP activates P_{2Y}-purinoceptors located on the endothelium in the thoracic aorta of rat. In addition, basilen blue did not change the response to EFS in capsaicin-pretreated preparations, providing evidence that involvement of ATP released from extraneuronal sites can be excluded. The fact that basilen blue only partially inhibited the response to EFS suggests the possibility that EFS may release other mediators together with ATP.

Pretreatment with P_1-purinoceptor antagonist (8-phenyltheophylline) or P_{2X}-purinoceptor antagonist (Evans blue) did not influence the response to EFS or exogenously added ATP, which excludes an involvement of P_1-purinoceptors or P_{2X}-purinoceptors.

If NO was derived from NANC nerve endings, the NO-mediated relaxation would be unaffected by endothelial cell removal. In our study, pretreatment with L-NAME significantly inhibited both the EFS-induced relaxation and the response to exogenously added ATP in endothelium-intact preparations, but had no influence in endothelium-denuded preparations. This suggests that NO is not derived from NANC nerve endings, but from endothelium by neuronally released ATP. This is consistent with the fact that ATP stimulates NO release from endothelium (Kelm et al 1988; Schmidt et al 1988). Moreover, the combination of capsaicin with L-NAME did not affect the response to EFS in endothelium-intact preparations. These findings, taken together, suggest that ATP released from sensory nerves only stimulates NO generation through P_{2Y}-purinoceptors located on the endothelium.

It appears from this study, that the endothelium plays an important role in the NANC relaxant response, since removal of the endothelium resulted in inhibition of EFS-induced relaxation. Prostanoid generation was not involved in this endothelium-dependent response since indomethacin had no effects on the EFS response. In addition, EFS-induced relaxation was attenuated by ODQ, a soluble guanylate cyclase specific inhibitor, confirming that NO released from endothelium activates soluble guanylate cyclase.

In summary, our findings suggest that the NANC nerve system is present in the thoracic aorta of rat, mediating vasodilatation by sensory nerves. ATP, as a neurotransmitter released from sensory nerves, activates P_{2Y}-purinoceptor located on the endothelium and stimulates the NO/cyclic GMP pathway, resulting in vasodilatation.

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