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Inhibitory and facilitatory presynaptic effects of endothelin on sympathetic cotransmission in the rat isolated tail artery

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1 The present study was undertaken to determine the modulatory effects of the endothelin peptides on the neurogenically-induced release of endogenous noradrenaline (NA) and the cotransmitter adenosine 5′-triphosphate (ATP) from the sympathetic nerves of endothelium-free segments of the rat isolated tail artery. The electrical field stimulation (EFS, 8 Hz, 0.5 ms, 3 min) evoked overflow of NA and ATP, in the absence of endothelins, was 0.035 ± 0.002 pmol mg^{-1} tissue and 0.026 ± 0.002 pmol mg^{-1} tissue, respectively.

2 Endothelin-1 (ET-1; 1–30 nM) significantly reduced the EFS evoked overflow of both NA and ATP. The maximum inhibitory effect was produced by a peptide concentration of 10 nM, the amount of NA overflow being 0.020 ± 0.002 pmol mg^{-1} and that of ATP overflow 0.015 ± 0.001 pmol mg^{-1}. Higher peptide concentrations (100 and 300 nM) reversed the EFS-evoked overflow of NA to control levels and that of ATP to above control levels. The inhibitory effect of ET-1 (10 nM) was resistant to the selective ET_A receptor antagonist cyclo-D-Trp-D-Asp(ONa)-Pro-D-Val-Leu (BQ-123) but was prevented by ET_B receptor desensitization with sarafotoxin S6c (StxS6c) or by ET_B receptor blockade with N, *cis*-2,6-dimethylpiperidinocarbonyl-L-gmethylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ-788).

3 StxS6c, upon acute application, exerted a dual effect on transmitter release. At concentrations of 0.001–0.3 nM the peptide significantly reduced the EFS-evoked NA overflow, whereas at concentrations of 1–10 nM it caused a significant increase in the evoked overflow of both ATP and NA. Both the maximum inhibitory effect of StxS6c at a concentration of 0.003 nM (approximately 85% reduction of NA overflow and 40% of ATP overflow) and the maximum facilitatory effect of the peptide at a concentration of 3 nM (approximately 400% increase of ATP overflow and 200% of NA overflow) were completely antagonized by either BQ-788 or by StxS6c-induced ET_B receptor desensitization.

4 ET-3 (10–100 nM) did not affect the EFS evoked overflow of either ATP or NA, but at a concentration of 300 nM significantly potentiated the release of both transmitters (0.118 ± 0.02 pmol mg^{-1} tissue ATP overflow and 0.077 ± 0.004 pmol mg^{-1} NA overflow). This effect was prevented either by BQ-123 or by BQ-788.

5 In summary, the endothelin peptides exerted both facilitatory and inhibitory effects on the neurogenically-induced release of the sympathetic cotransmitters ATP and NA in the rat tail artery. Both transmitters were modulated in parallel indicating that the endothelins do not differentially modulate the release of NA and ATP in this tissue.

Keywords: Endothelin-1; endothelin-3; sarafotoxin S6c (StxS6c); cotransmission; rat tail artery; noradrenaline (NA); ATP

Introduction

Endothelin, discovered as a potent vasoactive peptide produced by cultured endothelial cells (Yanagisawa *et al*., 1988a), comprises a family of peptides, endothelin-1 (ET-1), ET-2 and ET-3, that appear to function predominantly as local, rather than circulating hormones (Yanagisawa *et al*., 1988b; Inoue *et al*., 1989). Sarafotoxins, isolated from the venom of the snake *Atractaspis engaddensis*, possess structural features and pharmacological activities similar to those of the endothelins (Kloog *et al*., 1988).

Since their discovery, the biological activity of the endothelins has been exemplified by their profound actions on the cardiovascular system. It is quite likely that these endogenous peptides have physiological and pathological significance in cardiovascular homeostasis (see Rubanyi & Polokoff, 1944 for review). Besides their potent direct vasoconstrictor actions endothelin peptides may exert indirect control of vascular tone via modulation of sympathetic neurotransmission. Indeed, shortly after its identification, endothelin was shown to potentiate nerve-induced contractions in a number of vascular and non-vascular tissues (Hiley *et al*., 1989; Maggi *et al*., 1989; Tabuchi *et al*., 1989a; Wiklund *et al*., 1989; Mutafova-Yambolieva *et al*., 1992). Furthermore, ET-1 has been shown to reduce [^{3}H]-noradrenaline ([^{3}H]-NA) release in some sympathetically innervated tissues such as the rat mesenteric artery (Tabuchi *et al*., 1989b), guinea-pig femoral artery, pulmonary artery and vas deferens (Wiklund *et al*., 1988; 1989; 1990). Most experimental evidence indicates that endothelins (especially ET-1) inhibit NA release from sympathetic nerve terminals, but potentiate the biological actions of NA on postjunctional sites, thus leading Wiklund *et al*. (1991) to postulate that 'a general pattern in the neuromodulation by the endothelins is seen: a prejunctional inhibitory effect and a stimulatory postjunctional effect'. However, neurotransmission to various vascular and non-vascular sympathetically innervated tissues involves at least two cotransmitters, adenosine 5′-triphosphate (ATP) and NA (Sneddon & Westfall, 1984; Burnstock, 1990). When a substance affects the release of NA it has often been assumed that the release of ATP will be similarly influenced. However,

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the validity of this assumption has been questioned by findings which imply that the two cotransmitters are not always released or modulated in parallel (Burnstock, 1990; Msghina *et al*., 1992; Todorov *et al*., 1994; 1996; Driessen *et al*., 1996). Moreover, a differential modulation of the release of ATP and NA has been found for ET-3 in the guinea-pig vas deferens (Mutafova-Yambolieva & Westfall, 1995), suggesting that this may also be the case in blood vessels. Indirect evidence for modulatory effects of ET-1 on the purinergic component of the nerve-induced contraction has also been obtained in the rat vas deferens (Mutafova-Yambolieva & Radomirov, 1993) and the rabbit saphenous artery (Mutafova-Yambolieva & Radomirov, 1994). Although there is evidence for ET receptors in neuronal tissues (Jones *et al*., 1991; Takimoto *et al*., 1993), the functional role of these neuronal receptors in the modulation of neurotransmitter release by endothelin has not been investigated.

Therefore, in the present study we examined the effects of endothelin peptides and putative ET receptor antagonists on the neurogenically-induced release of NA and ATP in endothelium-free segments of rat tail artery—a model tissue for studying sympathetic cotransmission (Sneddon & Burnstock, 1985; Westfall *et al*., 1987; Bao *et al*., 1990; 1993).

Methods

Male Wistar rats (weighing 220 to 280 g) were killed by CO_2 overdose followed by exsanguination. As long a segment as possible of ventral caudal artery (generally about 10 cm) was removed and cleaned of connective tissue while being kept in oxygenated physiological salt solution (PSS). After being flushed with PSS the artery was cut into 3 mm ring segments. The lumen of each segment of artery was gently rubbed by passing a syringe needle back and forth. Following this procedure, the absence of endothelium was confirmed histologicaly in some segments with a silver nitrate staining technique (Abrol *et al*., 1984). Each experiment consisted of placing the denuded rings of one tail artery into a 200 μl plastic superfusion chamber. The top and the bottom of each chamber were fitted with platinum nets that served as the stimulating electrodes. The tissues were superfused at a rate of 1.85 ml min^{-1} with PSS of the following composition (mM): NaCl 110, KCl 4.6, $CaCl_2$ 2.5, $NaHCO_3$ 24.8, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 5.6. The solution was maintained at 35°C and continually bubbled with 95% O_2 and 5% CO_2. After 60 min equilibration, the tissues were subjected to a 'conditioning' stimulation, which consisted of a 3 min period of electrical field stimulation (EFS) with square wave pulses of 0.5 ms duration and a frequency of 8 Hz delivered from a Grass S48 stimulator. A second 3 min stimulation was applied 30 min later at which time superfusion solution was collected for the analysis of NA and ATP. Three minute samples of superfusion solution were collected before and during the EFS and analysed for the content of ATP by high-performance liquid chromatography (h.p.l.c.) fluorescent detection and for NA by h.p.l.c.-electrochemical detection, as described in detail previously (Sedaa *et al*., 1990).

ET-1, ET-3 or sarafotoxin S6c (StxS6c, acute administration) were added 20 min before the EFS. In some experiments the effects of ET-1, ET-3 or StxS6c were examined after pretreating tissues for 20 min either with the ET_A receptor antagonist (cyclo-(D-Trp-D-Asp(ONa)-Pro-D-Val-Leu) (BQ-123; 1 or 10 μM), with the ET_B receptor antagonist (N, *cis*-2,6-dimethylpiperidinocarbonyl-L-gmethyl-leucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine) (BQ-788, 1 μM) or with the combination of both BQ-123 and BQ-788. In other experiments the tissues were pretreated for 45 min with StxS6c (150 nM) to desensitize the ET_B receptors (Henry, 1993), then washed with PSS for 30 min before application of the endothelin peptides. The effect of only one concentration of endothelin was tested in a single tissue.

To evaluate the major source of electrically-evoked ATP overflow in this study separate sets of experiments were performed consisting of: (i) 30 min pretreatment with tetrodotoxin (TTX) at a concentration of 1 μM; (ii) 120 min pretreatment with guanethidine at a concentration of 10 μM; (iii) 20 min pretreatment with prazosin at a concentration of 1 μM; (iv) pretreatment with prazosin (1 μM) and suramin (300 μM) for 20 min; (v) pretreatment for 3–5 min with methoxamine at a concentration of 20 μM. In other experiments the animals were treated subcutaneously with reserpine, 48 h (5 mg kg^{-1}) and 24 h (2.5 mg kg^{-1}) before the experiment (Khoyi *et al*., 1988), in order to deplete noradrenaline. In some experiments tissues from reserpine-pretreated animals were exposed for 20 min to suramin (300 μM).

Data are presented as means ± s.e.mean. Means were compared by Student's unpaired *t* test. A probability value of less than 0.05 was considered significant. The results were obtained from 316 animals (respectively number of experiments, *n*).

Materials

Endothelin-1 (ET-1), endothelin-3 (ET-3), tetrodotoxin (TTX), prazosin, methoxamine, reserpine and guanethidine were obtained from Sigma Chemical Company (Saint Louis, MO, U.S.A.). Sarafotoxin S6c (StxS6c), cyclo(-D-Trp-D-Asp(ONa)-Pro-D-Val-Leu (BQ-123), N,*cis*-2,6-dimethylpiperidinocarbonyl-L-gmethylleucyl-D-1-methoxycarbonyltryptophanyl-D-norleucine (BQ-788) were obtained from Research Biochemical International (Natick, MA, U.S.A.), and suramin from Calbiochem (La Jolla, CA, U.S.A.). StxS6c, BQ-123 and BQ-788 were dissolved in 0.1% bovine serum albumin in saline and further diluted in PSS. Reserpine was dissolved in 20% ascorbic acid. All other drugs were initially dissolved in redistilled water and further diluted in PSS.

Results

Electrically-evoked overflow of NA and ATP

There were no detectable amounts of either ATP or NA in samples collected for 3 min before stimulation of endothelium-free rat tail artery (data not shown). The EFS-evoked overflow of both ATP and NA and the effects of different compounds on this overflow are shown in Figure 1. The overflow of both ATP and NA was significantly reduced after 1 μM TTX or 10 μM guanethidine (Figure 1a). In the preparations from reserpine-pretreated rats the overflow of NA was almost abolished but that of ATP was not affected (Figure 1b). The overflow of NA was enhanced after pretreatment with prazosin (1 μM), but the overflow of ATP was unchanged (Figure 1b). Addition of suramin (300 μM) to the prazosin-pretreated tissues was no more effective than was prazosin alone, i.e. increased NA overflow and unmodified ATP overflow (Figure 1c). Suramin applied to tissues from rats treated with reserpine did not affect the overflow of ATP

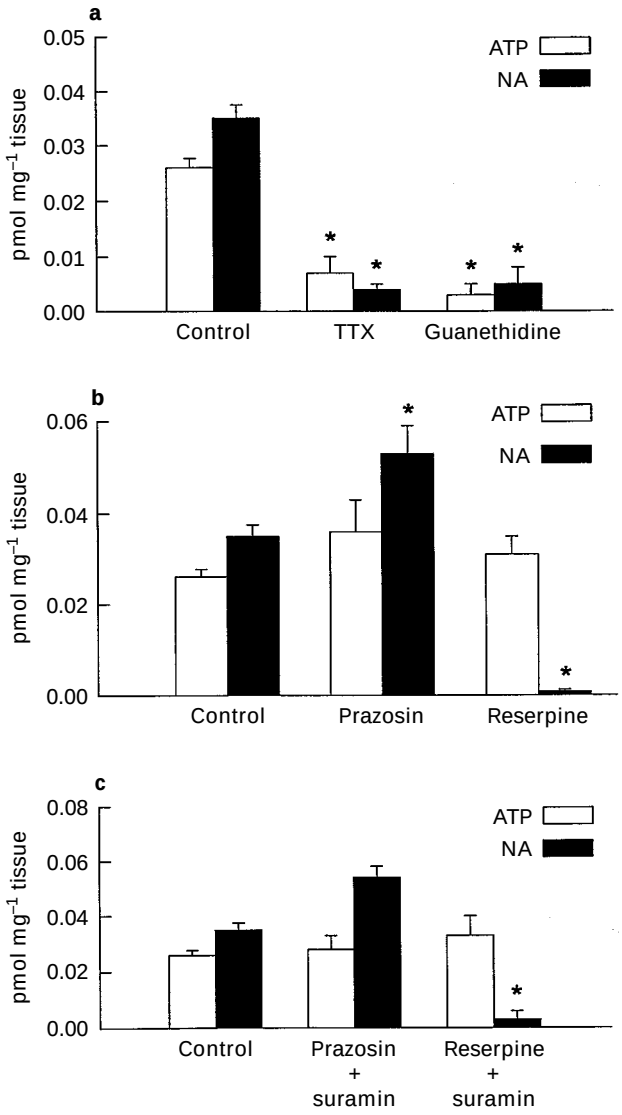

Figure 1 Overflow of ATP and noradrenaline (NA) in endothelium-free segments of the rat isolated tail artery evoked by electrical field stimulation (EFS) of 0.5 ms pulses at 8 Hz for 3 min. (a) The effects of treatment with tetrodotoxin (TTX; 1 μM) and guanethidine (10 μM) on the EFS evoked overflow of ATP and NA. (b) The effects of treatment with prazosin (1 μM) and pretreatment of animals with reserpine (5 mg kg^{-1} at 48 h and 2.5 mg kg^{-1} at 24 h before the experiment) on the EFS-evoked overflow of ATP and NA. (c) The effects of combined prazosin and suramin (300 μM) treatment and combined suramin and reserpine pretreatment on the EFS evoked overflow of ATP and NA. Each column shows the mean and s.e.means of 4–13 experiments. In this and subsequent figures the asterisk indicates a significant difference from control, $P<0.05$.

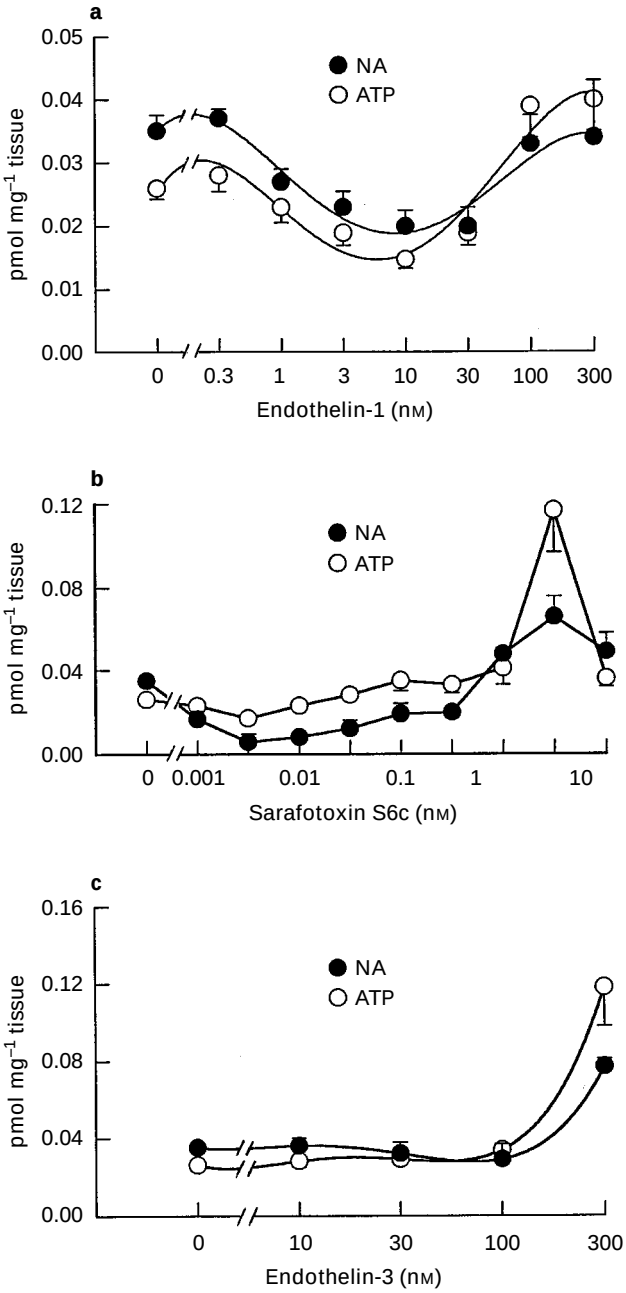

Figure 2 The effect of endothelin-1 (ET-1), sarafotoxin S6c (StxS6c) and endothelin-3 (ET-3) on the overflow of ATP and NA (pmol mg^{-1} tissue) in endothelium-free segments of the rat isolated tail artery evoked by EFS of 0.5 ms at 8 Hz for 3 min. (a) Concentration-dependent effects of ET-1. (b) The effect of acute application of StxS6c. (c) The effect of ET-3. Each point represents the mean of 4–9 experiments; vertical lines show s.e.mean.

(Figure 1c). Methoxamine (30 μM) did not evoke the appearance of measurable amounts of ATP or of NA in prestimulation samples (data not shown).

Effects of ET-1, ET-3 and StxS6c on electrically-evoked overflow of NA and ATP

In resting (no EFS) conditions ET-1, ET-3 or StxS6c did not cause release of ATP or NA (data not shown). The effects of increasing concentrations of ET-1 on the electrically-induced overflow of NA and ATP are shown in Figure 2a. ET-1 had a bell-shaped effect on the overflow of NA consisting of a reduction in NA overflow at concentrations of 1–30 nM and reversal of the NA overflow to control levels upon application of higher peptide concentrations (100 and 300 nM). ET-1 produced similar effects on the overflow of ATP, reaching the maximum inhibitory effect at a concentration of 10 nM. Higher peptide concentrations (100 and 300 nM) led to a significant elevation of ATP overflow. StxS6c (acute application) had a dual effect on the EFS-evoked overflow of NA consisting of a significant reduction of the overflow at peptide concentrations of 0.001–0.3 nM and significant potentiation at concentrations of 1–10 nM (Figure 2b). The maximum inhibitory effect was reached at a concentration of 0.003 nM and the maximum facilitatory effect at a concentration of 3 nM. The effects of StxS6c on

the overflow of ATP resembled those on the overflow of NA, though with regards to ATP overflow the inhibitory effect was less pronounced and the facilitatory effect more pronounced than that on NA overflow. Thus, in the presence of 0.003 nM StxS6c the overflow of ATP was reduced from 0.026 ± 0.001 pmol mg^{-1} tissue in controls ($n=10$) to 0.017 ± 0.002 pmol mg^{-1} tissue ($n=4$), $P<0.01$, whereas the overflow of NA was decreased from 0.035 ± 0.002 pmol mg^{-1} tissue ($n=13$) in controls to 0.005 ± 0.001 pmol mg^{-1} tissue ($n=4$) in the presence of StxS6c at 0.003 nM, $P<0.001$. ET-3 did not modify the EFS-induced overflow of either ATP or NA at concentrations of 10 to 100 nM, but did cause significant potentiation of both ATP and NA overflow at a concentration of 300 nM (Figure 2c).

Effect of BQ-123, BQ-788 or desensitization of ET_B receptors by StxS6c on the effects of ET-1, StxS6c and ET-3 on the overflow of ATP and NA

The most pronounced effects of the three peptides (i.e., ET-1 at a concentration of 10 nM, StxS6c at concentrations of 0.003 nM and 3 nM and ET-3 at a concentration of 300 nM) were further characterized by use of the selective ET_A receptor antagonist BQ-123 (Ihara *et al*., 1992) and the selective ET_B receptor antagonist BQ-788 (Ishikawa *et al*., 1994). Potential ET_B-like receptor-mediated effects were also studied by prolonged incubation of the tissue with StxS6c leading to desensitization of ET_B receptors (Henry, 1993).

Neither BQ-123, BQ-788 nor desensitization of ET_B receptors by StxS6c modified significantly the EFS-induced overflow of ATP (Table 1). The overflow of NA was unaffected by BQ-788 and BQ-123, but significantly inhibited by StxS6c-induced ET_B receptor desensitization. The inhibitory effect of ET-1 on the overflow of both ATP and NA was not modified by pretreatment with BQ-123 at concentrations of 1 or 10 μM but was completely prevented by BQ-788. StxS6c-induced desensitization of ET_B receptors abolished the effect of ET-1 on the overflow of ATP.

The inhibitory effect of StxS6c at a concentration of 0.003 nM on the electrically-induced overflow of NA was significantly reduced from 0.006 ± 0.001 pmol mg^{-1} tissue ($n=6$) in the absence of BQ-788 to 0.032 ± 0.04 pmol mg^{-1} tissue ($n=5$) in the presence of BQ-788 and to 0.046 ± 0.008 ($n=4$) after StxS6c-induced desensitization. The facilitatory effect of StxS6c at a concentration of 3 nM on the overflow of both ATP and NA was also completely prevented either by BQ-788 or by StxS6c-induced ET_B receptor densensitization (Figure 3).

The potentiating effect of ET-3 at a concentration of 300 nM on the EFS-induced overflow of both ATP and NA was prevented by either BQ-123 or BQ-788, and in fact BQ-123 reversed the action of ET-3 on NA overflow i.e. after treatment with BQ-123, ET-3 caused a significant inhibition of electrically-evoked release of NA (Figure 4).

Table 1 Effect of ET-1 (10 nM) and the ET receptor antagonists BQ-123 (10 μM), BQ-788 (1 μM) and StxS6c (150 nM)-induced desensitization (StxS6c des) on the electrically-evoked overflow of ATP and NA in the rat tail artery

	ATP overflow (fmol mg^{-1} tissue)	*NA overflow* (fmol mg^{-1} tissue)
Control	26±1 (10)	35±2 (13)
ET-1	15±1* (7)	20±2* (7)
BQ-123	26±3 (6)	43±6 (6)
BQ-123 + ET-1	17±1* (4)	23±3* (4)
BQ-788	29±3 (5)	27±2 (5)
BQ-788 + ET-1	30±2 (7)	24±3 (7)
StxS6c des	31±5 (7)	16±3* (7)
StxS6c des + ET-1	28±5 (6)	55±7* (6)

Data are expressed as means±s.e.mean. Number of experiments in parentheses. *Significant difference ($P<0.05$) in comparison to the corresponding control.

Discussion

Wiklund *et al*. (1988, 1990, 1991) and Tabuchi *et al*. (1989b) have shown that ET-1 causes a reduction of the nerve-induced release of NA. The present study confirms these observations

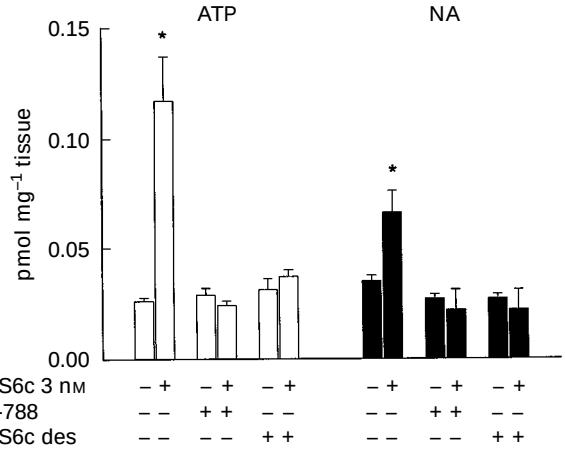

Figure 3 The effect of StxS6c (3 nM) on the overflow of ATP and NA (pmol mg^{-1} tissue) in endothelium-free segments of the rat isolated tail artery evoked by EFS of 0.5 ms at 8 Hz for 3 min before and after treatment with BQ-788 (1 μM) or desensitization (des) of ET_B receptors by StxS6c at 150 nM for 45 min. Each column shows the mean and s.e.means of 4–7 experiments.

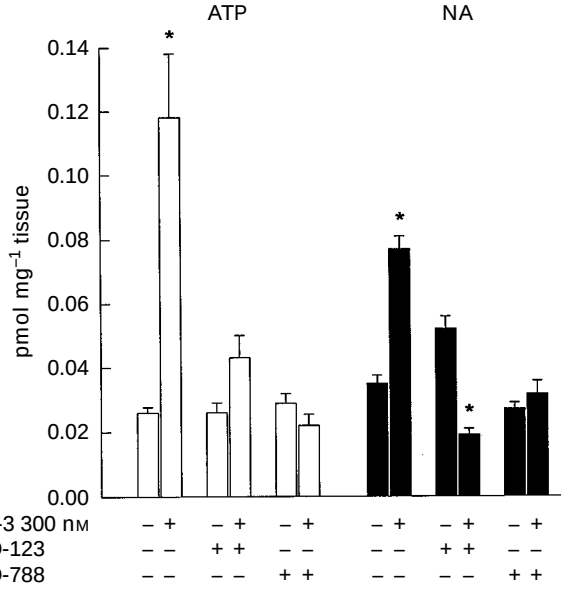

Figure 4 The effect of ET-3 (300 nM) on the overflow of ATP and NA (pmol mg^{-1} tissue) in endothelium-free segments of the rat isolated tail artery evoked by EFS of 0.5 ms at 8 Hz for 3 min before and after treatment with BQ-123 (1 μM) or BQ-788 (1 μM). Each column shows the mean and s.e.means of 4–7 experiments.

and, in addition, provides information about other endothelins, as well as describing a methodology that allows for the simultaneous evaluation of the release of both endogenous NA and ATP. Our results suggest that these peptides lead to both inhibitory and facilitatory effects on the release of NA and ATP and, also, indicate that endothelins do not differentially modulate the release of the cotransmitters in this tissue.

It is generally accepted that nerve stimulation-evoked release of NA originates exclusively from sympathetic neurones. However, ATP may originate from either neuronal or extraneuronal sources. The endothelium is considered to be the major extraneuronal source of ATP (Pearson & Gordon, 1979; Sedaa *et al*., 1990; Buxton *et al*., 1990; Shinozuka *et al*., 1991; 1994). Since the goal of the present study was to explore the effects of endothelins on the neuronal release of ATP, we employed endothelium-free arteries. Functional and histological evidence indicated that the endothelium had been removed. For example, methoxamine, an agent shown to cause the release of ATP predominantly from the endothelium (Sedaa *et al*., 1990; Shinozuka *et al*., 1991) failed to release measurable amounts of ATP in the samples collected before EFS. Further, the EFS-evoked overflow of ATP was almost equal to the overflow of NA—a finding indicating non-endothelium origin of ATP (Sedaa *et al*., 1990). Besides the endothelium, it has been suggested that ATP can originate from the smooth muscle cells upon contraction evoked either by neuronally released NA (NA-evoked ATP overflow) or by neuronally released ATP (ATP-evoked ATP overflow). However, in the present study the overflow of ATP was apparently independent of NA action, because the ATP overflow was not reduced either by depletion of NA following reserpine pretreatment or by α_1-adrenoceptor blockade with prazosin. Pretreatment with suramin, an antagonist of P2-postsynaptic purinoceptors, did not reduce ATP overflow either when suramin was applied alone or when it was administered in tissues from reserpine-treated animals, thus providing evidence that the EFS-evoked overflow of ATP was not an 'ATP-evoked' overflow. Finally, the electrically-induced overflow of both ATP and NA was significantly reduced after pretreatment with tetrodotoxin or guanethidine, indicating that the overflow of both substances was action potential induced. We conclude, therefore, that the ATP overflow in this study reflects primarily release from sympathetic nerves.

The present study confirms the observations that ET-1 inhibits NA release and, in addition, provides evidence that ET-1 also causes a reduction of the nerve-induced release of ATP. The inhibitory effect of ET-1 on the EFS-evoked overflow of cotransmitters was not antagonized by the ET_A-receptor antagonist BQ-123 but was completely sensitive to ET_B receptor blockade (BQ-788 or StxS6c induced ET_B receptor-desensitization), indicating that the inhibitory effect of ET-1 on the release of both ATP and NA may be mediated by ET_B-like receptors. If this is the case, one would expect the ET_B-specific receptor agonist StxS6c (acute application) to cause inhibition of transmitter release as well. Indeed, in the picomolar concentration range StxS6c caused inhibition of the evoked NA and ATP release. Moreover, this effect was abolished by both BQ-788 or by StxS6c-induced ET_B receptor desensitization, supporting the notion that the inhibitory effects on neurotransmitter release may be mediated by presynaptic ET_B-like receptors.

Interestingly, StxS6c exerted a dual effect. In addition to its inhibitory action at pmol concentrations this peptide facilitated the release of both ATP and NA at nanomolar concentrations. This facilitatory effect did not occur following ET_B receptor blockade, indicating that this effect of StxS6c may also be mediated by ET_B-like receptors. There is precedence for the idea that endothelins may increase transmitter release. For example, in studies with the rabbit isolated bronchus and rat iris sphincter, ET-3 potentiated cholinergic nerve induced contractions without affecting the postjunctional sensitivity to cholinoceptor agonists. Therefore, it was concluded that ET-3 enhanced transmitter release (McKay *et al*., 1993; Shinkai *et al*., 1994). In a previous study with guinea-pig vas deferens, we also found that endothelins produce both facilitatory and inhibitory effect on transmitter release (Mutafova-Yambolieva & Westfall, 1995). The present study provides another example of the existence of both facilitatory and inhibitory effects of the endothelins on transmitter release from a single tissue, in this case a blood vessel. Interestingly, the desensitization of the ET_B receptors by prolonged administration of StxS6c significantly reduced NA overflow and did not affect ATP overflow. The reason for this differential modulation of electrically-evoked overflow of the two neurotransmitters is unknown at present.

Considering the uniform agreement that ET_B receptors are nonselective (Sakurai *et al*., 1992), one would expect ET-3 to exert similar effects to those of ET-1. However, the effects of this peptide are unusual. ET-3 did not have any effect on transmitter release at concentrations up to 100 nM, but caused an increase in the evoked release of both ATP and NA at a concentration of 300 nM. We hypothesized that this facilitatory effect of ET-3 was mediated by the facilitatory ET_B-like (thus BQ-788-sensitive) receptors. Indeed, BQ-788 completely blocked this effect of ET-3. In addition, the potentiation was sensitive to the ET_A-selective antagonist BQ-123. Thus the receptors that mediate the facilitatory actions of ET-3 are sensitive to both BQ-123 and BQ-788. Whether or not this receptor is the same as that (presumably ET_B-like) which mediates the facilitatory effect of StxS6c was not determined. The presence of an atypical receptor, specific for ET-3, cannot be ruled out. It is interesting to note that these findings are similar to previous observations concerning the facilitatory effects of ET-3 on the evoked release of ATP in the guinea-pig vas deferens, in that the facilitatory effect was sensitive to both BQ-123 and desensitization of ET_B receptors with StxS6c (Mutafova-Yambolieva & Westfall, 1995).

The distinctive profiles of two receptor subtypes (ET_A and ET_B) for ET-1 and ET-3 has led to the ET-1/ET-3 potency ratio being used to classify the receptors which mediate the functional responses of the endothelins. However, the presence of both facilitatory and inhibitory actions of endothelin peptides in this tissue makes the characterization of the ET receptors difficult. In other words, the responses mediated by the one type of receptor (e.g. inhibitory) may be confounded by a concomitant affinity for a receptor subtype mediating the opposite (e.g. facilitatory) effect. The two opposing effects of the endothelin peptides appear to be mediated by at least two functionally distinct receptors, which pharmacologically could be characterized as: (1) an inhibitory receptor activated by ET-1 and StxS6c but not ET-3, which can be desensitized by StxS6c, antagonized by BQ-788 but is insensitive to BQ-123, and (2) an excitatory receptor activated by both ET-3 and StxS6c, but not by ET-1 which can be desensitized by StxS6c and is antagonized by both BQ-788 and BQ-123.

In conclusion, this study provides evidence that the endothelin peptides may exert both facilitatory and inhibitory effects on the neuronally-induced release of transmitters at the autonomic sympathetic neuroeffector junction in rat tail artery and that these neuromodulatory effects may be mediated by two subtypes of endothelin receptor. The peptide action,

therefore, is the result of two counteracting effects. Further, it appears that in the rat tail artery (in contrast to the guinea-pig vas deferens, e.g. Mutafova-Yambolieva & Westfall, 1995) the endothelins do not differentially modulate the release of the cotransmitters, ATP and NA.

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References

ABROL, R.P., HUGHES, V.M., KRUEGER, C.A. & COOK, D.A. (1984). Detection of endothelium in cerebral blood vessels. *J. Pharmacol. Methods*, **12,** 213–219.

BAO, J.X., ERIKSSON, I.E. & STJÄRNE, L. (1990). Neurotransmitters and pre- and post-junctional receptors involved in the vasoconstrictor response to sympathetic nerve stimulation in rat tail artery. *Acta Physiol. Scand.*, **140,** 467–479.

BAO, J.X., GONNON, F. & STJÄRNE, L. (1993). Kinetics of ATP- and noradrenaline-mediated sympathetic transmission in rat tail artery. *Acta Physiol. Scand.*, **149,** 503–519.

BURNSTOCK, G. (1990). Co-transmission. *Arch. Int. Pharmacodyn.*, **304,** 7–33.

BUXTON, I.L.O., WALTHER, J. & WESTFALL, D.P. (1990). Adrenergic receptor-stimulated release of ATP from cardiac endothelial cells in primary culture. In *Biological Actions of Extracellular ATP*. ed. Dubyak, G.R. & Fedan, J.S., pp. 503–506. *Ann. New York Acad. Sci.*, Vol 603.

DRIESSEN, B., BÜLTMANN, R., GONÇALVES, J. & STARKE, K. (1996). Opposite modulation of noradrenaline and ATP release in guinea-pig vas deferens through prejunctional beta-adrenoreceptors: evidence for the beta 2 subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **353,** 564–571.

HENRY, P.J. (1993). Endothelin-1 (ET-1)-induced contracton in rat isolated trachea: involvement of ET_A and ET_B receptors and multiple signal transduction systems. *Br. J. Pharmacol.*, **110,** 435–441.

HILEY, C.R., PELTON, J.T. & MILLER, R.C. (1989). Effects of endothelin on field stimulated rat vas deferens and guinea-pig ileum. *Br. J. Pharmacol.*, **96,** 104P.

IHARA, M., NOGUCHI, K., SAEKI, T., FUKURODA, T., TSUCHIDA, S., KIMURA, S., FUKAMI, T., ISHIKAWA, K., NISHIKABE, M. & YANO, M. (1992). Biological profiles of highly potent novel endothelin antagonists selective for the ET_A receptor. *Life Sci.*, **50,** 247–255.

INOUE, A., YANAGISAWA, M., KIMURA, S., KASUYA, Y., MIYAUCHI, T., GOTO, K. & MASAKI, T. (1989). The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. *Proc. Natl. Acad. Sci. U.S.A.*, **86,** 2863–2867.

ISHIKAWA, K., IHARA, M., NOGUCHI K., MASE, T., MINO, N., SAEKI, T., FUKURODA, T., FUKAMI, T., OZAKI, S.T., NISHIKABE, M. & YANO, M. (1994). Biochemical and pharmacological profile of a potent and selective endothelin b-receptor antagonist, BQ-788. *Proc. Natl. Acad. Sci. U.S.A.*, **91,** 4892–4896.

JONES, C.R., HILEY, C.R., PELTON, J.T. & MILLER, R.C. (1991). Endothelin receptor heterogeneity; structure activity, autoradiographic and functional studies. *J. Receptor Res.*, **11,** 299–310.

KHOYI, M.A., WESTFALL, D.P., BUXTON, I.L.O., AKHTAR-KHAVARI, F., REZAIE, E., SALAICES, M. & SANCHEZ-GARCIA, P. (1988). Norepinephrine and potassium induced calcium translocation in rat vas deferens. *J. Pharmacol. Exp. Ther.*, **246,** 917–923.

KLOOG, Y., AMBAR, I., SOKOLOVSKY, M., KOCHYA, E., WOLLBERG, Z. & BDOLAH, A. (1988). Sarafotoxin, a novel vasoconstrictor peptide: phosphoinositide hydrolysis in rat heart and brain. *Science*, **242,** 268–270.

MAGGI, C.A., GUILIANI, S., PATACCHINI, R., ROVERO, P., GIACHETTI, A. & MELI, A. (1989). The activity of peptides of the endothelin family in various mammalian smooth muscle preparations. *Eur. J. Pharmacol.*, **174,** 23–31.

MCKAY, K.O., ARMOUR, C.L. & BLACK, J.L. (1993). Endothelin-3 increases transmission in the rabbit pulmonary parasympathetic nervous system. *J. Cardiovasc. Pharmacol.*, **22** (Suppl. 8), S181–S184.

MSGHINA, M., MERMET, C., GONNON, F. & STJÄRNE, L. (1992). Electrophysiological and electrochemical analysis of the secretion of ATP and noradrenaline from the sympathetic nerves in rat tail artery: effects of α_2-adrenoceptor agonists and antagonists and noradrenaline reuptake blockers. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **346,** 173–186.

MUTAFOVA-YAMBOLIEVA, V., PETKOV, O., STANEVA-STOYTCHEVA, D. & LASOVA, L. (1992). Interactions between the effects of endothelin-1, clonidine and yohimbine on electrically-induced contractions in rat vas deferens. *Gen. Pharmacol.*, **23,** 529–534.

MUTAFOVA-YAMBOLIEVA, V.N. & RADOMIROV, R.G. (1994). Modulatory effects of endothelin-1 on purinergic and adrenergic components of sympathetically-mediated contractile activity of rabbit saphenous artery. *Br. J. Pharmacol.*, **112,** 1109–1117.

MUTAFOVA-YAMBOLIEVA, V. & RADOMIROV, R. (1993). Effects of endothelin-1 on postjunctionally-mediated purinergic and adrenergic components of rat vas deferens contractile responses. *Neuropeptides*, **24,** 35–42.

MUTAFOVA-YAMBOLIEVA, V.N. & WESTFALL, D.P. (1995). Endothelin-3 can both facilitate and inhibit transmitter release in the guinea-pig vas deferens. *Eur. J. Pharmacol.*, **285,** 213–216.

PEARSON, J.D. & GORDON, J.L. (1979). Vascular endothelial and smooth muscle cells in culture selectively release adenine nucleotides. *Nature*, **281,** 384–386.

RUBANYI, G.M. & POLOKOFF, M.A. (1994). Endothelins: molecular biology, biochemistry, pharmacology, physiology and pathophysiology. *Pharmacol. Rev.*, **46,** 325–415.

SAKURAI, T., YANAGISAWA, M. & MASAKI, T. (1992). Molecular characterization of endothelin receptors. *Trends Pharmacol. Sci.*, **13,** 103–108.

SEDAA, K.O., SHINOZUKA, K., BJUR, R.A. & WESTFALL, D.P. (1990). Nerve and drug-induced release of adenine nucleosides and nucleotides from rabbit aorta. *J. Pharmacol. Exp. Ther.*, **252,** 1060–1067.

SHINKAI, M., TSURUOKA, H., WAKABAYASHI, S., YAMAMOTO, Y. & TAKAYANAGI, I. (1994). Pre- and post-junctional actions of endothelin in the rat iris sphincter preparation. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **350,** 63–67.

SHINOZUKA, K., HASHIMOTO, M., MASUMURA, S., BJUR, R.A., WESTFALL, D.P. & HATTORI, K. (1994). *In vitro* studies of release of adenine nucleotides and adenosine from rat vascular endothelium in response to a1-adrenoceptor stimulation. *Br. J. Pharmacol.*, **113,** 1203–1208.

SHINOZUKA, K., SEDAA, K.O., BJUR, R.A. & WESTFALL, D.P. (1991). Participation by purines in the modulation of norepinephrine release by methoxamine. *Eur. J. Pharmacol.*, **192,** 431–434.

SNEDDON, P. & BURNSTOCK, G. (1985). ATP as a co-transmitter in rat tail artery. *Eur. J. Pharmacol.*, **106,** 149–152.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.*, **347,** 561–580.

TABUCHI, Y., NAKAMARU, M., RAKUGI, H., NAGANO, M., MIKAMI, H. & OGIHARA, T. (1989a). Endothelin enhances adrenergic vasoconstriction in perfused rat mesenteric arteries. *Biochem. Biophys. Res. Commun.*, **159,** 1304–1308.

TABUCHI, Y., NAKAMARU, M., RAKUGI, H., NAGANO, M., MIKAMI, H. OGIHARA, T. (1989b). Endothelin inhibits presynaptic adrenergic neurotransmission in rat mesenteric artery. *Biochem. Biophys. Res. Commun.*, **161,** 803–808.

TAKIMOTO, M., INUI, T., TOSHIKAZU, O. & URADE, Y. (1993). Contraction of smooth muscle by activation of endothelin receptors on autonomic neurons. *FEBS*, **324,** 277–282.

TODOROV, L., BJUR, R.A. & WESTFALL, D.P. (1994). Temporal dissociation of the release of the sympathetic cotransmitters ATP and noradrenaline. *Clin. Exp. Pharmacol. Physiol.*, **21,** 931–932.

TODOROV, L.D., MIHAYLOVA-TODOROVA, S., CRAVISO, G.L., BJUR, R.A. & WESTFALL, D.P. (1996). Evidence for the differential release of the cotransmitters ATP and noradrenaline from sympathetic nerves of the guinea-pig vas deferens. *J. Physiol.*, **496,** 731–748.

WESTFALL, D.P., SEDAA, K. & BJUR, R.A. (1987). Release of endogenous ATP from rat caudal artery. *Blood Vessels*, **24,** 125–127.

WIKLUND, N.P., ÖHLÉN, A. & CEDERQVIST, B. (1988). Inhibition of adrenergic neuroeffector transmission by endothelin in the guinea-pig femoral artery. *Acta Physiol. Scand.*, **134,** 311–312.

WIKLUND, N.P., ÖHLÉN, A. & CEDERQVIST, B. (1989). Adrenergic neuromodulation by endothelin in guinea pig pulmonary artery. *Neurosci. Lett.*, **101,** 269–273.

WIKLUND, N.P., ÖHLÉN, A., WIKLUND, C.U., HEDQVIST, P. & GUSTAFSSON, L.E. (1990). Endothelin modulation of neuroeffector transmission in rat and guinea pig vas deferens. *Eur. J. Pharmacol.*, **185,** 25–33.

WIKLUND, N.P., WIKLUND, C.U., CEDERQVIST, B., ÖHLÉN, A., HEDQVIST, P. & GUSTAFSSON, L.E. (1991). Endothelin modulation of neuroeffector transmission in smooth muscle. *J. Cardiovasc. Pharmacol.*, **17** (Suppl. 7), S335–S339.

YANAGISAWA, M., KURIHARA, H., KIMURA, S., TOMOBE, Y., KOBAYASHI, M., MITSUI, Y., YAZAKI, Y., GOTO, K. & MASAKI, T. (1988a). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, **332,** 411–415.

YANAGISAWA, M., INOUE, A., ISHIKAWA, T., KASUYA, Y., KIMURA, S., KUMAGAYE, S., NAKAJIMA, K., WATANABE, T., SAKAKIBARA, S., GOTO, K. & MASAKI, T. (1988b). Primary structure, synthesis and biological activity of rat endothelin, an endothelium derived vasoconstrictor peptide. *Proc. Natl. Acad. Sci. U.S.A.*, **84,** 6964–6967.

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