A study of ATP as a sympathetic cotransmitter in human saphenous vein

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1 Strips of human saphenous veins were superfused with Krebs-Henseleit solution at either 25°C or 37°C. Constrictor responses to electrical stimulation (10 Hz, 40 s) but not to exogenous noradrenaline (0.1, 1 µM) were abolished by guanethidine (10 µM) and tetrodotoxin (1 µM). Hence, responses to electrical stimulation are due to action potential-induced release of sympathetic neurotransmitters.

2 Constrictor responses to electrical stimulation and noradrenaline were reduced by the α_1 -adrenoceptor antagonist, prazosin (0.3 µM) as well as by the α_2 -adrenoceptor antagonist, rauwolscline (1 µM). The combination of prazosin and rauwolscline abolished constrictor responses to noradrenaline at 25°C and 37°C. However, constrictor responses to electrical stimulation were partly resistant to α -adrenoceptor blockade by prazosin and rauwolscline (at 25°C about 30%). Residual constrictor responses to electrical stimulation were also observed in the presence of the combination of prazosin (3 µM) and rauwolscline (10 µM) as well as in the presence of phenoxybenzamine (10 µM).

3 Veins, incubated with [³H]-noradrenaline, released tritium upon electrical stimulation (10 Hz, 40 s). Moreover, electrical stimulation also induced an overflow of ATP amounting to 4.8 ± 1.5 pmol g⁻¹ at 25°C and 2.0 ± 0.5 pmol g⁻¹ at 37°C. Both tritium and ATP overflow were abolished by tetrodotoxin (0.5 µM). The combination of prazosin (0.3 µM) and rauwolscline (1 µM) increased tritium overflow at either 25°C or 37°C by about 120%, but reduced ATP overflow by about 70%. Hence, a significant percentage of the electrically evoked ATP overflow seems to be released from non-neuronal cells upon activation of α -adrenoceptors by endogenous noradrenaline. The remaining ATP overflow, which was resistant to α -adrenoceptor blockade, may reflect neuronally released ATP.

4 ATP (300 µM) and α,β -methylene-ATP (1, 10 µM), both induced constrictor responses. The P₂-purinoceptor antagonist, suramin (300 µM) markedly inhibited constrictor responses to ATP and α,β -methylene-ATP, but not those to electrical stimulation and to noradrenaline. Moreover, suramin (300 µM) failed to diminish the α -adrenoceptor blockade-resistant constrictor response to 10 Hz.

5 In conclusion, constrictor responses to sympathetic nerve stimulation in human saphenous veins are mainly but not exclusively mediated by neuronally released noradrenaline. There is a concomitant release of ATP and noradrenaline. P₂-purinoceptors which mediate vasoconstriction are present; however, a role of neuronally released ATP in constrictor responses to electrical stimulation could not be established. Therefore, the nature of the sympathetic transmitter responsible for α -adrenoceptor blockade-resistant constrictor responses remains unknown.

Keywords: α -Adrenoceptors; P_{2X}-purinoceptors; noradrenaline-ATP cotransmission; ATP release; prazosin; rauwolscline; α,β -methylene-ATP; suramin; human saphenous vein

Introduction

Noradrenaline and ATP are cotransmitters in the postganglionic sympathetic nervous system. In numerous tissues of several animal species ATP or a related nucleotide mediates responses to sympathetic nerve stimulation, which are resistant to α -adrenoceptor blockade (see Burnstock, 1986; 1990; von Kügelgen & Starke, 1991a; Hoyle, 1992; Morris & Gibbins, 1992). α -Adrenoceptor blockade-resistant responses have also been observed in man (Taddei *et al.*, 1989; 1990; Kahan *et al.*, 1992). However, it is an open question whether or not ATP is involved in sympathetic neurotransmission in man and, hence, could be responsible for the reported α -adrenoceptor blockade-resistant responses (Pelleg & Burnstock, 1990; Taddei *et al.*, 1990; Stephens *et al.*, 1992).

The saphenous vein is a commonly used preparation to study sympathetic neurotransmission in man (see Docherty, 1987). The smooth muscle cells of these veins possess α_1 - and α_2 -adrenoceptors both of which mediate vasoconstrictor responses to endogenous and exogenous noradrenaline (Müller-Schweinitzer, 1984; Steen *et al.*, 1984; Docherty & Hyland, 1985a; Docherty, 1987). The same holds true for the saphenous vein of the dog (Sullivan & Drew, 1980; De Mey

& Vanhoutte, 1981; Pereira *et al.*, 1991). Vasoconstrictor responses to sympathetic nerve stimulation in the canine saphenous vein are at least partly resistant to blockade by α -adrenoceptor antagonists (Flavahan & Vanhoutte, 1986b). These non-adrenergic constrictor responses were even more prominent at a temperature of 24°C and seem to be mediated by neuronally released ATP (Flavahan & Vanhoutte, 1986b).

In order to study a possible cotransmitter role of ATP in man, constrictor responses to sympathetic nerve stimulation as well as ATP and noradrenaline overflow were measured in human saphenous veins.

Methods

The study was approved by the local ethics committee. Veins were obtained from patients (21 females, 51–76 years of age, mean age 65.1 ± 1.6 ; 90 males, 42–81 years of age, mean age 62.2 ± 0.9) undergoing open heart surgery for coronary bypass grafting. None of the patients had been treated with drugs known to interact with storage or release mechanism of noradrenaline. Portions of about 3–4 cm of the human saphenous vein were dissected free from surrounding connective tissue and cut into helical strips. The strips were placed

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between two platinum electrodes in a jacketed chamber maintained at either 25°C or 37°C and superfused with Krebs-Henseleit solution at a constant rate of 6 ml min⁻¹ (experiments with overflow measurements 1 ml min⁻¹). The strips were connected to a force-displacement transducer (Biegestab K30, Hugo Sachs Electronics, March-Hugstetten, Germany) for measuring isometric tension with a multi-pen recorder (Rikadenki, Freiburg i. Br., Germany). Resting tension was set at 19.6 mN. Vasoconstrictor responses were measured as the maximum increase in tension (mN) above resting tension. The superfusion fluid was gassed continuously with carbogen (95% O₂:5% CO₂) and passed through a heat exchanger before it reached the vessels. A Stimulator II (Hugo Sachs Electronics) operating in the constant current mode was used for electrical field-stimulation.

Protocol A (measurement of postjunctional responses)

After an equilibration period of 90 min vasoconstrictor responses were elicited by two periods of stimulation (60 min apart). In some experiments each stimulation period consisted of three consecutive electrical stimulations at 1, 4 and 10 Hz (40 s duration, 100 mA current strength, 0.5 ms pulse width; applied at intervals of 5 min). In most other experiments each stimulation period consisted of an electrical stimulation (S₁ or S₂, at either 2.5 or 10 Hz, 40 s) followed, after an interval of 5 min, by a stimulation elicited by exogenous noradrenaline (N₁ or N₂, 0.1–1.0 µM). In some further experiments either ATP (300 µM) or the stable ATP analogue, α,β-methylene-ATP (1–10 µM) was used instead of electrical field stimulation to elicit vasoconstrictor responses. Noradrenaline, ATP and α,β-methylene-ATP were added to the superfusion solution until the constrictor response had reached its maximum. The effect of other drugs on the vasoconstrictor responses was tested by adding them to the superfusion solution 50 min before the second stimulation period.

Further experimental details are given in the Results section.

Protocol B (overflow measurement)

The strips were incubated at 37°C for 30 min in 2 ml Krebs-Henseleit solution containing 0.4 µM [³H]-noradrenaline. After a washing period the strips were placed between two parallel platinum electrodes and superfused with Krebs-Henseleit solution. Drugs or solvent were administered by infusion (16 µl min⁻¹) into the superfusion stream. After an equilibration period of 60 min, a priming stimulation (10 Hz for 40 s, 100 mA, 0.3 ms) was given; 58 min after the priming stimulation the superfusate was collected in 1, 5 or 10 min periods. Then there were two electrical stimulations (S₁ and S₂), 60 min apart, at 10 Hz for either 10 or 40 s. The effect of drugs was tested by adding them to the superfusion solution 42 min before S₂. At the end of each experiment, the strips were weighed (104.1 ± 12.7 mg; *n* = 29) and solubilized in 1 ml Soluene-350 (Canberra Packard, Frankfurt a. M., Germany). The ATP content of the superfusate was measured with the luciferase technique (von Kügelgen & Starke, 1991b) using the ATP bioluminescence HS assay kit (Boehringer Mannheim, Mannheim, Germany) and a Biolumat LB 9500 C luminometer (Berthold, Wildbad, Germany). A blank value obtained with fresh solution was subtracted from each experimental value. Calibration curves for ATP were obtained using Krebs-Henseleit solution as solvent. None of the drugs used interfered with the assay. The amount of tritium in the superfusate samples and the solubilized tissues was measured by liquid scintillation counting. The outflow of ATP was expressed in pmol min⁻¹ g⁻¹ tissue. Outflow of tritium was expressed as a fraction of the amount of tritium present in the tissue at the onset of the respective collection period (fractional rate of outflow; min⁻¹). The electrically evoked overflow of ATP and tritium was calculated as the

difference between the total overflow during the 7 min after onset of stimulation, and the estimated basal outflow within these 7 min. The basal outflow of ATP was assumed to be the outflow in the 1 min interval before onset of stimulation, and the electrically evoked overflow of ATP (total minus basal) was expressed as pmol g⁻¹ tissue. The basal outflow of tritium was assumed to change linearly from the 1 min interval before to the interval 7–8 min after onset of stimulation, and the electrically evoked overflow of tritium (total minus basal; Bq) was expressed as a percentage of the tritium content (Bq) of the tissue at the start of stimulation.

Materials

The following drugs were used: neuropeptide Y (NPY, human) (Bachem, Heidelberg, Germany), suramin hexa-sodium salt (Bayer, Wuppertal, Germany), adenosine 5'-triphosphate disodium salt (ATP) (Boehringer Mannheim, Mannheim, Germany), (–)-[ring-2,5,6-³H]-noradrenaline, specific activity 1.62, 1.94 or 2.11 TBq mmol⁻¹ (Du Pont, Dreieich, Germany), prazosin HCl (Pfizer, Karlsruhe, Germany), phenoxybenzamine HCl (Röhm Pharma, Weiterstadt, Germany), rauwolszine HCl (Roth, Karlsruhe, Germany), guanethidine sulphate, α,β-methylene-ATP lithium salt, (–)-noradrenaline HCl, tetrodotoxin (Sigma, Deisenhofen, Germany). Solutions of drugs were prepared with distilled water (tetrodotoxin: sodium acetate buffer 0.1 M, pH 4.8). The Krebs-Henseleit solution had the following composition (mM): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 0.45, NaHCO₃ 25, KH₂PO₄ 1.03, glucose 11.1, disodium EDTA 0.07, ascorbic acid 0.07.

Statistics

Means ± s.e.mean are given throughout. Differences between means were tested for statistical significance by Student's *t* test. *P* < 0.05 or lower was taken to be statistically significant.

Results

Human saphenous veins were cut into helical strips and superfused with Krebs-Henseleit solution. Constrictor re-

Table 1 Constrictor responses to electrical field stimulation, exogenous noradrenaline, ATP and α,β-methylene-ATP in human saphenous vein

Stimulus	Constrictor responses (mN) at 25°C	Constrictor responses (mN) at 37°C
2.5 Hz	22.2 ± 4.3 (13)	29.3 ± 7.9 (8)
10 Hz	22.4 ± 1.7 (73)	33.7 ± 3.2 (45)
Noradrenaline 0.1 µM	31.2 ± 2.7 (57)	33.6 ± 3.1 (33)
1 µM	45.9 ± 4.7 (29)	56.3 ± 3.7 (49)
ATP 300 µM		8.8 ± 1.6 (16)
α,β-methylene-ATP 1 µM		20.6 ± 3.6 (10)
10 µM		36.3 ± 17.0 (3)

Strips of human saphenous veins were superfused with Krebs-Henseleit solution at 25°C or 37°C. Constrictor responses were elicited by electrical stimulation (2.5 or 10 Hz for 40 s), exogenous noradrenaline, ATP or α,β-methylene-ATP. Means ± s.e.mean of (*n*) experiments.

sponses to electrical field stimulation, exogenous noradrenaline, ATP and α,β -methylene-ATP in the absence of drugs at 25°C and 37°C are shown in Table 1.

Effect of phenoxybenzamine on constrictor responses to either 1, 4 and 10 Hz or to exogenous noradrenaline

There were two series (60 min apart) of consecutive electrical stimulations at 1, 4 and 10 Hz (each for 40 s) at 37°C. Vasoconstrictor responses in the first series were 1.8 ± 1.1 mN, 5.8 ± 2.1 mN and 12.2 ± 1.6 mN, respectively ($n = 4$). Phenoxybenzamine ($10 \mu\text{M}$), added to the superfusion solution 50 min before the second series of electrical stimulation at 1, 4 and 10 Hz reduced the respective vasoconstrictor responses to 2.5 ± 0.9 , 16.3 ± 1.4 and $21.1 \pm 1.9\%$ of control ($n = 4$). The residual responses to electrical stimulation at 4 and 10 Hz in the presence of phenoxybenzamine were almost abolished by further addition of tetrodotoxin ($1 \mu\text{M}$; $n = 4$; not shown). Constrictor responses to exogenous noradrenaline ($1 \mu\text{M}$) were abolished by phenoxybenzamine ($10 \mu\text{M}$; $n = 5$; not shown).

Figure 1 Effects of guanethidine (Gua) and tetrodotoxin (TTX) on constrictor responses to electrical stimulation and exogenous noradrenaline (NA) in human saphenous veins. Veins were superfused with Krebs-Henseleit solution at 25°C (a) or 37°C (b). There were two stimulation periods (60 min apart). Each period consisted of an electrical stimulation (S₁ or S₂; at 10 Hz, 40 s; open columns) followed by a stimulation elicited by noradrenaline (N₁ or N₂; $0.1 \mu\text{M}$, cross-hatched columns or $1 \mu\text{M}$, hatched columns). Guanethidine ($10 \mu\text{M}$), tetrodotoxin ($1 \mu\text{M}$) or their solvents (control, Con) were added to the superfusion solution 50 min before S₂. Constrictor responses in the second stimulation period (S₂ or N₂) are expressed as a percentage of that in the first stimulation period (S₁ or N₁, respectively). Means $\pm$ s.e.mean of 3–15 experiments. *Significant differences from corresponding control (solvent; $P < 0.05$).

Effects of guanethidine and tetrodotoxin on constrictor responses to either 10 Hz or exogenous noradrenaline

Guanethidine ($10 \mu\text{M}$) and tetrodotoxin ($1 \mu\text{M}$), when added to the superfusion solution 50 min before the second stimulation period, almost abolished the vasoconstrictor responses to electrical stimulation at 25°C and 37°C (Figure 1). Vasoconstrictor responses to exogenous noradrenaline were not significantly altered by either tetrodotoxin or guanethidine (Figure 1).

Effects of prazosin and rauwolscine on constrictor responses to either 10 Hz or exogenous noradrenaline

Prazosin ($0.3 \mu\text{M}$) as well as rauwolscine ($1 \mu\text{M}$) reduced constrictor responses to electrical stimulation and to exogenous noradrenaline ($0.1, 1 \mu\text{M}$) at 25°C and 37°C (Figure 2). The combination of prazosin and rauwolscine abolished constrictor responses to noradrenaline ($0.1, 1 \mu\text{M}$) at both temperatures. However, constrictor responses to electrical

Figure 2 Effects of prazosin (Pra), rauwolscine (Rau) and the combination of prazosin and rauwolscine on constrictor responses to electrical stimulation and exogenous noradrenaline (NA) in human saphenous veins. Veins were superfused with Krebs-Henseleit solution at 25°C (a) or 37°C (b). There were two stimulation periods (60 min apart). Each period consisted of an electrical stimulation (S₁ or S₂; at 10 Hz, 40 s; open columns) followed by a stimulation elicited by noradrenaline (N₁ or N₂; $0.1 \mu\text{M}$, cross-hatched columns or $1 \mu\text{M}$, hatched columns). Prazosin ($0.3 \mu\text{M}$ or $3 \mu\text{M}$), rauwolscine ($1 \mu\text{M}$ or $10 \mu\text{M}$) or their solvent (control, Con) were added to the superfusion solution 50 min before S₂. Constrictor responses in the second stimulation period (S₂ or N₂) are expressed as a percentage of that in the first stimulation period (S₁ or N₁, respectively). Means $\pm$ s.e.mean of 3–15 experiments. *Significant differences from corresponding control (solvent; $P < 0.05$).

stimulation were further inhibited but not abolished by the combination of prazosin and rauwolscline (Figure 2). Even ten fold higher concentrations of prazosin and rauwolscline ($3 \mu\text{M}$ and $10 \mu\text{M}$, respectively) failed to attenuate further the constrictor responses to electrical stimulation (25°C ; Figure 2). Tetrodotoxin ($1 \mu\text{M}$) added in addition to prazosin ($3 \mu\text{M}$) and rauwolscline ($10 \mu\text{M}$) markedly inhibited the residual responses to electrical stimulation ($n = 5$; not shown). Residual constrictor responses to electrical stimulation obtained in the presence of prazosin and rauwolscline were greater at 25°C than at 37°C and were about 30% of control at 25°C (Figure 2; see also below Figure 4).

Effects of prazosin and rauwolscline on constrictor responses to either 2.5 Hz or exogenous noradrenaline

Similar results were obtained when the veins were electrically stimulated for 40 s at 2.5 Hz instead of at 10 Hz. The combination of prazosin ($0.3 \mu\text{M}$) and rauwolscline ($1 \mu\text{M}$)

abolished constrictor responses to exogenous noradrenaline ($0.1 \mu\text{M}$) at 25°C and 37°C (not shown). Constrictor responses to electrical stimulation were abolished at 37°C , but there were residual constrictor responses (about 22% of control) at 25°C . These residual constrictor responses to electrical stimulation tended to be smaller than those obtained with stimulation at 10 Hz.

Effects of tetrodotoxin, prazosin and rauwolscline on electrically evoked constrictor responses, tritium and ATP overflow

Human saphenous veins were preincubated with [^3H]-noradrenaline and then superfused with Krebs-Henseleit solution at 25°C or 37°C . Electrical stimulation elicited constrictor

Figure 3 Time course of ATP outflow (a), tritium outflow (b) and smooth muscle tension (c). Human saphenous veins were incubated with [^3H]-noradrenaline and then superfused with Krebs-Henseleit solution at 37°C . There were two electrical stimulations (S_1 and S_2 , 120 and 180 min after the start of superfusion, each at 10 Hz, 40 s). Solvent was added to the superfusion solution 42 min before S_2 . Figure shows ATP and tritium outflow per minute as means $\pm$ s.e.mean of 6 experiments and representative smooth muscle tension tracings from one experiment.

Figure 4 Effects of tetrodotoxin (TTX) and a combination of prazosin and rauwolscline (Pra + Rau) on electrically evoked ATP overflow (a), tritium overflow (b) and constrictor responses (c) in human saphenous veins. Veins were superfused with Krebs-Henseleit solution at 25°C (cross-hatched) or 37°C (open columns). There were two stimulations (S_1 or S_2 ; 60 min apart, each at 10 Hz, 40 s). Tetrodotoxin ($0.5 \mu\text{M}$), prazosin ($0.3 \mu\text{M}$), rauwolscline ($1 \mu\text{M}$) or their solvents (control, Con) were added to the superfusion solution 42 min before S_2 . Responses in the second stimulation period (S_2) were expressed as a percentage of that in the first stimulation period (S_1). Means $\pm$ s.e.mean of 4–6 experiments. *Significant differences from corresponding control (solvent; $P < 0.05$).

Table 2 Basal ATP and tritium outflow (b_1) from human saphenous vein, and electrically evoked ATP and tritium overflow and constriction (S_1)

Stimulus	ATP outflow ($\text{pmol min}^{-1} \text{g}^{-1}$)	b_1 Tritium outflow (min^{-1})	ATP overflow (pmol g^{-1})	S_1 Tritium overflow (% of tissue tritium)	Constriction (mN)	n
Temperature: 25°C						
10 Hz, 40 s	0.149 ± 0.039	0.00044* ± 0.00005	4.745 ± 1.522	0.319 ± 0.079	20.1 ± 3.6	10
Temperature: 37°C						
10 Hz, 10 s	0.124 ± 0.023	0.00083* ± 0.00006	0.782 ± 0.291	0.071* ± 0.016	13.1 ± 2.3	5
10 Hz, 40 s	0.094 ± 0.016	0.00064* ± 0.00004	1.949 ± 0.496	0.331 ^b ± 0.076	20.2 ± 3.5	14

After incubation with [^3H]-noradrenaline, human saphenous veins were superfused with Krebs-Henseleit solution at 25°C or 37°C. S_1 was applied after 120 min of superfusion and consisted of either 100 pulses (10 Hz, 10 s) or 400 pulses (10 Hz, 40 s). b_1 refers to the 1 min period immediately before S_1 . Means $\pm$ s.e. mean of n experiments.

*Corresponding to $36.8 \pm 5.3 \text{ Bq min}^{-1}$; ^bcorresponding to $159.0 \pm 32.7 \text{ Bq}$.

*Significant differences from experiments with 10 Hz, 40 s at 37°C ($P < 0.05$).

responses as well as an overflow of tritium and ATP. The time course is shown for one experimental group in Figure 3 and mean values of the responses elicited by the first stimulation period in the absence of drugs are summarized in Table 2. Electrical stimulation with 400 pulses (10 Hz for 40 s) induced greater constrictor responses, tritium and ATP overflow than stimulation with 100 pulses (10 Hz for 10 s; Table 2; 37°C). Constrictor responses and tritium overflow at either 25°C or 37°C did not differ. The mean value of ATP overflow obtained at 25°C was about twice that obtained at 37°C; however, this difference was not significant ($P = 0.059$). Constrictor responses as well as tritium and ATP overflow to 400 pulses (10 Hz) were well reproducible after an interval of 60 min (Figures 3 and 4). Tetrodotoxin ($0.5 \mu\text{M}$) added 42 min before the second stimulation period abolished constrictor responses as well as tritium and ATP overflow (Figure 4). The combination of prazosin ($0.3 \mu\text{M}$) and rauwolscine ($1 \mu\text{M}$) reduced but did not abolish constrictor responses and ATP overflow at 25°C and 37°C, whereas the combination increased tritium overflow by about 120% at either temperature (Figure 4). In the presence of prazosin and rauwolscine the basal outflow of tritium was increased by about 60% at both temperatures; the basal outflow of ATP remained unchanged (not shown).

Effects of suramin on constrictor responses to 10 Hz, noradrenaline, ATP and α, β -methylene-ATP at 37°C

Exogenous ATP ($300 \mu\text{M}$) and α, β -methylene-ATP ($1-10 \mu\text{M}$) elicited fast constrictor responses (mean values of the maximum increase in tension are shown in Table 1). The responses to ATP were transient; the smooth muscle tended to return to baseline within 5 min after addition of ATP. The responses to α, β -methylene-ATP, in contrast, were more sustained (most prominently at 25°C, not shown). Suramin ($300 \mu\text{M}$), when added to the superfusion solution 50 min before the second stimulation period, almost abolished the constrictor responses to ATP ($300 \mu\text{M}$) and α, β -methylene-ATP ($1 \mu\text{M}$) (Figure 5). Suramin did not significantly reduce constrictor responses to either 10 Hz or noradrenaline ($0.1 \mu\text{M}$) (Figure 5).

Effects of suramin on α -adrenoceptor blockade-resistant constrictor responses to 10 Hz at 25°C

The combination of prazosin ($0.3 \mu\text{M}$) and rauwolscine ($1 \mu\text{M}$) abolished the constrictor responses to noradrenaline, but only partly reduced the constrictor responses to electrical stimulation at 10 Hz (Figure 2). Suramin ($300 \mu\text{M}$), given in

addition to prazosin and rauwolscine, did not further diminish the α -adrenoceptor blockade-resistant constrictor responses to 10 Hz (Figure 6).

Constrictor responses to exogenous neuropeptide Y at 37°C

Neuropeptide Y (0.01 , 0.1 and $1 \mu\text{M}$) induced concentration-dependent constrictor responses of $2.5 \pm 1.0 \text{ mN}$ ($n = 13$), $3.3 \pm 1.1 \text{ mN}$ ($n = 14$) and $8.0 \pm 3.6 \text{ mN}$ ($n = 11$), respectively.

Figure 5 Effects of suramin (Sur) on constrictor responses (R) to electrical stimulation, exogenous noradrenaline (NA), ATP and α, β -methylene-ATP (mATP) in human saphenous veins. Veins were superfused with Krebs-Henseleit solution at 37°C. There were two stimulation periods (60 min apart). Each period consisted of a stimulation elicited by electrical pulses (10 Hz, 40 s; open columns), ATP ($300 \mu\text{M}$; horizontally striped columns) or mATP ($1 \mu\text{M}$; vertically striped columns) followed by a stimulation elicited by noradrenaline ($0.1 \mu\text{M}$, cross-hatched columns). Suramin ($300 \mu\text{M}$) or its solvent (control, Con) was added to the superfusion solution 50 min before S_2 . Constrictor responses in the second stimulation period (R_2) are expressed as a percentage of that in the first stimulation period (R_1). Means $\pm$ s.e. mean of 4–12 experiments. *Significant differences from corresponding control (solvent; $P < 0.05$).

Figure 6 Effects of suramin (Sur) on constrictor responses to electrical stimulation and exogenous noradrenaline (NA) in the presence of prazosin (Pra) and rauwolscline (Rau). Veins were superfused with Krebs-Henseleit solution at 25°C. There were two stimulation periods (60 min apart). Each period consisted of an electrical stimulation (S₁ or S₂; at 10 Hz, 40 s; open columns) followed by a stimulation elicited by noradrenaline (N₁ or N₂; 0.1 µM, hatched columns). Suramin (300 µM) was added to the superfusion solution together with prazosin (0.3 µM) and rauwolscline (1 µM) 50 min before S₂. Constrictor responses in the second stimulation period (S₂ or N₂) are expressed as a percentage of that in the first stimulation period (S₁ or N₁, respectively). Means ± s.e.mean of 6–11 experiments.

Discussion

ATP plays an important role as a cotransmitter of noradrenaline in sympathetically innervated tissues of various animals species (Burnstock, 1990; von Kugelgen & Starke, 1991a; Hoyle, 1992). The aim of the present study was to investigate whether this may also be the case in human saphenous veins. Electrical stimulation elicited constrictor responses of human saphenous veins; these responses were sensitive to tetrodotoxin and guanethidine and thus mediated by transmitter substances released from sympathetic nerve varicosities (see Burnstock, 1990; von Kugelgen & Starke, 1991a).

α-Adrenoceptor-mediated constrictor responses

In human saphenous veins constrictor responses to exogenous noradrenaline were inhibited by both the preferential α₁-adrenoceptor antagonist, prazosin and the preferential α₂-adrenoceptor antagonist, rauwolscline but only a combination of both drugs abolished these responses. This is in accord with previous findings that smooth muscle cells of the human saphenous vein possess both α₁- and α₂-adrenoceptors (Müller-Schweinitzer, 1984; Steen *et al.*, 1984; Docherty & Hyland, 1985a; Docherty, 1987). When the human saphenous veins were stimulated with a frequency of 10 Hz, the constrictor responses to electrical stimulation were also diminished by prazosin as well as by rauwolscline. Moreover, the combination of prazosin and rauwolscline caused a greater reduction of constrictor responses to electrical stimulation at 10 Hz than each of the antagonists alone. This confirms previous

findings that in the human saphenous vein both smooth muscle α₁-adrenoceptors and smooth muscle α₂-adrenoceptors are activated by endogenous noradrenaline and mediate a portion of the effector responses to sympathetic nerve stimulation (Docherty & Hyland, 1985a).

α-Adrenoceptor blockade resistant constrictor responses

The combination of prazosin and rauwolscline as well as phenoxybenzamine abolished constrictor responses to exogenous noradrenaline (0.1 µM and 1 µM). Even though the constrictor responses to exogenous noradrenaline were higher than those to electrical stimulation (Table 1), the α-adrenoceptor antagonists failed to cause complete blockade of the responses to electrical stimulation. A ten fold increase in the concentrations of prazosin and rauwolscline did not further attenuate the constrictor responses to electrical stimulation. This, in addition, excludes the possibility that the α-adrenoceptor antagonists failed to block completely the responses to electrical stimulation due to the use of too low concentrations. Hence, these findings indicate that in the human saphenous vein some components of neurogenic constriction are resistant to blockade of α-adrenoceptors.

Responses to sympathetic nerve stimulation which are resistant to α-adrenoceptor blockade are well known in sympathetically innervated smooth muscle tissues of animals including the canine saphenous vein and have also been observed in human tissues (see Introduction). These α-adrenoceptor blockade-resistant constrictor responses vary with frequency of stimulation (Burnstock, 1990; von Kugelgen & Starke, 1991a). Therefore, the frequency-dependence was tested in the present study. When all α-adrenoceptors were blocked by phenoxybenzamine, constrictor responses to lower stimulation frequencies were abolished (1 Hz) or markedly inhibited (4 Hz). In contrast, at higher stimulation frequency (10 Hz) there were significant residual constrictor responses. A similar pattern of α-adrenoceptor blockade-resistant constrictor responses to stimulation at high but not at low frequencies has been observed in the canine saphenous vein (Flavahan & Vanhoutte, 1986b). The more pronounced α-adrenoceptor blockade resistant constrictor responses at 10 Hz as compared to 1 and 4 Hz may be due to a greater 'per pulse' release of sympathetic cotransmitters at higher frequencies. An increase in the release of the sympathetic cotransmitters ATP and neuropeptide Y with increasing frequency has been demonstrated in guinea-pig vas deferens (Kirkpatrick & Burnstock, 1987; von Kugelgen & Starke, 1991b), canine gracilis muscle (Pernow, 1988), pig nasal mucosa (Lacroix *et al.*, 1989) and pig spleen (Lundberg *et al.*, 1989). Since the present study in human saphenous veins is focussed on possible non-adrenergic mechanisms of sympathetic neurotransmission, most of the experiments discussed were performed at a stimulation frequency of 10 Hz.

Cooling from 37°C to 25°C augmented α-adrenoceptor blockade-resistant constrictor responses to electrical stimulation in human saphenous veins. A temperature lower than 37°C may be a more physiological temperature for superficial veins such as the vena saphena. Thus, the importance of non-adrenergic components observed at 37°C in these tissues is likely to be underestimated compared with the *in vivo* situation. An augmented residual constrictor response at 24°C as compared to 37°C has also been observed in the canine saphenous vein (Flavahan & Vanhoutte, 1986b) and, in addition, not only in venous preparations but also in the rat isolated mesenteric vasculature (Yamamoto *et al.*, 1992). It is not known why cooling enhances α-adrenoceptor blockade-resistant constrictor responses. α-Adrenoceptor responsiveness may be reduced at lower temperatures (Flavahan & Vanhoutte, 1986a). Another likely explanation is that sympathetic cotransmitters are inactivated more slowly at lower temperatures as has been shown for ATP in the guinea-pig vas deferens (Cunnane & Manchanda, 1988).

ATP-overflow and P_2 -purinoceptors

If ATP is the transmitter responsible for the observed α -adrenoceptor blockade-resistant constrictor responses, then ATP should be released upon electrical stimulation and should be a vasoconstrictor agent in the human saphenous veins. This was investigated further. Electrical stimulation of veins preincubated with [3 H]-noradrenaline induced an overflow of tritium. Tetrodotoxin abolished this tritium overflow. This in agreement with previous findings indicates that the tritium overflow represents neuronal release of [3 H]-noradrenaline in the human saphenous vein (Janssens & Verhaeghe, 1983; Göthert *et al.*, 1984; Docherty & Hyland, 1985b; see Starke, 1977). Electrical stimulation caused not only an overflow of tritium but also an overflow of ATP. A concomitant release of noradrenaline and ATP upon electrical stimulation has previously been demonstrated mainly in vasa deferentia: guinea-pig (Kirkpatrick & Burnstock, 1987; Lew & White, 1987; Kasakov *et al.*, 1988; von Kügelgen & Starke, 1991b; Vizi *et al.*, 1992), rat (Vizi & Burnstock, 1988) and mouse (Drake & Petersen, 1992) but also in arterial blood vessels: rat caudal artery (Westfall *et al.*, 1987) and rabbit aorta (Sedaa *et al.*, 1990).

Tetrodotoxin abolished both ATP and tritium overflow which seems to support the idea that ATP released from human saphenous veins is neuronal in origin. However, the sources of ATP released upon stimulation of nerve fibres are not exclusively neuronal. The combination of prazosin and rauwolscine markedly reduced the overflow of ATP. This indicates that a major part of the electrically evoked ATP overflow derives from non-neuronal cells upon activation of postjunctional α -adrenoceptors by endogenous noradrenaline. Accordingly, ATP release from smooth muscle or endothelium cells induced by endogenous noradrenaline contributes to the total electrically evoked ATP overflow in the rat caudal artery (Westfall *et al.*, 1987), the rabbit aorta (Sedaa *et al.*, 1990) and the guinea-pig (von Kügelgen & Starke, 1991b; Vizi *et al.*, 1992) and rat vas deferens (Vizi & Burnstock, 1988). The physiological consequences of this non-neuronal ATP release are not known but it has been proposed that ATP released from non-neuronal sources may contribute to the constrictor responses (Vizi *et al.*, 1992). The remaining overflow of ATP in the presence of α -adrenoceptor blockade by prazosin and rauwolscine may reflect neuronally released ATP (see Sedaa *et al.*, 1990).

Are there vasoconstriction-mediating P_2 -purinoceptors which could be activated by released ATP? Exogenous ATP and the metabolically stable and P_{2X} -purinoceptor selective analogue α,β -methylene-ATP caused constrictor responses of the human saphenous vein. The P_2 -purinoceptor antagonist, suramin (Dunn & Blakeley, 1988; Schlicker *et al.*, 1989; von Kügelgen *et al.*, 1989) markedly attenuated the responses to

ATP and α,β -methylene-ATP. These findings indicate that the human saphenous vein smooth muscle possesses P_{2X} -purinoceptors as has been shown for many blood vessels of animal species (see Kennedy, 1990; Hoyle, 1992) as well as for human pial (Hardebo *et al.*, 1987), pulmonary (Liu *et al.*, 1989), subcutaneous and omental arteries (Martin *et al.*, 1991).

If neuronally released ATP plays a role in neurogenic constriction of the human saphenous vein, blockade of P_{2X} -purinoceptors by suramin should attenuate the effector responses to electrical stimulation as has been shown, for example, in mouse vas deferens (von Kügelgen *et al.*, 1989), guinea-pig submucosal arterioles (Evans & Surprenant, 1992) and the pithed rat (Schlicker *et al.*, 1989). However, this was not the case. Suramin did not change the constrictor responses to electrical stimulation in the absence of other drugs and in the presence of α -adrenoceptor blockade by prazosin and rauwolscine. Desensitization of P_{2X} -purinoceptors by prolonged addition of α,β -methylene-ATP has also been used to identify P_{2X} -purinoceptor-mediated components of neurogenic vasoconstriction in isolated blood vessels (von Kügelgen & Starke, 1985; Flavahan & Vanhoutte, 1986b) and perfused organs such as the rat isolated kidney (Schwartz & Malik, 1989; Rump *et al.*, 1990; 1992). In human saphenous veins, α,β -methylene-ATP caused long-lasting increases in resting tone. At 25°C the smooth muscle tone did not return to baseline within 60 min after addition of α,β -methylene-ATP. Therefore, in our study this approach could not be used.

In conclusion, constrictor responses to sympathetic nerve stimulation in human saphenous veins are mainly but not exclusively mediated by neuronally released noradrenaline. Sympathetic nerve stimulation induces a concomitant release of ATP and noradrenaline. There are vasoconstriction-mediating P_2 -purinoceptors in the human saphenous vein, but they seem not to be activated by endogenous ATP. Hence, our study failed to identify ATP as the responsible transmitter for α -adrenoceptor blockade-resistant constrictor responses. The nature of this sympathetic transmitter remains unknown. Neuropeptide Y is a vasoconstrictor agent in the human saphenous vein and therefore a likely candidate.

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