Modulation of noradrenaline release from the sympathetic nerves of the human saphenous vein and pulmonary artery by presynaptic EP₃- and DP-receptors

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1 Spirally cut strips of the human saphenous vein and pulmonary artery were used to determine the pharmacological properties of the presynaptic prostanoid receptors involved in the modulation of sympathetic [³H]-noradrenaline release. Strips preincubated with [³H]-noradrenaline were superfused with physiological salt solution containing inhibitors of uptake₁ and uptake₂ and rauwolscine to eliminate involvement of presynaptic α_2 -adrenoceptors. Tritium overflow was evoked by transmural electrical stimulation (standard frequency: 2 Hz).

2 In the saphenous vein, prostaglandin E₂ (PGE₂) inhibited the electrically-evoked tritium overflow; at the highest concentration investigated, tritium overflow was inhibited by more than 75% and the pEC₅₀ value was 7.00. These effects were mimicked by prostaglandin E₁, the EP₁/EP₃ receptor agonist, sulprostone and the EP₂/EP₃ receptor agonist, misoprostol with the rank order (pEC₅₀): sulprostone (8.60) > PGE₁ (7.25) > misoprostol (6.96). This rank order of potency suggests that the inhibitory effect of the drugs is mediated by presynaptic EP₃-receptors. In contrast, PGF_{2 α} did not inhibit evoked tritium overflow; the IP/EP₁ receptor agonist iloprost and the stable thromboxane A₂ analogue U 46619 (9, 11-dideoxy-11 α ,9 α -epoxy-methanoprostaglandin F_{2 α}) produced inhibition only at concentrations above 1 μ M.

3 The EP₁-receptor antagonist, AH 6809 (6-isopropoxy-9-oxoxanthene-2-carboxylic acid) had no effect on the evoked tritium overflow nor did it modify the inhibitory effect of PGE₂, further excluding involvement of inhibitory presynaptic EP₁-receptors.

4 PGD₂ caused a facilitation of evoked tritium overflow in the saphenous vein; this facilitation is probably mediated by presynaptic DP-receptors, since it was abolished by the selective DP-receptor antagonist, BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin).

5 In the pulmonary artery, sulprostone (pEC₅₀ value 8.35), misoprostol (7.70) and PGE₂ (6.80) inhibited electrically-evoked tritium overflow. This rank order of potency is consistent with the involvement of inhibitory presynaptic EP₃-receptors.

6 These results suggest that the sympathetic nerve fibres of both human saphenous vein and pulmonary artery are endowed with presynaptic inhibitory EP₃ receptors. The EP₃-receptors do not interact with the α_2 -autoreceptors. In addition, the human saphenous vein seems to be endowed with presynaptic facilitatory DP-receptors.

Keywords: Human saphenous vein; human pulmonary artery; EP₃-receptors; DP-receptors; presynaptic receptors; noradrenaline release

Introduction

Presynaptic prostanoid EP-receptors mediate inhibition of noradrenaline release from sympathetic nerve fibres in a variety of tissues from different species (for reviews, see Güllner, 1983; Malik & Sehic, 1990). These presynaptic inhibitory EP-receptors belong to the EP₃-subtype in guinea-pig atria and vas deferens (Coleman *et al.*, 1987; Mantelli *et al.*, 1991), rat trachea, stomach and vena cava (Racké *et al.*, 1991; Möhlig *et al.*, 1992; Molderings *et al.*, 1992a) as well as in human iris-ciliary body (Ohia & Jumblatt, 1991). Other studies suggest that presynaptic facilitatory DP-receptors may also exist, for example, on the sympathetic nerves in human corpus cavernosum (Molderings *et al.*, 1992b) and canine mesenteric artery (Nakajima & Toda, 1984).

Few reports have so far described the modulation of noradrenaline release via EP-receptors in human blood vessels and none has characterized precisely the EP-receptor subtype involved. Therefore, the aims of the present study were (1) to investigate whether modulatory presynaptic EP- and DP-receptors are present on the sympathetic nerves of the human saphenous vein and pulmonary artery, and (2) if

present, to determine the subtype (EP₁, EP₂, EP₃) to which the EP-receptors belong. A preliminary account of this study has been given at the 34th Spring Meeting of the Deutsche Gesellschaft für Pharmakologie und Toxikologie (Molderings *et al.*, 1993).

Methods

Segments of macroscopically intact human saphenous veins and pulmonary arteries were obtained from normotensive 35 to 70 year-old male or female patients undergoing surgery for aorta-coronary bypass grafting or lung surgery, respectively. The patients were not treated with adrenoceptor agonists or antagonists or with drugs influencing the storage or release of noradrenaline. Meperidine and promethazine were used for premedication. Patients were anaesthetized (both induction and maintenance) with flunitrazepam and fentanyl. During maintenance of anaesthesia, they were ventilated with mixtures of oxygen and air. Pancuronium was administered for neuromuscular blockade.

Vascular segments were cut into spiral strips (3 × 20 mm) and incubated for 60 min in 1.5 ml physiological salt solution

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(37°C; composition see below) containing (–)-[ring-2,5,6-³H]noradrenaline 0.2 µM (specific activity 52.3 Ci mmol⁻¹). Subsequently, the strips were mounted vertically in an organ bath (tension adjusted to 2.0 g) between two parallel platinum electrodes and superfused with physiological salt solution of 37°C at a rate of 2 ml min⁻¹. The composition of the solution was (mM): NaCl 118, NaH₂PO₄ 1.2, NaHCO₃ 25, KCl 4.7, CaCl₂ 1.6, MgSO₄ 1.2, glucose 11.0, ascorbic acid 0.3, Na₂ EDTA 0.03 (aerated with 95% O₂ and 5% CO₂). Throughout superfusion this solution contained desipramine 0.6 µM and corticosterone 40 µM to block neuronal and extraneuronal uptake of noradrenaline, respectively.

The superfusate was collected in 4-min fractions. Five (in some experiments three or four) 3 min periods of transmural electrical stimulation (2 Hz, rectangular pulses of 150 mA and 0.3 ms) were applied to each vascular strip after 94 (S₁), 126 (S₂), 158 (S₃), 190 (S₄) and 222 (S₅) min of superfusion. In a few experiments, the vascular strips were stimulated at 0.66 Hz for 9 min (otherwise identical time schedule). Antagonist drugs were introduced into the superfusion fluid from 4 min before S₁ until the end of the experiment, naproxen from 18 min before S₁ until the end of the experiment and agonist drugs at concentrations increasing by a factor of 10 from 12 min before S₃, S₄ and S₅ until 20 min after the onset of the respective stimulation period. Separate time-matched control experiments (if relevant, in the presence of the respective antagonist) were carried out for each series of experiments. At the end of superfusion the strips were solubilized with Soluene, and the radioactivity in the superfusate samples and blood vessels was determined by liquid scintillation counting.

Tritium efflux was calculated as the fraction of tritium present in the strip at the start of the respective collection period. Tritium content in the strips at the start of each period of superfusate sampling was calculated by adding the radioactivity in the respective and subsequent superfusate samples to that in the solubilized blood vessel. Basal tritium efflux was expressed as the ratio of tritium efflux per min during the collection period immediately before S₃, S₄ or S₅ (t₃, t₄, t₅) over that immediately before S₂ (t₂). Stimulation-evoked tritium overflow was calculated by subtraction of the basal efflux from the total efflux during stimulation and in the subsequent 13 min (basal efflux decreased linearly from the collection period before to that 16–20 min after the start of stimulation; not shown). The tritium overflow evoked by the five stimulation periods is given as a percentage of tissue tritium at the start of stimulation and is denoted as S₁, S₂, S₃,

S₄ and S₅ after the respective stimulation period (as generally accepted for studies of presynaptic receptors; see e.g. papers quoted in Westfall, 1977; Langer, 1981; Starke *et al.*, 1989). The ratios of the overflow evoked by S₃, S₄ or S₅ over that evoked by S₂ were determined. The ³H overflow ratios (S₃/S₂, S₄/S₂ and S₅/S₂) obtained in the experiments with the prostanoid under study, were expressed as percentage of the overflow ratios in the respective control experiments (performed in the absence of the prostanoid).

The negative logarithm of the agonist concentration at which the evoked tritium overflow was inhibited by 50% (pEC₅₀) was determined as an estimate of the potency of the compound from the concentration-response curves shown in the figures. Results are given as the mean ± s.e.mean. Student's *t* test was used for comparison of the mean values.

Drugs used

(–)-[ring-2,5,6-³H]-noradrenaline (New England Nuclear, Dreieich, Germany); desipramine hydrochloride (Ciba-Geigy, Wehr, Germany); corticosterone, prostaglandins D₂, E₁, E₂, F_{2α} (Sigma, München, Germany); AH 6809 (6-isopropoxy-*p*-oxoxanthene-2-carboxylic acid; Glaxo, Ware, England); iloprost, sulprostone (Schering, Berlin, Germany); misoprostol (Searle, Skokie, IL, U.S.A.), rauwolsine hydrochloride (Roth, Karlsruhe, Germany); U46619 (9, 11-dideoxy-11α,9α-epoxy-methano-prostaglandin F_{2α}; Biosigma, Munich, Germany); BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin; Wellcome Research Laboratories, Kent, England). Stock solutions were prepared with water (AH 6809, desipramine, rauwolsine), saline (iloprost), 1,2-propanediol (corticosterone), ethanol (misoprostol, sulprostone, PGE₁, PGE₂, PGD₂, PGF_{2α}, BW A868C) or dimethylsulphoxide (U46619) and diluted with physiological salt solution to the concentration required. The solvents did not affect basal or evoked tritium overflow.

Results

Common features of basal and stimulation-evoked tritium-overflow in saphenous veins and pulmonary arteries

In strips of human saphenous vein and pulmonary artery preincubated with [³H]-noradrenaline, basal tritium efflux under control conditions decreased with time, as reflected by

Table 1 Effect of rauwolsine, AH 6809, naproxen and BW A868C on the electrically-evoked tritium overflow from strips of human saphenous vein (A) and pulmonary artery (B)

Drugs (µM)	Frequency of stimulation (Hz)	S ₂ ^a	S ₃ /S ₂ ^b	S ₄ /S ₂ ^b	S ₅ /S ₂ ^b
A None	2	0.30 ± 0.04 ^c	1.02 ± 0.12	0.97 ± 0.10	0.91 ± 0.07
Rauwolsine 1	2	0.98 ± 0.20 ^d	1.03 ± 0.06	0.97 ± 0.05	0.95 ± 0.03
Rauwolsine 1	0.66	0.34 ± 0.12	1.00 ± 0.09	1.02 ± 0.17	0.93 ± 0.11
Rauwolsine 1 + AH 6809 10	2	0.65 ± 0.11	0.94 ± 0.04	0.89 ± 0.05	0.82 ± 0.04
Rauwolsine 1 + naproxen 10	2	0.60 ± 0.08	1.04 ± 0.13	1.03 ± 0.17	1.01 ± 0.15
Rauwolsine 1 + BW A868C 0.1	2	1.05 ± 0.28	0.89 ± 0.04	0.83 ± 0.05	0.90 ± 0.11
B Rauwolsine 1	2	0.43 ± 0.07 ^e	1.02 ± 0.10	0.93 ± 0.08	1.00 ± 0.08

The values were obtained in a representative series of control experiments. Five 3- or 9-min periods (2 and 0.66 Hz, respectively) of transmural electrical stimulation (S₁, S₂, S₃, S₄ and S₅) were applied in each preparation. Data are means ± s.e.means of 5–9 experiments. Representative absolute values for basal efflux and tritium overflow evoked by S₂ (in nCi) are given below.

^aEvoked tritium overflow by S₂ above basal efflux, expressed as percentage of tissue tritium. ^bTritium overflow evoked by S₃, S₄ and S₅ is given as ratio over the respective tritium overflow evoked by S₂. ^cCorresponding to 3.4 ± 0.8 nCi (basal efflux amounted to 1.3 ± 0.2 nCi min⁻¹). ^dCorresponding to 7.8 ± 2.4 nCi (basal efflux amounted to 1.1 ± 0.2 nCi min⁻¹). ^eCorresponding to 2.3 ± 0.4 nCi (basal efflux amounted to 1.1 ± 0.2 nCi min⁻¹).

a decrease of the ratios t_3/t_2 to t_5/t_2 (all ratios <1.0 ; not shown). Basal tritium efflux was not significantly altered by rauwolscline ($1\text{ }\mu\text{M}$; see e.g. Table 1, footnotes c and d) or by the other drugs applied at the concentrations investigated (results not shown).

Transmural electrical stimulation of strips of human saphenous vein and pulmonary artery elicited an overflow of tritium above basal efflux. In the presence of rauwolscline $1\text{ }\mu\text{M}$ from 4 min before S_1 until the end of the experiments, the tritium overflow evoked by S_2 at 2 Hz (360 impulses) was increased about 3 fold compared to the experiments without rauwolscline (Table 1) whereas overflow evoked by S_2 at 0.66 Hz (360 impulses as well) was in a similar range as in the experiments without previous α -adrenoceptor blockade at 2 Hz (Table 1). At both frequencies of stimulation and under all experimental conditions studied, the evoked overflow either slightly decreased from S_2 to S_5 or remained approximately constant, as reflected by S_n/S_2 ratios close to unity (Table 1).

Saphenous veins

PGE₂ produced a concentration-dependent inhibition of electrically-evoked tritium overflow that was unaffected by rauwolscline ($1\text{ }\mu\text{M}$; Figure 1). The pEC₅₀ of PGE₂ was 6.80 in the presence of rauwolscline (Table 2) and 7.00 in its absence. All further experiments were performed in the presence of rauwolscline $1\text{ }\mu\text{M}$, to eliminate any possible drug interactions with α_2 -autoreceptors. This manipulation enhanced evoked tritium overflow by about 3 fold (Table 1), facilitating quantitation of inhibitory drug effects.

Since modulation of [³H]-noradrenaline release by presynaptic receptors has been shown to increase with decreasing frequency of stimulation (see Starke *et al.*, 1989), it was of interest to examine whether such an effect also occurred with PGE₂. When the frequency of stimulation was decreased from 2 Hz to 0.66 Hz, PGE₂ was more potent as reflected by leftward shift of the concentration-response curve (pEC₅₀ of PGE₂ at 0.66 Hz, 7.85; Figure 2).

Naproxen $10\text{ }\mu\text{M}$ did not significantly modify the tritium overflow evoked by electrical impulses at the standard frequency of 2 Hz (compare relevant S_2 values in Table 1). In the presence of this drug, PGE₂ was clearly more potent (about 7 fold) in inhibiting the evoked tritium overflow than in the absence of the cyclo-oxygenase inhibitor (Figure 3a; pEC₅₀ value 7.62). On the other hand, the inhibitory effect of

PGE₂ on tritium overflow was not affected by a high concentration ($10\text{ }\mu\text{M}$) of the selective EP₁-receptor antagonist, AH 6809 (Figure 3b; pEC₅₀ value 6.85), which given alone did also not significantly modify the evoked tritium overflow (compare relevant S_2 values in Table 1).

Further experiments were carried out to determine the EP-receptor subtype which mediates the inhibitory effect of PGE₂. The EP₁/EP₃-receptor agonist sulprostone was about 63 fold and PGE₁ 3 fold more potent than PGE₂; the EP₂/EP₃-receptor agonist, misoprostol, exhibited about the same potency as PGE₂ (Figure 4a, for pEC₅₀ values determined from these concentration-response curves, see Table 2). The thromboxane analogue, U46619 and the IP/EP₁ receptor agonist, iloprost, inhibited the electrically-evoked tritium overflow only at concentrations of 1 and $10\text{ }\mu\text{M}$, respectively (by less than 40%), and PGF_{2 α} at concentrations of up to $1\text{ }\mu\text{M}$ failed to affect the evoked overflow (Figure 4b). Taken together, the following rank order of potency of the inhibitory drugs was obtained: sulprostone $\gg$ PGE₁ $>$ misoprostol = PGE₂ $\gg$ U46619 and iloprost (see also Table 2).

In contrast, PGD₂ enhanced the evoked tritium overflow (Figure 5). The facilitatory effect of PGD₂ on evoked

Table 2 Potencies of prostanoids (given as their pEC₅₀ values) for their inhibitory effect on the electrically evoked tritium overflow from superfused strips of human saphenous vein and pulmonary artery preincubated with [³H]-noradrenaline and superfused in the presence of rauwolscline $1\text{ }\mu\text{M}$

Prostanoid	pEC ₅₀ ^a	
	Saphenous vein	Pulmonary artery
Sulprostone	8.60 (10)	8.35 (3)
Prostaglandin E ₁	7.25 (6)	
Misoprostol	6.96 (8)	7.70 (3)
Prostaglandin E ₂	6.80 (8)	6.80 (6)
U46619	<6.00 (5)	
Iloprost	<5.20 (7)	

Number of experiments in parentheses. ^apEC₅₀ values were determined from the concentration-response curves shown in Figures 1, 4 and 6.

Figure 1 Inhibitory effect of prostaglandin E₂ (PGE₂) in the absence (●) or presence (○) of rauwolscline $1\text{ }\mu\text{M}$ on the electrically (2 Hz)-evoked tritium overflow from strips of the human saphenous vein preincubated with [³H]-noradrenaline. Ordinate scale: ³H overflow ratios, expressed as percentage of ratios in prostaglandin E₂-free control experiments. Means $\pm$ s.e. means of 8 experiments each. * $P < 0.05$; ** $P < 0.025$; *** $P < 0.001$ (compared with the corresponding controls).

Figure 2 Frequency-dependence of prostaglandin E₂ (PGE₂) induced inhibition of electrically-evoked tritium overflow in strips of human saphenous vein. α_2 -Autoreceptors were blocked with rauwolscline $1\text{ }\mu\text{M}$. Ordinate scale: ³H overflow ratios, expressed as percentage of ratios in prostaglandin E₂-free control experiments at 0.66 Hz (○) and 2 Hz (□). Means $\pm$ s.e. means of 6 to 8 experiments. ** $P < 0.025$; *** $P < 0.001$ (compared with the corresponding controls).

overflow was abolished in the presence of the selective DP-receptor antagonist, BW A868C (0.1 μM ; Figure 5). The antagonist itself did not affect evoked tritium overflow (compare relevant S_2 values in Table 1).

Figure 3 Influence of naproxen (1 μM , a) and AH 609 (10 μM , b) on the inhibitory effect of prostaglandin E_2 (PGE_2) (in the presence of rauwolscline 1 μM) on electrically (2 Hz)-evoked tritium overflow from strips of the human saphenous vein. Experiments without naproxen and AH 6809 (open columns); experiments with naproxen 1 μM or AH 6809 10 μM (cross-hatched columns). Ordinate scale: ^3H overflow ratios, expressed as percentage of ratios in PGE_2 -free control experiments. Means $\pm$ s.e. means of 6 to 7 experiments. ** $P < 0.025$ (compared with the response to PGE_2 in the experiments without naproxen).

Pulmonary arteries

All experiments using pulmonary artery strips were performed in the presence of rauwolscline (1 μM). Electrically-evoked tritium overflow was concentration-dependently inhibited by PGE_2 , misoprostol and sulprostone (Figure 6). As in the saphenous vein, sulprostone was considerably more potent in inhibiting tritium overflow than PGE_2 . Misoprostol exhibited a lower potency than sulprostone but a higher one than PGE_2 (Figure 6). The pEC_{50} values of sulprostone and PGE_2 were in a similar range to those in the saphenous vein and that of misoprostol was moderately higher than in the latter blood vessel (Table 2).

Discussion

The aim of the present study was to identify and characterize presynaptic prostanoid receptors on sympathetic neurones in the human saphenous vein and pulmonary artery. Under the conditions applied here (blockade of neuronal and extra-neuronal uptake), electrically evoked tritium overflow from these blood vessels has been shown to be tetrodotoxin-sensitive and Ca^{2+} -dependent (Göthert *et al.*, 1984; Göthert & Hentrich, 1985). The electrically-evoked tritium overflow consists mainly of [^3H]-noradrenaline and reflects action potential-induced release of unlabelled noradrenaline from

Figure 4 Effects of prostanoids in the presence of rauwolscline 1 μM on the electrically (2 Hz)-evoked tritium overflow from strips of human saphenous vein preincubated with [^3H]-noradrenaline. (a) Prostaglandin E_2 (●); prostaglandin E_1 (◇); sulprostone (○); misoprostol (□); (b) prostaglandin E_2 (●); iloprost (◆); prostaglandin $\text{F}_{2\alpha}$ (■); U 46619 (▲). Ordinate scale: ^3H overflow ratios, expressed as percentage of ratios in prostanoid-free control experiments. Means $\pm$ s.e. means from 5 to 10 experiments. ** $P < 0.025$; *** $P < 0.001$ (compared with the corresponding controls).

the sympathetic neurones contained in the blood vessels (for discussion of this point, see Göthert *et al.*, 1984; Göthert & Hentrich 1985).

Both in the saphenous vein and in the pulmonary artery, PGE₂ inhibited the electrically-evoked noradrenaline release from the sympathetic neurones. It has been suggested that this inhibitory effect of PGE₂ (which is in agreement with previous findings obtained in a variety of other sympathetically innervated tissues from various species) may be mediated by presynaptic EP-receptors (for references, see introduction). The substantial increase of the potency of PGE₂ in the human saphenous vein when the frequency of stimulation was decreased from 2 Hz to 0.66 Hz is a typical feature of responses mediated by presynaptic receptors (Starke *et al.*, 1989) and, therefore, is compatible with the involvement of presynaptic EP receptors in the PGE₂-induced inhibition.

In view of the possibility that presynaptic EP receptors might interact with the presynaptic α_2 -autoreceptors, our experiments on both blood vessels were, as a rule, carried out

in the presence of rauwolscline in the superfusion fluid. Such an interaction occurs in peripheral (Ohia & Jumblatt, 1990) and central noradrenergic neurones (Allgaier *et al.*, 1989). When in those studies, the autoreceptors which are tonically activated by endogenous noradrenaline were blocked by an α_2 -adrenoceptor antagonist, the inhibitory effect of PGE₂ was increased. However, in the present experiments on human saphenous veins, blockade of the α_2 -adrenoceptor by rauwolscline did not enhance the inhibitory effect of PGE₂. Thus, the possibility was ruled out that activation of α_2 -autoreceptors attenuates the inhibitory effect mediated by the presynaptic EP-receptors. This finding in the human saphenous vein resembles that previously obtained in the rat vena cava (Molderings *et al.*, 1992a). Two further advantages can be derived from the routine presence of rauwolscline in the superfusion fluid: firstly, in view of the increased noradrenaline release in its presence, a more exact quantitative evaluation of the inhibitory effect of drugs was possible, and secondly, the theoretical possibility of an action of any of these drugs on α_2 -adrenoceptors was excluded.

The pharmacological characteristics of the EP receptor mediating inhibition of noradrenaline release in both human blood vessels clearly conform to an EP₃-receptor according to the classification criteria of Coleman and coworkers (1990). Thus, the effect of PGE₂ was mimicked with greater potency by the non-selective agonist PGE₁ in the saphenous vein, and, in both blood vessels, by the EP₂/EP₃ receptor agonist, misoprostol and the EP₁/EP₃-receptor agonist, sulprostone. In the pulmonary artery, misoprostol was considerably more potent than in the saphenous vein. This difference cannot be explained satisfactorily on the basis of our data. The potency of the drugs in inhibiting noradrenaline release in the human saphenous vein is in good agreement with their potency in inhibiting neurotransmitter release from guinea-pig vas deferens, a preparation recognised to possess EP₃-receptors (Coleman *et al.*, 1987; 1990). In the latter preparation, potency ratios of PGE₁ and sulprostone relative to PGE₂ (= 1) were 0.8 and 0.02, respectively. In the human saphenous vein they were 0.35 and 0.02, respectively, and in the human pulmonary artery, the potency ratio of sulprostone related to PGE₂ was 0.03. Iloprost, a stable analogue of prostacyclin (the main product of arachidonic acid released in response to sympathetic nerve stimulation in blood vessels; see Malik & Sehic, 1990), was only a weak agonist at the presynaptic EP-receptor in human saphenous vein. This drug has been shown to be equipotent with PGE₂ at EP₁-receptors and markedly less potent than PGE₂ at EP₂- and EP₃-receptors (Sheldrick *et al.*, 1988). Moreover, the EP₁-receptor antagonist, AH 6809, at a concentration which is at least 30 fold higher than its pA₂ value at EP₁-receptors (Coleman *et al.*, 1985), failed to affect that PGE₂-induced inhibition. Taken together, it may be concluded that the effect of PGE₁ and PGE₂ in both human blood vessels is mediated via EP₃-receptors. This is in agreement with findings for the presynaptic EP-receptors on postganglionic sympathetic nerves innervating other vascular and non-vascular tissues, namely the rat vena cava (Molderings *et al.*, 1992a), the rat trachea (Racké *et al.*, 1991), the guinea-pig vas deferens (Coleman *et al.*, 1987; 1990) and atrium (Beckman & Knirk, 1991; Mantelli *et al.*, 1991), and the human iris-ciliary body (Ohia & Jumblatt, 1991).

Experiments with the cyclo-oxygenase inhibitor, naproxen, were performed to determine whether or not, under the present experimental conditions, [³H]-noradrenaline release is also inhibited by endogenously formed prostanoids. Gryglewski (1979) has shown that naproxen (1 μ M) effectively inhibits cyclo-oxygenase. If the EP receptors were tonically activated by endogenous arachidonic acid metabolites, the exogenous prostanoid would compete with the endogenously formed prostanoids resulting in an apparently lower potency of the exogenous agonist. In such circumstances, inhibition of the formation of these endogenous prostanoids with naproxen would be expected to enhance the inhibitory effect of

Figure 5 Effect of prostaglandin D₂ (PGD₂) and the DP-receptor antagonist, BW A868C, on electrically (2 Hz)-evoked tritium overflow from strips of the human saphenous vein (in the presence of rauwolscline 1 μ M). Experiments without BW A868C, (○); experiments with BW A868C 0.1 μ M, (●). Ordinate scale: ³H overflow ratios, expressed as percentage of ratios in PGD₂-free control experiments. Means $\pm$ s.e. means of 8 to 9 experiments. ***P* < 0.025 (compared with the corresponding controls).

Figure 6 Inhibitory effects of prostanoids on electrically (2 Hz)-evoked tritium overflow from strips of the human pulmonary artery (in the presence of rauwolscline 1 μ M). Prostaglandin E₂ (●); sulprostone (○); misoprostol (□). Ordinate scale: ³H overflow ratios, expressed as percentage of ratios in prostanoid-free control experiments. Means $\pm$ s.e. means of 3 to 6 experiments. **P* < 0.05; ***P* < 0.025; ****P* < 0.001 (compared with the corresponding controls).

exogenous PGE₂ on [³H]-noradrenaline release. Indeed, such an enhancement was found in the present study. This finding is in agreement with results reported for several other vascular preparations (for review, see e.g. Malik & Sehic, 1990). Interestingly, naproxen might be expected to facilitate noradrenaline release by itself, by abolishing the inhibitory effect produced by endogenous prostanoids. However, such an enhancement of evoked noradrenaline release was not observed in our experiments, a discrepancy which may be related to the simultaneous inhibition of the synthesis of prostanoids with facilitatory action on noradrenaline release.

In the human saphenous vein, PGD₂ increased the evoked noradrenaline release. An enhancement of noradrenaline release has also been found in human corpus cavernosum (Molderings *et al.*, 1992b) and canine mesenteric artery (Nakajima & Toda, 1984), but not e.g. in rat vena cava (Molderings *et al.*, 1992a). The facilitatory effect of PGD₂ on noradrenaline release was abolished by a low concentration of the highly selective DP-receptor antagonist, BW A868C (Giles *et al.*, 1989; Trist *et al.*, 1989). Therefore, the data suggest that presynaptic DP-receptors are involved in PGD₂-

induced facilitation of electrically-evoked noradrenaline release.

The lack of an effect of the FP receptor agonist, PGF_{2α} (at concentrations up to 1 μM) on noradrenaline release and the low potency and efficacy of the stable thromboxane analogue, U46619, in inhibiting noradrenaline release suggest that the sympathetic nerve endings in the human saphenous vein are devoid of FP- and thromboxane receptors.

In conclusion, the present data indicate that the sympathetic nerves of the human saphenous vein and pulmonary artery are endowed with presynaptic inhibitory EP₃-receptors and presynaptic facilitatory DP receptors, respectively. The EP₃-receptors may be tonically activated by endogenous PGE₂ and do not interact with the presynaptic α₂-autoreceptors.

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