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Mechanism of prostaglandin E_2-, $F_{2\alpha}$- and latanoprost acid-induced relaxation of submental veins

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Abstract

The mechanism of prostaglandin E_2-, prostaglandin $F_{2\alpha}$- and latanoprost acid (13,14-dihydro-17-phenyl-18,19,20-trinor-prostaglandin $F_{2\alpha}$)-induced relaxation of the rabbit submental vein was studied. Prostaglandin E_2 caused maximum relaxation of endothelin-1 precontracted vessels (EC_{50}: 1.8×10^{-8} M). Much of the relaxation could be abolished by denuding the endothelium with the nitric oxide synthase inhibitor, L-NAME (N^G-Nitro-L-arginine methylester). CGRP-(8–37) (calcitonin gene-related peptide fragment (8–37)), a calcitonin gene-related peptide receptor antagonist, exhibited a partial blocking effect, whereas the tachykinin NK_1 receptor blocker, GR 82334 ([D-Pro^9[Spiro-γ-Lactam]Leu^{10},Trp^{11}]physalaemin (1–11)), markedly attenuated the response. Both prostaglandin $F_{2\alpha}$ and the relatively selective FP receptor agonist, latanoprost acid, caused relaxation of the veins to about 50% of the precontracted state in the presence of GR 32191B ([1*R*-[1α(*Z*),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid), a thromboxane receptor antagonist (EC_{50}: for prostaglandin $F_{2\alpha}$ 7.9×10^{-9} M, and for latanoprost acid 4.9×10^{-9} M). L-NAME, as well as denuding the endothelium, completely abolished the effect. In addition, most or at least a large part of the relaxation was also blocked by CGRP-(8–37) as well as GR 82334. These results indicate that the FP receptor-mediated relaxation of veins is based on release of nitric oxide in addition to involvement of calcitonin gene-related peptide and substance P, or some other tachykinin, probably released from perivascular sensory nerves. The more pronounced relaxation induced by prostaglandin E_2 could be due to vasodilator EP receptors in the smooth muscle layer of the veins. © 1997 Elsevier Science B.V.

Keywords: Prostaglandin E_2; Prostaglandin $F_{2\alpha}$; Latanoprost acid; Veins relaxation; (Rabbit); FP receptor; Nitric oxide (NO); CGRP (Calcitonin gene-related peptide); Substance P

1. Introduction

Latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-prostaglandin $F_{2\alpha}$-isopropyl ester) is a new intraocular pressure reducing agent developed for the treatment of glaucoma (Stjernschantz and Resul, 1992; Resul et al., 1993; Stjernschantz and Alm, 1996). Many prostaglandins cause conjunctival hyperemia when applied topically on the eye, e.g. prostaglandin $F_{2\alpha}$ and prodrugs of prostaglandin $F_{2\alpha}$ (Alm and Villumsen, 1989; Stjernschantz et al., 1989), but latanoprost has been found to induce significantly less hyperemia (Astin et al., 1994; Stjernschantz et al., 1995; Stjernschantz and Alm, 1996). High doses of latanoprost, however, cause hyperemia in rabbits (Resul et al., 1993), but without significant effect on the arterial blood flow to the eye (Astin et al., 1994). This prompted us to investigate the effects of latanoprost and some other prostaglandins on veins, since engorgement of veins could also result in macroscopic hyperemia.

The rabbit isolated saphenous vein has been shown to contain relaxant IP, DP, as well as EP receptors, possibly of the EP_4 subtype, and contractile TP receptors (Lydford et al., 1996). Relaxant EP_4 receptors have also been described in the pig saphenous vein (Coleman et al., 1994a; Milne et al., 1995) and in the rabbit jugular vein (Milne et al., 1995). Furthermore, prostaglandin $F_{2\alpha}$ has been shown to induce relaxation in the isolated human hand vein (Arner et al., 1994) as well as in the piglet saphenous vein (Coleman et al., 1994a). It has been suggested that these relaxant effects induced by prostaglandin $F_{2\alpha}$ are not mediated by FP receptors. However, somewhat surprisingly, relaxant FP receptors have been described in the rabbit jugular vein (Chen et al., 1995).

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Small veins have a thin muscle layer making precise in vitro measurements difficult. We applied a myographic technique with the vessels used as ring segments, allowing small vessels to be studied (Mulvany and Halpern, 1977). The purpose of the present study was to investigate the mechanism of the relaxant effects of latanoprost acid, prostaglandin $F_{2\alpha}$ and prostaglandin E_2, in small veins.

2. Material and methods

2.1. Preparation of blood vessels

New Zealand white rabbits of either sex weighing 2.5–3.5 kg were killed by a blow to the head. The submental veins were identified, removed, quickly dissected, and placed in physiological salt solution of the following composition: NaCl 119 mM, KCl 4.7 mM, $CaCl_2$ 1.5 mM, $MgSO_4$ 1.17 mM, KH_2PO_4 1.18 mM, $NaHCO_3$, 25 mM, EDTA 0.027 mM and glucose 11 mM. The veins were rinsed of blood and the connective tissue was removed. The vessels were mounted as 1–2 mm long intact ring segments in a small vessel myograph (J.P. Trading, Denmark) (Mulvany and Halpern, 1977). The preparations were allowed to equilibrate in oxygenated (95% O_2, 5% CO_2) physiological salt solution at 37°C, pH 7.4, for approximately 60 min, and were then stretched stepwise to a resting tension of approximately 2 mN. The physiological salt solution contained atropine (10^{-7} M), propranolol (10^{-6} M), and phentolamine (2×10^{-6} M) for elimination of cholinergic and adrenergic tone as well as indomethacin (3×10^{-6} M) to prevent synthesis of endogenous prostaglandins. In a few experiments the vessels were dissected 24 h before use. These preparations were kept in the refrigerator until used. There was no difference in response between these preparations and fresh preparations that were used immediately after dissection.

The study was approved by the local Ethics Committee for Animal Experiments.

2.2. Experimental procedure

The smooth muscle responses of the vessel preparations were recorded isometrically. To study the relaxant effects, the ring segments were precontracted with endothelin-1, $1–3 \times 10^{-9}$ M. Concentration-response curves were made by adding the prostaglandins cumulatively to the baths. At the end of each experiment, papaverine, 10^{-4} M, was added to determine maximal relaxation. For evaluating the blocking effect of L-NAME (N^G-Nitro-L-arginine methylester), 10^{-4} M, a nitric oxide synthase inhibitor (Rees et al., 1990); GR 82334 ([D-Pro^9[Spiro-γ-lactam]Leu^{10},Trp^{11}] physalaemin (1–11)), 5×10^{-6} M, a tachykinin NK_1 receptor antagonist (Hagan et al., 1991; Beattie et al., 1993) or CGRP-(8–37) (calcitonin gene-related peptide fragment (8–37)), 5×10^{-6} M, a calcitonin gene-related peptide receptor antagonist (Chiba et al., 1989; Hughes and Brain, 1991), the blockers were incubated with the tissue preparations for about 30 min before addition of the prostaglandins. Furthermore, GR 32191B ([1*R*-[1α-(*Z*),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid), 10^{-6} M, a thromboxane (TP) receptor antagonist (Lumley et al., 1989), was added about 30 min before addition of the prostanoids in order to eliminate the vasoconstriction resulting from interaction with TP receptors.

In some experiments the endothelium of the veins was removed by gently rubbing the inside of the ring segments with a hair. Successful removal of the endothelium was confirmed by checking that the relaxing effect of acetylcholine (10^{-6} M) was absent in preparations precontracted with endothelin-1. This was done before atropine was added to the physiological salt solution.

Each preparation was used for one or maximally two different prostaglandins. If two analogues were tested on the same preparation, the sequence in which the drugs were tested did not affect the results.

2.3. Drugs

Atropine sulphate, indomethacin, papaverine hydrochloride, phentolamine hydrochloride, and (D,L)-propranolol were purchased from Sigma Chemical Company, USA. N^G-Nitro-L-arginine methylester (L-NAME), and [D-Pro^9[Spiro-γ-Lactam]Leu^{10},Trp^{11}]physalaemin (1-11) (GR 82334) were purchased from Research Biochemicals International, USA and human calcitonin gene-related peptide fragment (8–37) (CGRP-(8–37)) and endothelin-1 from Peninsula laboratories Europe, UK. [1*R*-[1α(*Z*),2β,3β,5α]]-(+)-7-[5-([1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid, (GR 32191B) was a gift from Glaxo Group Research, UK. Prostaglandin E_2, prostaglandin $F_{2\alpha}$, and latanoprost acid (used as the lithium salt) were obtained from the Department of Medicinal Chemistry, Glaucoma Research Laboratories, Pharmacia and Upjohn, Uppsala, Sweden.

2.4. Analysis of data

The relaxant effects were compared to the difference in tension between the endothelin-1 precontraction and papaverine relaxation and expressed as percentage. The data are presented as the mean values ± standard error of the mean (S.E.M.) and log-concentration curves were plotted using the GraphPAD Prism Program (Graph PAD Software, San Diego, CA). The relative potencies (EC_{50}) were calculated from the graphs with the computer programme.

Statistical significance was determined by means of Student's *t*-test for unpaired data. A P value of 0.05 or less was considered statistically significant.

3. Results

3.1. Vasorelaxant effect of prostaglandin E_2

The vasorelaxant effect of prostaglandin E_2 in endothelin-1 precontracted rabbit submental vein preparations is shown in Fig. 1. Prostaglandin E_2 at a concentration of 10^{-5} M induced maximum relaxation, similar to that with papaverine, the concentration-response curve ranging over 5 log units (EC_{50} value 1.8×10^{-8} M). Removal of the endothelium and pretreatment with L-NAME significantly reduced the vasorelaxant effect to about half, L-NAME being somewhat less effective (Fig. 1A). The NK_1 receptor antagonist, GR 82334, caused a significant inhibition of the prostaglandin E_2-induced relaxation and displaced the concentration-response curve about 2 log units to the right (Fig. 1B). The neuropeptide antagonist, CGRP-(8–37), had a smaller but still significant blocking effect at higher concentrations of prostaglandin E_2 (Fig. 1B). Somewhat unexpectedly, the TP receptor antagonist, GR 32191B, displaced the prostaglandin E_2 concentration-response curve by about one log unit to the right (Fig. 1C).

3.2. Vasorelaxant effect of prostaglandin $F_{2\alpha}$

The vasorelaxant effect of prostaglandin $F_{2\alpha}$ in endothelin-1 precontracted submental vein preparations in the presence of GR 32191B is shown in Fig. 2. The maximum relaxation was somewhat less than 50% of that with papaverine and prostaglandin E_2, the EC_{50} value being 7.9×10^{-9} M. Removal of the endothelium as well as pretreatment with L-NAME completely abolished the relaxant effect (Fig. 2A). During blockade with L-NAME there was a tendency to a slight increase in tension at higher concentrations of prostaglandin $F_{2\alpha}$ despite the presence of the TP receptor antagonist. GR 82334 significantly attenuated the vasorelaxant effect of prostaglandin $F_{2\alpha}$ and, in addition, CGRP-(8–37) reduced the prostaglandin $F_{2\alpha}$-induced relaxation (Fig. 2B). In the absence of the TP receptor antagonist (GR 32191B) prostaglandin $F_{2\alpha}$ caused slight contraction at concentrations exceeding 10^{-7} M (Fig. 2C).

3.3. Vasorelaxant effect of latanoprost acid

The vasorelaxant effect of latanoprost acid on the endothelin-1-precontracted submental vein preparations in the presence of GR 32191B is shown in Fig. 3. The maximum relaxation induced by the drug was about 50% of that in response to papaverine and prostaglandin E_2, the EC_{50} value being 4.9×10^{-9} M. The relaxation was completely abolished both by removal of the endothelium and by L-NAME (Fig. 3A). In the presence of L-NAME, latanoprost acid showed a tendency to produce a slight

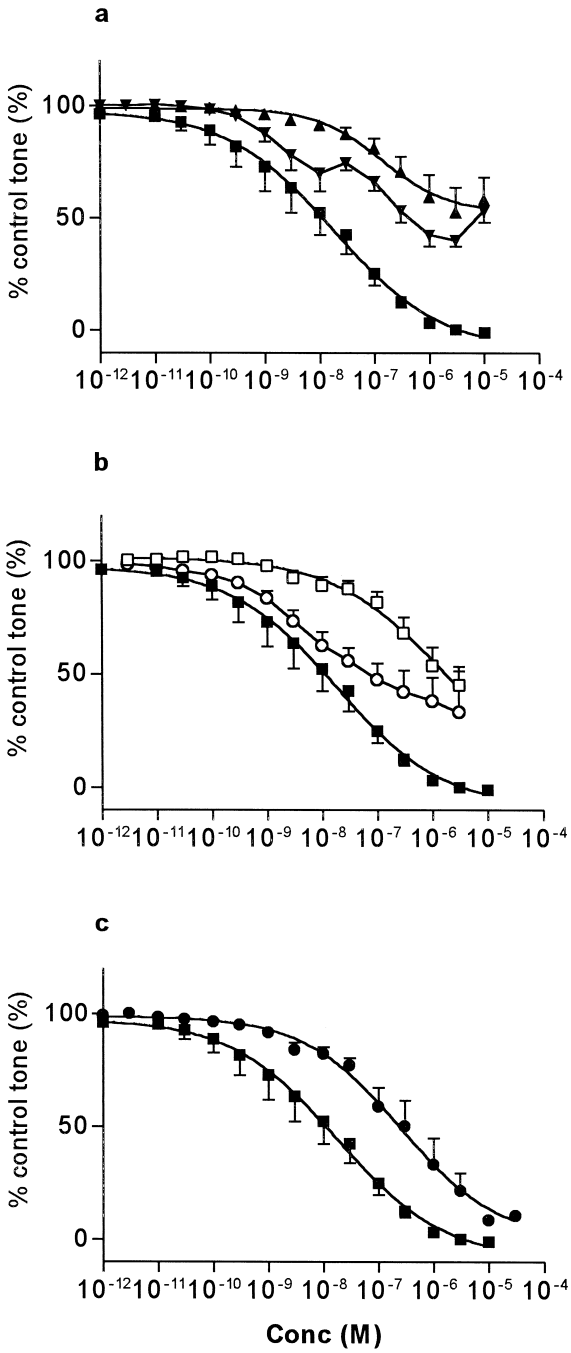

Fig. 1. Cumulative concentration-response curves for the relaxant effects of prostaglandin E_2 in the endothelin-1 precontracted rabbit submental vein. (a) Prostaglandin E_2 (■), prostaglandin E_2 in the presence of 1×10^{-4} M L-NAME (▼) and prostaglandin E_2 in endothelium-denuded preparations (▲). (b) Prostaglandin E_2 (■), prostaglandin E_2 in the presence of 5×10^{-6} M CGRP-(8–37) (○) and prostaglandin E_2 in the presence of 5×10^{-6} M GR 82334 (□). (c) Prostaglandin E_2 (■) and prostaglandin E_2 in the presence of 1×10^{-6} M GR 32191B (●). Relaxation is expressed as percentage of the difference between precontracted and papaverine relaxed values and the data are shown as means ± S.E.M., $n = 4–7$.

contraction (Fig. 3A). The vasorelaxant action of latanoprost acid was significantly reduced by CGRP-(8–37) and also diminished by GR 82334 (Fig. 3B). In the absence of the TP receptor antagonist (GR 32191B) latanoprost acid caused a biphasic concentration-response curve (Fig. 3C); doses lower than 10^{-8} M caused relaxation, whereas doses of 10^{-6} M and higher caused contraction of the smooth muscle compared to baseline. (Fig. 3C).

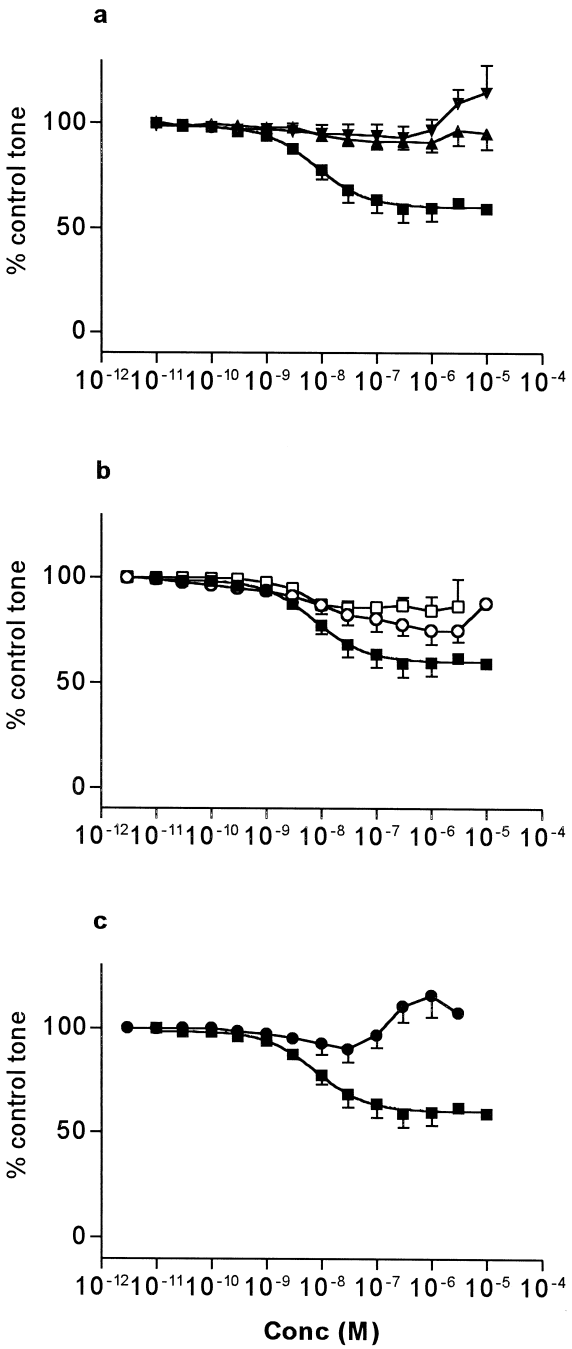

Fig. 2. Cumulative concentration-response curves for the relaxant effects of prostaglandin $F_{2\alpha}$ in the endothelin-1 precontracted rabbit submental vein in the presence of 1×10^{-6} M GR 32191B. (a) Prostaglandin $F_{2\alpha}$ (■), prostaglandin $F_{2\alpha}$ in the presence of 1×10^{-4} M L-NAME (▼) and prostaglandin $F_{2\alpha}$ in endothelium-denuded preparations (▲). (b) Prostaglandin $F_{2\alpha}$ (■), prostaglandin $F_{2\alpha}$ in the presence of 5×10^{-6} M CGRP-(8–37) (○) and prostaglandin $F_{2\alpha}$ in the presence of 5×10^{-6} M GR 82334 (□). (c) Prostaglandin $F_{2\alpha}$ (■) and prostaglandin $F_{2\alpha}$ in the absence of GR 32191B (●). Relaxation is expressed as percentage of the difference between precontracted and papaverine relaxed values and the data are shown as means ± S.E.M., $n = 4–6$.

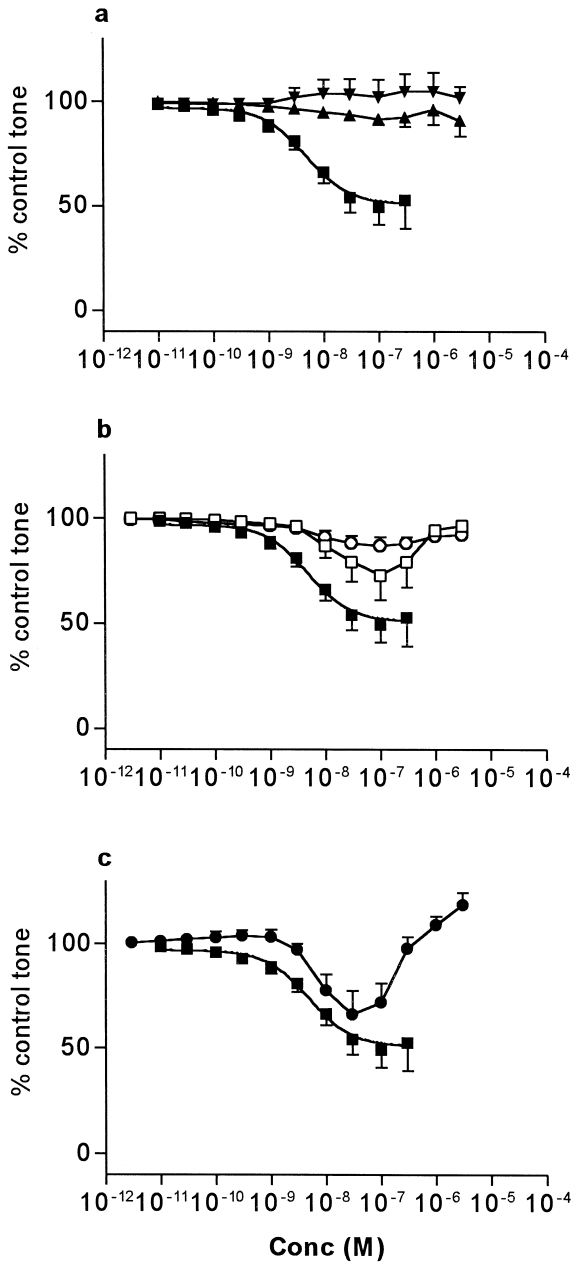

Fig. 3. Cumulative concentration-response curves for the relaxant effects of latanoprost acid in the endothelin-1 precontracted rabbit submental vein in the presence of 1×10^{-6} M GR 32191B. (a) Latanoprost acid (■), latanoprost acid in the presence of 1×10^{-4} M L-NAME (▼) and latanoprost acid in endothelium-denuded preparations (▲). (b) Latanoprost acid (■), latanoprost acid in the presence of 5×10^{-6} M CGRP-(8–37) (○) and latanoprost acid in the presence of 5×10^{-6} M GR 82334 (□). (c) Latanoprost acid (■) and latanoprost acid in the absence of GR 32191B (●). Relaxation is expressed as percentage of the difference between precontracted and papaverine relaxed values and the data are shown as means ± S.E.M., $n = 4–9$.

3.4. Effect of receptor antagonists and L-NAME on tone of precontracted vessels

The tachykinin NK_1 receptor antagonist, GR 82334, and the calcitonin gene-related peptide receptor antagonist, CGRP-(8–37), as well as the nitric oxide synthase inhibitor, L-NAME, slightly increased the basal tone with approximately 5–10%, and augmented the contractile response to endothelin-1 (data not shown). The TP receptor antagonist, GR 32191B, did not affect the basal precontracted tone.

4. Discussion

The veins of the surface structures of the rabbit eye are too small for in vitro studies and we, therefore, chose the rabbit submental vein as a suitable model for studying the effects of prostaglandins on small to medium-sized veins. Although the primary objective of the study was to investigate the vasorelaxant effect of prostaglandins with special emphasis on ocular veins, the results of the study may also be of interest from a general pharmacological point of view, since relatively little is known about the effects of prostaglandins on veins.

The results indicate that prostaglandin E_2 and prostaglandin $F_{2\alpha}$ as well as latanoprost acid, in the presence of the TP receptor antagonist, GR 32191B, can exert a vasorelaxant effect in small veins such as the rabbit submental vein under in vitro conditions. The three prostaglandins tested have very different receptor profiles. Prostaglandin E_2 is a potent agonist on the prostaglandin E (EP) receptors, and in addition has a considerable agonistic effect on the prostaglandin F (FP) receptor (Coleman et al., 1989). Prostaglandin $F_{2\alpha}$ is a potent but not very selective FP receptor agonist, since it also exerts some agonistic activity on EP receptors (Coleman et al., 1989; Stjernschantz et al., 1995), whereas latanoprost acid is a relatively selective FP receptor agonist (Stjernschantz et al., 1995). Since some prostaglandins, e.g. prostaglandin $F_{2\alpha}$, at higher concentrations are known to spill over on the TP receptor (Coleman et al., 1989), we have used the TP receptor blocker, GR 32191B, to avoid smooth muscle contracting effects brought about by co-stimulation of TP receptors. It can indeed be seen in Fig. 2C and Fig. 3C that in the absence of the TP receptor antagonist both prostaglandin $F_{2\alpha}$ and latanoprost acid at higher concentrations tended to cause contraction of the vascular smooth muscle, which disturbs the interpretation of the vasorelaxant effects. No such effect was observed with prostaglandin E_2.

The data obtained show clearly that the endothelium plays a significant role in the mediation of the vasorelaxant effect of prostaglandin $F_{2\alpha}$ and latanoprost acid. The endothelium-dependent relaxation in response to the FP receptor agonists agrees well with the results of Chen et al. (1995), although the vasorelaxant response was smaller in the submental vein than in the rabbit jugular vein. Furthermore, the prostaglandin $F_{2\alpha}$-induced relaxation in the hand vein, although attributed to the stimulation of EP_2 or IP receptors, was documented to be endothelium-dependent (Arner et al., 1994). Much of the vasorelaxant effect of prostaglandin E_2 was dependent on the endothelium, which also has been demonstrated for the human hand vein (Arner et al., 1994). This is, however, in contrast to the endothelium-independent vasorelaxation of prostaglandin E_2 demonstrated in the rabbit jugular vein (Chen et al., 1995; Milne et al., 1995). These divergent results may be explainable by the existence of different EP receptor subpopulations in different types and sizes of veins. Our results thus indicate that endothelial nitric oxide mediates the vasorelaxant effect of FP receptor agonists entirely or almost entirely, whereas the effect of prostaglandin E_2 seems to be only partly mediated by the release of endothelial nitric oxide in the rabbit submental vein.

The finding that the tachykinin NK_1 receptor antagonist, GR 82334, virtually completely abolished the vasorelaxant effect of prostaglandin $F_{2\alpha}$, inhibited the latanoprost acid-induced relaxation, and further caused about 2 log units rightward shift of the concentration-response curve of prostaglandin E_2 suggests that a tachykinin, possibly substance P, is involved in the relaxant effect (Maggi, 1995). The fact that the vasorelaxant effect induced by prostaglandin $F_{2\alpha}$ and latanoprost acid could be completely abolished by L-NAME as well as by denuding of the endothelium suggests that nitric oxide and the neuropeptide are coupled in series rather than in parallel. It has been shown that prostaglandin $F_{2\alpha}$-isopropyl ester increased the blood flow to the surface structures of the rabbit eye, and this effect could be abolished by L-NMMA (N^G-monomethyl-L-arginine monoacetate), a nitric oxide synthase blocker (Astin et al., 1994). Recently we also showed that sensory denervation abolishes the prostaglandin $F_{2\alpha}$-isopropyl ester-induced increase in blood flow to the surface structures of the rabbit eye (Astin and Stjernschantz, 1997). Although these in vivo data reflect changes in arterioles, they nevertheless support the finding of the present study that the vasodilator effect of the prostaglandins was based on release of both nitric oxide and neuropeptides. The sequence of release of these mediators could not be determined from the present experiments but the in vivo data described above would suggest that perivascular nerves are stimulated first to release e.g. substance P which in turn releases nitric oxide from the vascular endothelium. Tachykinin-induced vasodilation has been reported to be endothelium-dependent (Maggi, 1995). It cannot be totally excluded, however, that the vasorelaxant effect is mediated directly by prostaglandin receptors in the endothelium, and somehow in parallel by neuropeptides, because of the artificial in vitro conditions.

Pretreatment with CGRP-(8–37) also suppressed the prostanoid-induced relaxant responses, at least those to prostaglandin $F_{2\alpha}$ and latanoprost acid, which suggests the

involvement of calcitonin gene-related peptide in the relaxation. Calcitonin gene-related peptide-induced vasodilatation has been found to be endothelium-dependent as well as endothelium-independent (Maggi, 1995). Substance P and calcitonin gene-related peptide are known to be co-localized in perivascular sensory nerves (Maggi, 1995). Therefore, on the basis of the results it is reasonable to believe that the vasorelaxant effect of the two FP receptor agonists is at least in part mediated by the release of substance P and calcitonin gene-related peptide from perivascular nerves.

The fact that the relatively flat concentration-response curve of prostaglandin E_2 extends over 5 log units suggests that several components are involved in the vasorelaxation, such as nitric oxide, tachykinins, calcitonin gene-related peptide, and probably direct smooth muscle effects of prostaglandin E_2. Since prostaglandin E_2 is also a potent agonist on the EP_1 and EP_3 receptors which are associated with calcium mobilization and linked to the phosphoinositol pathway (Coleman et al., 1989, 1994b), it may be that the smooth muscle relaxing effect of prostaglandin E_2 is the sum of contractile and relaxant forces leading to a flat concentration-response curve. Indeed, the increase in tension seen at around 5×10^{-8} and 10^{-5} M in the prostaglandin E_2 concentration-response curve during pretreatment with L-NAME could reflect stimulation of smooth muscle receptors and subsequent contraction. It is well known that synthesis of nitric oxide by constitutive nitric oxide synthase is Ca^{2+}-dependent (Moncada et al., 1991). Since synthesis and release of nitric oxide seem to be important for a large part of the vasorelaxant effect induced by prostaglandin E_2 in the rabbit submental vein, the EP receptor subtype associated with this response could be EP_1 and/or EP_3. However, the results indicate that the relaxation in response to prostaglandin E_2 is partly endothelium-independent in this preparation. Endothelium-independent relaxation in response to prostaglandin E_2 has also been shown in the rabbit jugular vein (Chen et al., 1995; Milne et al., 1995). The EP receptor responsible for this relaxation seems to be of the EP_4 receptor subtype (Milne et al., 1995). The EP_2 and EP_4 receptors are coupled to adenylate cyclase stimulating G-proteins (Coleman et al., 1989, 1994a). Therefore, it is reasonable to suggest that the rabbit submental vein contains, in addition to EP_1 and/or EP_3 receptors, EP receptors linked to stimulation of adenylate cyclase.

Interestingly, whereas the blocking effect of the NK_1 receptor antagonist (GR 82334) was very efficent, the corresponding effect of CGRP-(8–37) was rather modest and parallel displacement was not observed. Thus, it would appear that the mechanism of the neurokinin-induced vasorelaxation differs from that of calcitonin gene-related peptide. The calcitonin gene-related peptide effect is perhaps a direct one whereas the neurokinin effect is based on release of nitric oxide (Maggi, 1995).

The fact that the concentration-response curve of prostaglandin E_2 was displaced to the right by about one log unit during TP receptor antagonism indicates that the antagonist (GR 32191B) is probably a weak antagonist of some of the EP receptors, probably the EP_4 receptor (Coleman et al., 1994b), which has recently been documented for other TP receptor antagonists in piglet (Coleman et al., 1994a) and rabbit (Lydford et al., 1996) saphenous veins.

In general it appeared that in the presence of the TP receptor blocker, prostaglandin $F_{2\alpha}$ induced somewhat less relaxation than did latanoprost acid. The reason for this could be that latanoprost acid is a more selective FP receptor agonist than prostaglandin $F_{2\alpha}$. The effect of removal of the endothelium and L-NAME was consistent and very similar for both prostaglandin $F_{2\alpha}$ and latanoprost acid. With both drugs there was a slight tendency to contraction in the presence of L-NAME, which could have been due to a spill over on contractile receptors, possibly EP_1 or EP_3 receptors. In the absence of GR 32191B both drugs caused contraction of the vascular smooth muscle, although this was preceded by a relaxant effect of latanoprost acid at lower concentrations.

In the rabbit jugular vein both prostaglandin $F_{2\alpha}$ and fluprostenol were shown to induce almost maximal relaxation (Chen et al., 1995). In the submental vein the vasorelaxation induced by the FP receptor agonists, latanoprost acid and prostaglandin $F_{2\alpha}$, was less pronounced. In both these vein preparations the relaxant responses of the FP receptor agonists were dependent on the presence of an intact endothelium. However, whereas in the rabbit jugular vein with intact endothelium the concentration-response curve for prostaglandin $F_{2\alpha}$ after pretreatment with L-NAME was significantly shifted to the right, the relaxation induced by prostaglandin $F_{2\alpha}$ in the rabbit submental vein was completely abolished after pretreatment with the same inhibitor. These divergent results are difficult to explain. However, one possibility could be that the veins differ because of size and location. Another possibility could be differences in the techniques used. In the present study, endothelin-1 was used to induce precontraction and papaverine to induce maximal relaxation of the vessel preparations, whereas in the experiments with the jugular vein histamine was used as contractile agent and prostaglandin E_2 to induce maximal relaxation. Moreover, a different resting tension was used during the equilibration period in the two studies. Also, different TP receptor antagonists were used in the two studies. Finally, the physiological salt solution used in the present study contained EDTA and receptor antagonists for elimination of cholinergic (atropine) and adrenergic (propranolol and phentolamine) tone, whereas neither of these receptor antagonists nor EDTA were used in the experiment with the jugular vein.

In the submental vein with intact endothelium, the neuropeptide receptor blockers, CGRP-(8–37) and GR 82334, as well as the nitric oxide synthase inhibitor, L-NAME, caused a small increase in basal endothelin-1

precontracted tone. This could reflect a continuous physiological release of calcitonin gene-related peptide, a tachykinin and nitric oxide in the rabbit submental vein.

In conclusion, the results of the present study demonstrate that stimulation of the FP prostanoid receptor in veins results in vasorelaxation which seems to be due to release of nitric oxide, calcitonin gene-related peptide and probably a tachykinin. The vascular endothelium is necessary for this relaxation. Prostaglandin E_2 induced a stronger vasorelaxation than did the FP receptor agonists, and the mechanism was at least partly different in that in addition to the effect of nitric oxide and the neuropeptides, direct stimulation of EP receptors in the vascular smooth muscle layer may occur. Our data indicate that in vivo prostaglandin E_2 most likely induces dilatation of veins, whereas the in vivo effects of prostaglandin $F_{2\alpha}$ and latanoprost acid probably depend on the contractile state of the veins, and possibly on the stimulatory threshold of the perivascular nerves.

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