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Regulation of human penile smooth muscle tone by prostanoid receptors

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1 We have characterized the prostanoid receptors involved in the regulation of human penile arterial and trabecular smooth muscle tone.

2 Arachidonic acid induced relaxation of human corpus cavernosum strips (HCCS) that was blocked by the cyclo-oxygenase inhibitor, indomethacin, and augmented by the thromboxane receptor (TP) antagonist, SQ29548, suggesting that endogenous production of prostanoids regulates penile smooth muscle tone.

3 TP-receptors mediate contraction of HCCS and penile resistance arteries (HPRA), since the agonist of these receptors, U46619, potently contracted HCCS (EC_{50} 8.3 ± 2.8 nM) and HPRA (EC_{50} 6.2 ± 2.2 nM), and the contractions produced by prostaglandin $F_{2\alpha}$ at high concentrations (EC_{50} 6460 ± 3220 nM in HCCS and 8900 ± 6700 nM in HPRA) were inhibited by the selective TP-receptor antagonist, SQ29548 (0.02 μM).

4 EP-receptors are responsible for prostanoid-induced relaxant effects in HCCS because only prostaglandin E_1 (PGE_1), prostaglandin E_2 and the EP_2/EP_4-receptor agonist, butaprost, produced consistent relaxation of this tissue (EC_{50} 93.8 ± 31.5, 16.3 ± 3.8 and 1820 ± 1284 nM, respectively). In HPRA, both prostacyclin and PGE_1 (EC_{50} 60.1 ± 18.4 and 109.0 ± 30.9 nM, respectively) as well as the selective IP receptor agonist, cicaprost, and butaprost (EC_{50} 25.2 ± 15.2 and 7050 ± 6020 nM, respectively) caused relaxation, suggesting co-existence of IP- and EP-receptors (EP_2 and/or EP_4).

5 In summary, endogenous production of prostanoids may regulate penile smooth muscle contractility by way of specific receptors. TP-receptors mediate contraction in HCCS and HPRA, while the relaxant effects of prostanoids are mediated by EP_2- and/or EP_4-receptors in HCCS and by EP- and IP-receptors in HPRA.

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Abbreviations: DP, D-prostanoid; EP, E-prostanoid; FP, F-prostanoid; HCCS, human corpus cavernosum strips; HPRA, human penile resistance arteries; IP, I-prostanoid; PGD_2, prostaglandin D_2; PGE_1, prostaglandin E_1; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; PGI_2, prostacyclin; TP, T-prostanoid; TXA_2, thromboxane A_2

Introduction

Derivatives of arachidonic acid generated through the cyclo-oxygenase pathway are local mediators involved in many regulatory processes such as inflammation, platelet aggregation, control of vascular tone, etc. The large number and the importance of the actions exerted by prostanoids confer pharmacological relevance to the modulation of the effects induced by these compounds. Prostanoid receptors are grouped into five families: DP receptors, EP receptors (including four subtypes with different actions), FP receptors, IP receptors and TP receptors. The distribution of prostanoid receptors differs substantially between tissues and the coexistence of receptors mediating antagonistic actions is frequently observed (Baxter *et al*., 1995; Lydford *et al*., 1996; Qian *et al*., 1994).

Production of thromboxane A_2 (TXA_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2) and prostaglandin E_2 (PGE_2) has been reported in rabbit (Daley *et al*., 1996) and human corpus cavernosum tissue (Jeremy *et al*., 1986; Moreland *et al*., 2001), and prostanoids have been shown to induce both contractile and relaxant effects in human trabecular and arterial smooth muscle (Hedlund & Andersson, 1985).

As relaxation of penile smooth muscle is needed to achieve and maintain penile erection (Saenz de Tejada *et al*., 1991), the interest in prostanoids for the treatment of erectile dysfunction has focused on the use of those with relaxant properties. PGE_1 has been shown to produce penile trabecular smooth muscle relaxation and penile erection, and has been extensively used as intracavernosal therapy for impotence (Porst, 1996). It is possible, however, that pharmacological antagonism of the action of constrictor prostanoids may also facilitate penile smooth muscle relaxation and therefore erection. It has been recognized that the balance in the actions of prostanoids can be altered by disease. Excessive production of contractile prostanoids (Davi *et al*., 1997; Koltai *et al*., 1990) or enhanced contractile pathways (Hattori *et al*., 1999) have been

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described in other vascular tissues and the kidney (McCarty, 1998).

The aims of this study were to characterize the prostanoid-receptors which mediate contraction and relaxation of human corpus cavernosum and resistance penile arteries.

Methods

Human corpus cavernosum tissues

Human corpus cavernosum specimens were obtained from impotent men at the time of penile prosthesis insertion. Tissues were maintained at 4–6°C in M-400 solution (composition per 100 ml in g) manitol 4.19, KH_2PO_4, 0.205, $K_2HPO_4{\cdot}3H_2O$ 0.97, KCl 0.112, $NaHCO_3$, 0.084, until used, which was between 2 and 16 h from extraction (Simonsen *et al*., 1997).

Vascular reactivity of resistance penile arteries

Penile small arteries, helicine arteries (lumen diameter 150–400 μm), which are the terminal branches of deep penile arteries, were dissected by carefully removing the adhering trabecular tissue, and arterial ring segments (2 mm long) were subsequently mounted on two 40 μm wires on microvascular double Halpern-Mulvany myographs (J.P. Trading, Aarhus, Denmark) for isometric tension recordings. The vessels were allowed to equilibrate for 30 min in physiological salt solution (PSS) of the following composition (mM): NaCl 119, KCl 4.6, $CaCl_2$ 1.5, $MgCl_2$ 1.2, $NaHCO_3$ 24.9, glucose 11, KH_2PO_4 1.2, EDTA 0.027 at 37°C continuously bubbled with 95% O_2/5% CO_2 mixture to maintain a pH of 7.4. Passive tension and internal circumference of vascular segments when relaxed *in situ* under a transmural pressure of 100 mmHg (L_{100}), were determined. The arteries were then set to an internal circumference equivalent to 90% of L_{100}, at which the force development was close to maximal (Mulvany & Halpern, 1977). The preparations were then exposed to 125 mM K^+ (KPSS, equimolar substitution of NaCl for KCl in PSS) and the contractile response was measured. Contractile responses were evaluated by adding increasing cumulative concentrations of compounds on unstimulated arterial rings. For the relaxation studies, the arteries were contracted with 1 μM norepinephrine (80% of KPSS induced contraction approximately) and relaxation responses were evaluated by cumulative additions of compounds to the chambers.

Organ chamber studies

Strips of corpus cavernosum tissue ($3 \times 3 \times 7$ mm) were immersed in 8 ml organ chambers containing PSS, maintained at 37°C and aerated with 5% CO_2/95% O_2, pH 7.4. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 μM phenylephrine (Azadzoi *et al*., 1992; Kim *et al*., 1991). Contractile responses were evaluated by adding increasing cumulative concentrations of compounds on unstimulated strips. For the relaxation studies, tissues were contracted with 0.5–3 μM phenylephrine (80% of KPSS induced contraction) and relaxation responses were evaluated by cumulative additions of compounds to the chambers.

Measurement of cyclic nucleotides in human corpus cavernosum tissue

Corpus cavernosum strips were immersed in 8 ml organ chambers containing PSS, maintained at 37°C and aerated with 5% CO_2/95% O_2, pH of 7.4. Each tissue strip was incrementally stretched to optimal isometric tension, as determined by maximal contractile response to 1 μM phenylephrine. Then each tissue was given 0.5 μM phenylephrine, 30 μM zaprinast and 100 μM IBMX and allowed to incubate for 15 min; after which time tissues were treated with drug or vehicle. Tissues were allowed to incubate for another 5 min then immediately frozen in liquid nitrogen and stored at −80°C until extraction for cyclic nucleotide assay. Tissues were extracted by homogenization in 6% trichloroacetic acid followed by ether (H_2O-saturated) extraction and lyophilization. Cyclic nucleotides were determined by ELISA using a kit from Cayman Chemical Co. (Ann Arbor, MI, U.S.A.).

Protein determinations

Proteins were determined using the Bio-Rad Protein Assay Kit microtiter plate assay procedure (Bio-Rad, Hercules, CA, U.S.A.) with bovine serum albumin as standard.

Drugs and materials

Arachidonic acid, phenylephrine, norepinephrine (arterenol), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), 9,11-dideoxy-9α,11α-epoxymethano $PGF_{2\alpha}$ (U46619) and indomethacin were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Sulprostone, fluprostenol, prostaglandin E_2 (PGE_2), prostacyclin (PGI_2) and prostaglandin D_2 (PGD_2) were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). [1S-[1α,2α(Z),3α,4α]]-7-[3-[[2-[(Phenylamino)carbonyl]hydrazino]methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid (SQ29548) and 4-imidazolidine-heptenoic acid (BW245C) were obtained from Research Biochemical International (Natick, MA, U.S.A.). Prostaglandin E_1 (PGE_1)-α-cyclodextrin was provided by Schwarz-Pharma. Butaprost methyl ester was a gift from Bayer PLC and cicaprost was a gift from Schering AG.

Prostanoid derivatives were dissolved at 10 mM concentration in ethanol. Dilutions were made in distilled water at the time of the experiment. Ethanol diluted as for prostanoid curves, was used for the vehicle curves. PGE_1-αCD and non-prostanoid drugs were dissolved in distilled water. Indomethacin was dissolved in 1.5 mM $NaCO_3$.

Data analysis

Contractile effects produced by drugs are expressed as percentage of contraction elicited by KPSS. Relaxation responses are expressed as percentage of total relaxation (loss in tone) induced by the addition of 0.1 mM papaverine HCl to the chambers at the end of the experiment. All data are expressed as mean ± s.e. Complete concentration-response curves were obtained and compared by a two-factor

analysis of variance (ANOVA) statistical test using StatView software for Apple computers. Statistical analysis of tissue cyclic nucleotide levels was performed by one-factor ANOVA followed by a Student-Newman-Keuls *post hoc* test using GraphPad InStat software. Individual EC_{50} values were graphically calculated with the Cricket Graph software by plotting the individual concentration-response curve and interpolating the concentration value corresponding to the 50% of the maximum effect induced by the compound. The values obtained were grouped and expressed as mean $\pm$ s.e.

The pA_2 values for SQ29548 to antagonize U46619-induced contractions were estimated by applying the following equation (Lydford *et al*., 1996):

$$pA_2 = \log(r - 1) - \log[SQ29548]$$

Where r is the ratio EC_{50} for U46619 in the presence of SQ29548/EC_{50} for U46619 in the control curve in the same preparation (paired curve data). The concentration of SQ29548 was 2×10^{-8} M.

Results

Effect of endogenous production of prostanoids induced by arachidonic acid on trabecular smooth muscle tone

One-hundred μM arachidonic acid (AA) added to phenylephrine-contracted strips of human trabecular tissue caused modest relaxations, which were inhibited in tissues previously treated with the cyclo-oxygenase inhibitor, indomethacin (10 μM), and markedly enhanced in tissues treated with the TP receptor antagonist, SQ29548 (0.02 μM) (Figure 1).

Prostanoid-induced contractions of human penile smooth muscle

Contractions in response to agonists for TP, FP, EP_1 and EP_3 receptors were evaluated in human corpus cavernosum strips and penile resistance arteries.

The thromboxane analogue, U46619 (0.01 nM – 3 μM), produced potent contractile responses of human trabecular smooth muscle (EC_{50} 8.3 $\pm$ 2.8 nM). $PGF_{2\alpha}$ (1 nM – 100 μM), the endogenous agonist of FP receptors, induced contractions of human trabecular tissue with a maximal response (108.4 $\pm$ 37.7% of KPSS) not significantly different from that elicited by U46619 (128.2 $\pm$ 12.5%), but the concentrations required were markedly higher (EC_{50} 6460 $\pm$ 3220 nM). The specific synthetic agonist for FP receptors, fluprostenol (1 nM – 100 μM), was even less potent than $PGF_{2\alpha}$ in causing contractions of human trabecular smooth muscle (EC_{50} 29540 $\pm$ 14040 nM). Moreover, the EP_1 and EP_3 receptor agonist, sulprostone (1 nM – 100 μM), failed to contract trabecular smooth muscle (Figure 2).

The TP receptor antagonist, SQ29548 (0.02 μM), did not modify basal tone, but shifted to the right the concentration-response curves to U46619 in human corpus cavernosum (EC_{50} 115.2 $\pm$ 39.9 nM), without change of the maximal response (Figure 3A). The pA_2 value estimated for SQ29548 in human corpus cavernosum was 9.0 $\pm$ 0.1. In addition, TP receptor blockade with SQ29548 (0.02 μM) significantly inhibited the contractile responses to $PGF_{2\alpha}$ and fluprostenol (Figure 3B, C).

Prostanoid-induced contractions in human penile resistance arteries were very similar to those observed in trabecular tissue. U46619 (0.01 nM – 10 μM) evoked potent contractile responses of penile arteries (EC_{50} 6.2 $\pm$ 2.2 nM) while, among the other tested agonists, only $PGF_{2\alpha}$ (0.1 nM to 100 μM)

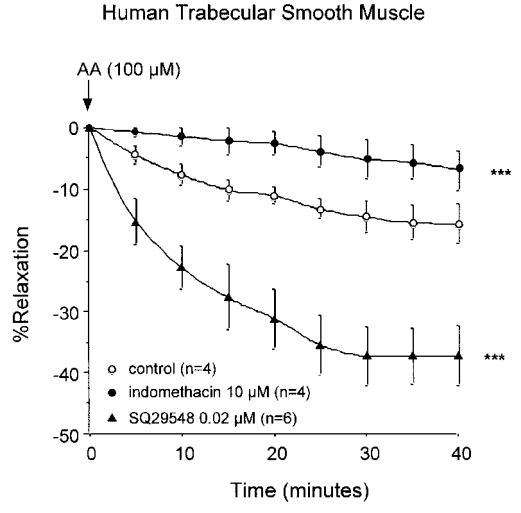

Figure 1 Effects of the treatment with indomethacin (10 μM) or SQ29548 (0.02 μM) on loss in tone induced by the addition of arachidonic acid (AA; 100 μM) in human trabecular smooth muscle strips contracted with phenylephrine. Data are expressed as mean $\pm$ s.e.mean of the percentage of total relaxation induced by 0.1 mM papaverine. *n* indicates the number of patients from whom the tissues were collected for the experiments. ***Indicates $P < 0.005$ vs control responses by a two-factors ANOVA test.

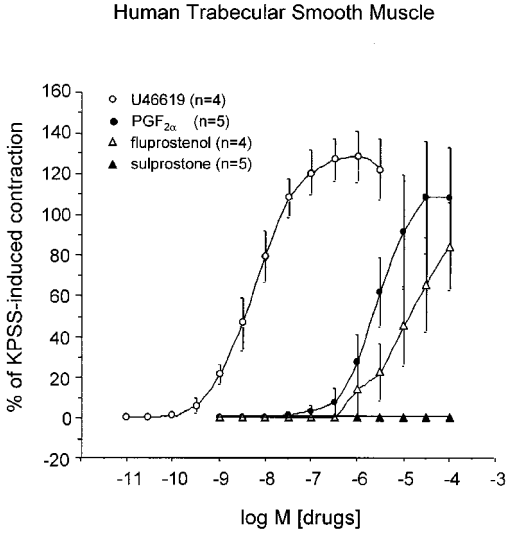

Figure 2 Contractile responses induced by the thromboxane analogue, U46619, by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), by the FP receptor agonist, fluprostenol, and by the agonist for EP_1 and EP_3 receptors, sulprostone, in human trabecular smooth muscle strips. Data are expressed as mean $\pm$ s.e.mean of the percentage of contraction induced by 125 mM K^+ (KPSS). *n* indicates the number of patients from whom the tissues were collected for the experiments.

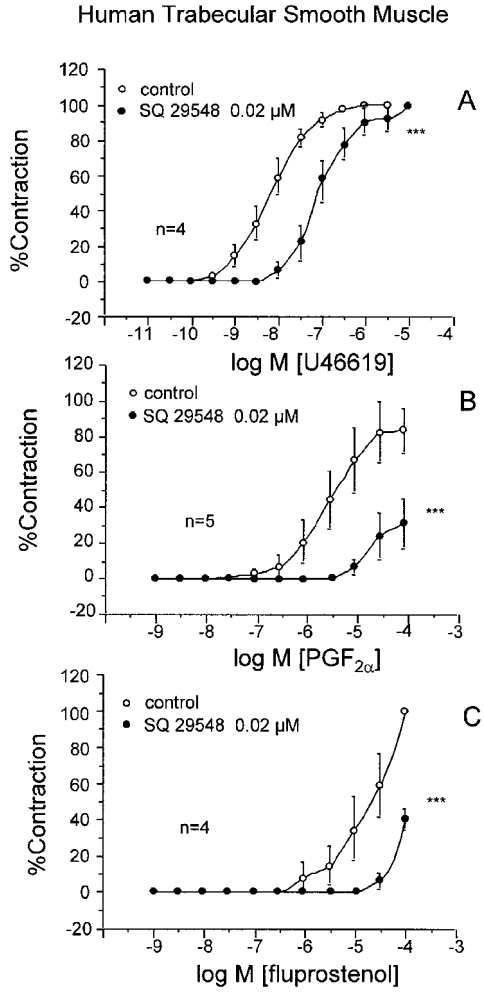

Figure 3 Effects of the treatment with the TP receptor antagonist, SQ29548 (0.02 μM), on the contractile responses elicited by the thromboxane analogue, U46619 (A), by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (B), and by the FP receptor agonist, fluprostenol (C), in human trabecular smooth muscle strips. Data are expressed as mean$\pm$ s.e.mean of the percentage of maximum contraction obtained in absence of SQ29548. *n* indicates the number of patients from whom the tissues were collected for the experiments. ***Indicates $P<0.005$ vs control responses by a two-factors ANOVA test.

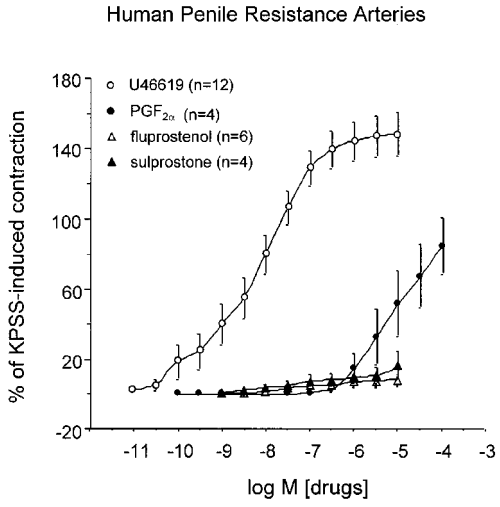

Figure 4 Contractile responses induced by the thromboxane analogue, U46619, by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), by the FP receptor agonist, fluprostenol, and by the agonist for EP_1 and EP_3 receptors, sulprostone, in human penile resistance arteries. Data are expressed as mean$\pm$s.e.mean of the percentage of contraction induced by 125 mM K^+ (KPSS). *n* indicates the number of patients from whom the tissues were collected for the experiments.

produced appreciable contractions, but requiring high concentrations (EC_{50} 8900$\pm$6700 nM) (Figure 4).

The TP receptor antagonist, SQ29548 (0.02 μM) did not alter basal tone, but, as observed in cavernosal tissue, inhibited, in a competitive manner, the contractile responses to U46619 (245.9$\pm$85.9 nM) in human penile arteries (Figure 5A). The pA_2 value estimated for SQ29548 in these arteries was 9.1$\pm$0.1. The contractions elicited by $PGF_{2\alpha}$ were significantly reduced by the treatment of penile arteries with SQ29548 (0.02 μM) (Figure 5B).

Relaxant responses elicited by prostaglandins in human penile smooth muscle

On phenylephrine-contracted human corpus cavernosum strips, administration of the endogenous agonists of DP and IP receptors, PGD_2 (1 nM – 10 μM) and PGI_2 (1 nM – 10 μM), respectively, did not induce significant relaxant responses and provoked weak contractions at high concentrations. In contrast, the EP receptor agonists, PGE_2 (1 nM – 3 μM) and PGE_1 (1 nM – 3 μM), and the EP_2/EP_4 subtype receptor agonist, butaprost (1 nM – 100 μM), induced consistent relaxations of trabecular smooth muscle with the potency order of $PGE_2 > PGE_1 > >$ butaprost (EC_{50} 16.3$\pm$3.8, 93.8$\pm$31.5 and 1820$\pm$1284 nM, respectively) (Figure 6).

In human penile resistance arteries, in contrast to corpus cavernosum strips, PGI_2 (1 nM – 10 μM) as well as PGE_1 (1 nM to 10 μM) produced consistent relaxant responses, being the potency order $PGI_2 > PGE_1$ (EC_{50} 60.1$\pm$18.4 and 109.0$\pm$30.9 nM, respectively). PGD_2 (1 nM to 1 μM) exerted a dual effect on contracted human penile arteries, inducing relaxations comparable to those obtained with PGE_1 at concentrations lower than 0.3 μM and producing an increase in contractile tone at higher concentrations (Figure 7A). When relaxations to more selective agonists of IP, EP_2/EP_4 and DP receptors, cicaprost (1 nM – 10 μM), butaprost (1 nM – 100 μM) and BW245C (1 nM – 10 μM), respectively, were tested, cicaprost and butaprost induced consistent relaxations, although cicaprost was several orders of magnitude more potent than butaprost (EC_{50} 25.2$\pm$15.2 and 7050$\pm$6020 nM, respectively). However, the selective agonist of DP receptors, BW245C, did not induce significant relaxations of human penile arteries (Figure 7B).

Effect of PGE_1 on cyclic nucleotide levels in human corpus cavernosum tissue

Addition of PGE_1 (0.1 – 10 μM) produced a significant increase of cyclic AMP content in human corpus cavernosum tissue, which was concentration-dependent, and was statistically significant at the 1 μM and higher concentrations (Figure 8A). Nevertheless, the levels of cyclic GMP in cavernosal tissue were not affected by the treatment with PGE_1 (Figure 8B).

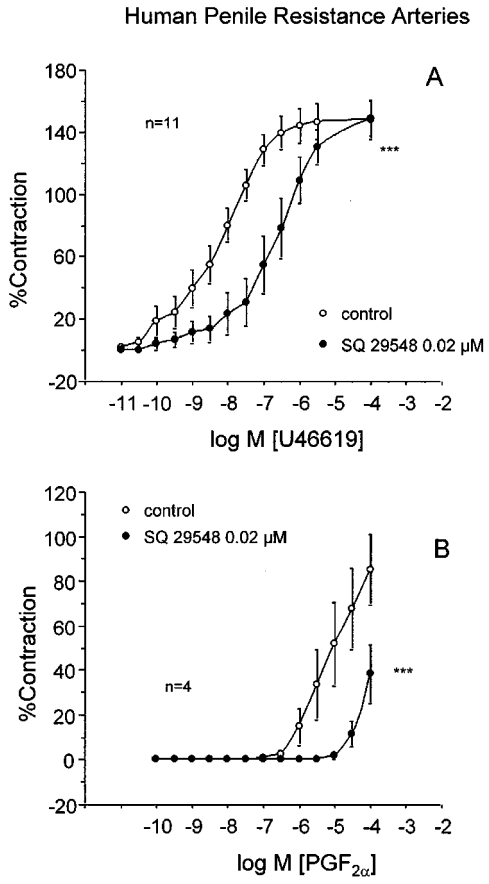

Figure 5 Effects of the treatment with the TP receptor antagonist, SQ29548 (0.02 μM), on the contractile responses elicited by the thromboxane analogue, U46619 (A) and by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (B) in human penile resistance arteries. Data are expressed as mean$\pm$s.e.mean of the percentage of maximum contraction obtained in absence of SQ29548. *n* indicates the number of patients from whom the tissues were collected for the experiments. ***Indicates $P<0.005$ vs control responses by a two-factors ANOVA test.

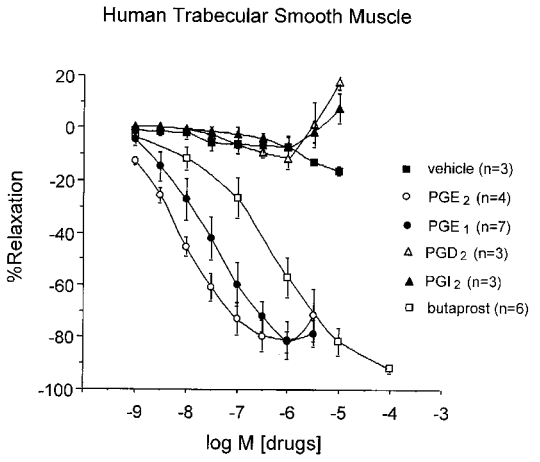

Figure 6 Responses elicited by vehicle, prostaglandin D_2 (PGD_2), prostacyclin (PGI_2), prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2) and the selective EP_2 receptor agonist, butaprost, on human trabecular smooth muscle strips contracted with phenylephrine. Data are expressed as mean$\pm$s.e.mean of the percentage of total relaxation induced by 0.1 mM papaverine. *n* indicates the number of patients from whom the tissues were collected for the experiments.

Discussion

The changes in tone following the addition of arachidonic acid, which are prevented by indomethacin, show the capacity of corpus cavernosum tissue to synthesize cyclo-oxygenase products that affect contractility of penile smooth muscle. Arachidonic acid promotes the synthesis of relaxant prostanoids, since its addition produces a moderate relaxation of trabecular smooth muscle which is blocked by the treatment with indomethacin. However, the metabolism of arachidonic acid in this tissue also involves the generation of contractile prostanoids, as demonstrated by the significant increase of relaxations following arachidonic acid administration when the strips were treated with the TP-receptor antagonist, SQ29548. Thus, these results suggest that contractile and relaxant prostanoids are generated from arachidonic acid, producing counteracting effects on contractility.

The TP, FP, EP_1 and EP_3 are the subtypes of prostanoid receptors which have been shown to produce contraction in human smooth muscle preparations (Baxter *et al.*, 1995; Qian *et al.*, 1994; Senior *et al.*, 1991; 1992). The results of the

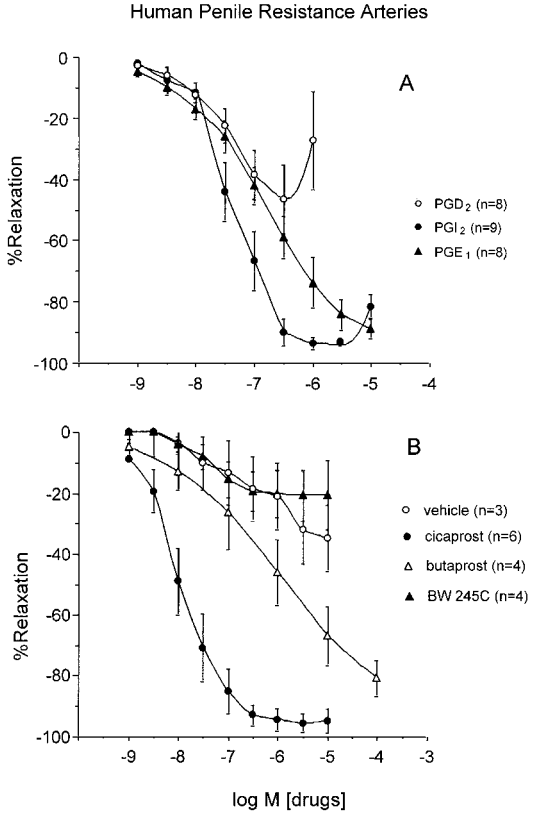

Figure 7 (A) Shows the responses elicited by prostaglandin D_2 (PGD_2), prostacyclin (PGI_2) and prostaglandin E_1 (PGE_1) on human penile resistance arteries contracted with norepinephrine. (B) shows the relaxations induced by the vehicle, by the selective DP receptor agonist, BW245C, by the selective IP receptor agonist, cicaprost and the selective EP_2 receptor agonist, butaprost, on human penile resistance arteries contracted with norepinephrine. Data are expressed as mean$\pm$s.e.mean of the percentage of total relaxation induced by 0.1 mM papaverine. *n* indicates the number of patients from whom the tissues were collected for the experiments.

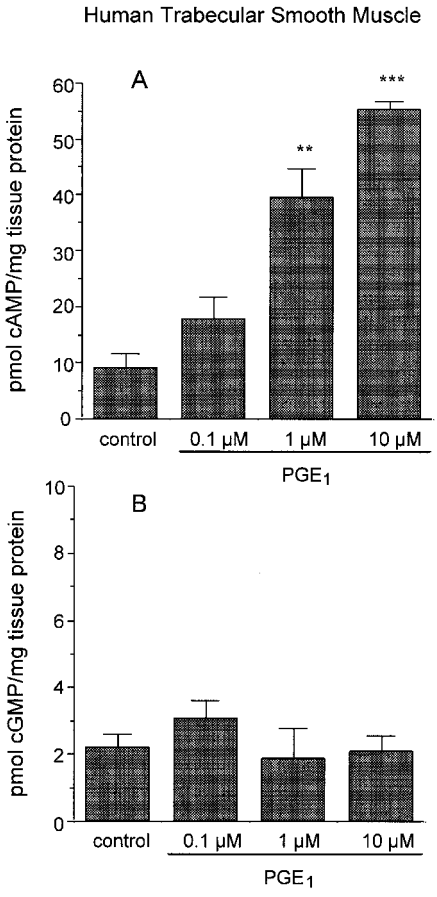

Figure 8 Cyclic AMP (A) and cyclic GMP (B) tissue content of human corpus cavernosum after exposure to prostaglandin E_1 (PGE_1). Data are expressed as mean ± s.e.mean of pmol cyclic AMP or cyclic GMP per mg of tissue protein content. *n* indicates the number of patients from whom the tissues were collected for the determinations. $^{**}P<0.01$, $^{***}P<0.005$ vs control. (Student-Newmann-Keuls *post hoc* test).

present study show that TP receptors are the prostanoid receptors which mediate contraction of human trabecular smooth muscle and penile arteries. This observation is supported by the high potency of the selective agonist of TP receptors, U46619, to induce contractions of human corpus cavernosum strips and penile arteries, in contrast to the low potency of FP agonists, $PGF_{2\alpha}$ and fluprostenol (Coleman, 1987), and the lack of response to sulprostone, an agonist of EP_1 and EP_3 receptors (Bunce *et al*., 1990). The EC_{50} values obtained for U46619 in human penile smooth muscle are in the same range as those obtained by other authors in preparations with functional TP receptors, including rat aorta (6.8 nM) (Kromer & Tippins, 1998), rabbit saphenous vein (9.8 nM) (Lydford *et al*., 1996), human bronchial smooth muscle (12 nM) (Coleman & Sheldrick, 1989) and human intrapulmonary (3.1 nM) (Jino *et al*., 1996) and uterine arteries (3.5 nM) (Baxter *et al*., 1995). In addition, although our pA_2 calculations for the antagonist of TP receptors, SQ29548, are only estimative, these are included in the range of pA_2 previously reported for this compound (Ogletree *et al*., 1985). The lack of functionally relevant FP receptors in our tissues is supported by the fact that in preparations having this type of receptors, including cultured cells (Griffin *et al*., 1998) and rabbit uterus (Chen *et al*., 1998), the EC_{50} values for $PGF_{2\alpha}$ (30.9 and 4 nM, respectively) are several orders of magnitude lower than those obtained in our study. In addition, in those studies (Griffin *et al*., 1998; Chen *et al*., 1998), the more selective FP receptor agonist, fluprostenol, was found to be equally or more potent than $PGF_{2\alpha}$ (4.4 and 6 nM, respectively), while it was 4 fold less potent in trabecular tissue and inactive in penile arteries. Furthermore, in preparations which bear TP but not FP receptors, $PGF_{2\alpha}$ was 300 fold less potent than U46619 in human bronchial smooth muscle (Coleman & Sheldrick, 1989) and 743 fold less potent in human uterine artery (Baxter *et al*., 1995). In agreement with these findings, PGF2α was 778 and 1435 fold less potent than U46619 in human trabecular tissue and penile arteries, respectively. The contractions elicited by FP receptor agonists seem to be produced by their interaction with TP receptors at high concentrations, since treatment with the TP-receptor blocker, SQ29548 (Hedberg *et al*., 1988), significantly reduced the responses to $PGF_{2\alpha}$ and fluprostenol.

With respect to the prostanoid receptors involved in relaxation of human penile smooth muscle it is necessary to distinguish between trabecular and arterial tissues. The type of prostanoid receptor that induces relaxation of human trabecular smooth muscle is the EP receptor, as supported by the consistent relaxations elicited by the prostaglandins of the E series, PGE_2 and PGE_1 as well as by the weak but selective EP_2/EP_4 receptor subtype agonist, butaprost (Gardiner, 1986). The potency of this agonist is more than one order of magnitude lower than PGE_2, but this low potency of butaprost at EP_2 receptors is commonly observed (Coleman *et al*., 1994b; Regan *et al*., 1994). In studies with cells expressing different prostanoid receptors from various animal species, butaprost only binds to EP_2 subtype presenting no activity on the closely related subtype EP_4 (Kiriyama *et al*., 1997; Castleberry *et al*., 2001; Jensen *et al*., 2001). However, butaprost methyl ester did not show selectivity between EP_2 and EP_4 human recombinant receptors (Abramovitz *et al*., 2000). Furthermore, the treatment of human cavernosal tissue with PGE_1 caused a concentration-dependent increase of cyclic AMP tissue levels, whereas the cyclic GMP levels remained unchanged. This fact agrees with the interaction of PGE_1 with an EP_2 receptor, which is known to be associated to a G_S-adenylate cyclase pathway (Coleman *et al*., 1994b). Nevertheless, this fact also does not preclude the existence of EP_4 receptors which are also coupled to Gs proteins. Thus, we cannot firmly conclude that EP_2 is the only prostanoid receptor mediating relaxation of trabecular tissue, as EP_4 may be also present. However, in support of a predominant functional role of EP_2 receptors is the observation that smooth muscle preparations with functional EP_4 subtypes usually yield EC_{50} values to PGE_2 below 1 nM (Lydford *et al*., 1996; Coleman *et al*., 1994a), while the values obtained in human trabecular tissue are notably higher and closer to those described in preparations expressing EP_2 receptors. The existence of DP and IP receptors, which have shown relaxant properties in other tissues (Baxter *et al*., 1995; Senior *et al*., 1992), can be excluded since PGD_2 and PGI_2 failed to produce significant relaxation of human trabecular smooth muscle. The activation of TP receptors was responsible for contractile effects at high concentrations of PGD_2 and PGI_2 while the blockade of these receptors (TP receptors) did not

influence the relaxation of human trabecular smooth muscle produced by PGE_1, as confirmed by experiments with SQ29548 (data not shown).

In human penile arteries, PGI_2 was the relaxant agent with the highest potency, although PGE_1 produced consistent relaxations too, with an EC_{50} similar to that obtained in trabecular smooth muscle. The presence of IP receptors is confirmed by the potent relaxant effect elicited in penile arteries by cicaprost, a selective agonist of these receptors (Stürzbecher *et al*., 1986). The relaxation effects of PGI_2 suggesting the presence of IP receptors, has already been described in large penile arteries (Hedlund & Andersson, 1985). Our findings show that IP receptors are also present in small resistance penile arteries. The EC_{50} values for PGI_2 obtained in our preparations are slightly higher than those observed in other human arterial preparations (15 nM; 12.7 nM) (Hadhazy *et al*., 1986; Baxter *et al*., 1995), but within the same order of magnitude. PGE_1 can interact with IP receptors (Dutta-Roy & Sinha, 1987), which could explain the relaxation of human penile resistance arteries induced by this molecule. However, the relaxation exerted by butaprost suggests the coexistence of IP and EP receptors (EP_2 and/or EP_4) in arterial penile smooth muscle. Although PGD_2 produced modest relaxation of penile arteries, the lack of response to BW245C, a selective agonist of DP receptors (Whittle *et al*., 1983) indicates the absence of this type of receptors in arterial smooth muscle. Thus, the relaxant effect of PGD_2 could be attributed to its interaction with other receptors. While PGD_2 does not seem to interact with IP receptors (Siegl *et al*., 1979; Dutta-Roy & Sinha, 1987), it has been proposed to induce relaxation of rabbit jugular vein induced by way of EP_2 receptors (Giles *et al*., 1989). The lack of relaxation effect of BW245C, that can interact with EP receptors (Giles *et al*., 1989) could be explained by the relatively higher affinity of this compound for EP_4 receptors (Wright *et al*., 1998; Davis & Sharif, 2000). This would suggest that penile arteries have predominantly functional EP_2 rather than EP_4 receptors.

In conclusion, endogenous and exogenous prostanoids regulate penile smooth muscle contractility *via* specific receptors. EP receptors (EP_2 and/or EP_4) in trabecular tissue and IP and probably EP receptors (EP_2 and/or EP_4) in penile arteries mediate relaxation of human penile smooth muscle, being the TP receptors responsible for prostanoid-induced contraction of penile smooth muscle (arterial and trabecular). The possible clinical relevance of these findings relies on the generation of new therapeutic targets which could enhance the action of relaxant prostanoids and/or block TP receptor activation, mainly in diseases where an excessive activity of these receptors could exist.

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