British Journal of Pharmacology (2003) 139, 1409–1416
www.nature.com/bjp

Pharmacological characterization of prostanoid receptors mediating vasoconstriction in human umbilical vein

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1 This study was undertaken to characterize pharmacologically the prostanoid receptor subtypes mediating contraction in human umbilical vein (HUV).

2 HUV rings were mounted in organ baths and concentration–response curves to U-46619 (TXA_2 mimetic) were constructed in the absence or presence of SQ-29548 or ICI-192,605 (TP receptor antagonists). U-46619 was a potent constrictor (pEC_{50}: 8.03). SQ-29548 and ICI-192,605 competitively antagonized responses to U-46619 with pK_B values of 7.96 and 9.07, respectively.

3 Concentration–response curves to EP receptor agonists: PGE_2, misoprostol and 17-phenyl-trinor-PGE_2 gave pEC_{50} values of 5.06, 5.25 and 5.32, respectively. Neither pEC_{50} nor maximum of PGE_2 and 17-phenyl-trinor-PGE_2 concentration–response curves were modified by the $DP/EP_1/EP_2$ receptor antagonist AH 6809 (1 μM). However, ICI-192,605 produced a concentration-dependent antagonism of the responses to all the EP receptor agonists. The pA_2 estimated for ICI-192,605 against PGE_2 or misoprostol were 8.91 and 9.22, respectively.

4 Concentration–response curves to FP receptor agonists: $PGF_{2\alpha}$ and fluprostenol gave pEC_{50} values of 6.20 and 5.82, respectively. ICI-192,605 (100 nM) was completely ineffective against $PGF_{2\alpha}$ or fluprostenol. In addition, lack of antagonistic effect of AH 6809 (1 μM) against $PGF_{2\alpha}$ was observed.

5 In conclusion, the findings obtained with TP-selective agonist and antagonists provide strong evidence of the involvement of TP receptors promoting vasoconstriction in HUV. Furthermore, the action of the natural and synthetic EP receptor agonists appears to be mediated *via* TP receptors. On the other hand, the results employing FP receptor agonists and antagonists of different prostanoid receptors suggest the presence of FP receptors mediating vasoconstriction in this vessel.
British Journal of Pharmacology (2003) **139,** 1409–1416. doi:10.1038/sj.bjp.0705375

Keywords: Human umbilical vein; prostanoid receptors; vasoconstriction; TP receptors; FP receptors; U-46619; SQ-29548; ICI-192,605; prostaglandin $F_{2\alpha}$; fluprostenol

Abbreviations: 5-HT, serotonin; HUV, human umbilical vein; HUVEC, human umbilical vein endothelial cell; PG, prostaglandin; TXA_2, thromboxane A_2

Introduction

Prostanoids are autacoids that exert diverse physiological and pathophysiological effects in various systems. These involve modulation of neuronal activity, alteration in platelet aggregation, regulation of ion and water transport in kidneys and gastrointestinal motility and secretion. However, the best-characterized actions are relaxation and contraction of smooth muscle (Wright *et al*., 2001). Prostanoids exert their effects by acting on five major subtypes of receptors including DP, EP, FP, IP and TP, which are named in accordance with their selectivity for the natural prostanoids, prostaglandin (PG) D_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane A_2 (TXA_2), respectively. EP receptors are further subdivided into EP_1, EP_2, EP_3 and EP_4. Of these receptors, EP_1, EP_3, FP and TP generally mediate contraction in smooth muscle, while DP, EP_2, EP_4 and IP predominantly mediate relaxation (Coleman *et al*., 1994).

The human umbilical vein (HUV) transports the oxygenated blood from the placenta to the foetus; therefore, a normal blood flow is crucial for its growth. Umbilical and placental vessels lack autonomic innervation (Reilly & Russell, 1977; Fox & Khong, 1990) and regulation of its vascular tone depends on the release of vasoactive substances, which are locally produced or conveyed through the blood stream. PGE_2, $PGF_{2\alpha}$, TXA_2 and/or their major circulating metabolites have been measured in umbilical cord plasma obtained immediately after full-term vaginal or caesarean deliveries (Mitchell *et al*., 1978; Dubin *et al*., 1981; Liu *et al*., 1998). These prostanoids have been also detected *in vitro* in the output from HUV perfused (Bjoro *et al*., 1986) and in the incubates of human umbilical vein endothelial cells (HUVEC, Watanabe *et al*., 1997; Camacho *et al*., 1998).

HUV contracts in response to PGE_2 and $PGF_{2\alpha}$ (Altura *et al*., 1972) and also to the TXA_2 mimetic, U-46619 (Boura *et al*., 1986). While the effect of U-46619 appears to be mediated *via* prostanoid TP receptors, since it is blocked by a selective TP receptor antagonist (Boura *et al*., 1986), the site of action of the PGE_2 and $PGF_{2\alpha}$ is unknown.

The aim of the present study was the pharmacological characterization of the prostanoid receptors mediating

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Advance online publication: 30 June 2003

contraction in HUV rings using both natural and synthetic agonists and antagonists.

Methods

HUV preparations

Approximately 15–35 cm segments were excised from human umbilical cords ($n=56$) midway between the placenta and newborn. These cords were collected from healthy and normotensive patients after full-term vaginal or caesarean deliveries. Written informed consent was obtained from each parturient. Cords were immediately placed at 4°C in modified Krebs solution of the following composition (mM): NaCl 119, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.0, EDTA 0.004 and D-glucose 11. Indomethacin (30 μM) was added to the bath solution 30 min before starting the concentration–response curves in order to avoid any effect induced by the release of endogenous prostanoids.

Functional studies

HUV samples were placed onto dissecting dishes containing Krebs solution. The vein (internal diameter approximately 5 mm) was carefully dissected free from Warthon's jelly using microdissecting instruments. Vascular preparations with intact endothelium were cut as rings of approximately 3 mm width. The preparations were suspended in 10 ml organ baths and stretched with an initial tension of 3–5 g as described previously (Errasti *et al*., 1999). The time from delivery until the tissue was set up in the organ baths was approximately 3 h. Changes in tension were measured with Grass isometric transducers (FT 03C, Grass Instrument, Quincy, MA, U.S.A.) and displayed on Grass polygraphs (Model 7D). During the equilibration period, Krebs solution was maintained at 37°C and at pH 7.4 by constant bubbling with 95% O_2/5% CO_2. The bath solution was replaced every 15 min with fresh warmed Krebs. After 70 min of equilibration period, each preparation was contracted with 40 mM KCl in order to test the functional state of the tissue. Optimal passive tension was adjusted throughout the equilibration period.

Concentration–response curves to U-46619 (TP receptor selective agonist), PGE_2 (endogenous agonist of EP receptors), misoprostol (EP_3 receptor agonist), 17-Phenyl-trinor-PGE_2 (EP_1 receptor agonist), $PGF_{2\alpha}$ (endogenous agonist of FP receptors) and fluprostenol (FP receptor selective agonist) were obtained after a 120 min equilibration period by cumulative addition of agonists in 0.25 log increments. Serotonin (5-HT, 10 μM) was applied at the end of each experiment in order to determine the tissue maximum response (Sardi *et al*., 1997). The TP receptor selective antagonists SQ-29548 and ICI-192,605 and the DP/EP_1/EP_2 receptor antagonist AH 6809 were applied 30 min before the cumulative addition of agonists. The lowest concentrations used of the selective TP receptor antagonist were in the range of pK_B or pA_2 previously published in other human smooth muscle tissues (Senchyna & Crankshaw, 1996; Boersma *et al*., 1999; Angulo *et al*., 2002). In the case of AH 6809, the concentration used was approximately 10-fold higher than the one published in tissues containing EP_1 receptors (Coleman *et al*., 1994). Experiments were performed in parallel in rings from the same tissue.

Data analysis and statistics

All data are presented as means ± s.e. mean. The number (n) of rings and veins is denoted: number rings/number veins, where each vein is obtained from a different umbilical cord and typically four or eight rings of each vein were employed. The responses are expressed as g of developed contraction.

The concentration–response curves were fitted to a four-parameter logistic model, where estimates of EC_{50} value, the agonist concentration that produces 50% of the maximum and the slope factor (n_H) were obtained using ALLFIT (DeLean *et al*., 1978). The EC_{50} values were transformed into pEC_{50} ($-\log EC_{50}$). Agonist log concentration ratio (r) was determined by subtracting the pEC_{50} value of the agonist in the presence of the antagonist from the pEC_{50} in control preparation. When the criteria for competitive antagonism were satisfied, that is the antagonist produced a parallel rightward shift of the agonist curve without attenuation in the maximum response over a wide range of concentrations (1–2 log units), antagonist pA_2 values and slope of Schild regressions were calculated as described by Arunlakshana & Schild (1959). In those cases, where the slope of Schild's plot was not significantly different from unity, the regression was recalculated with Schild's slope constrained to unity and the affinity value obtained was then referred as pK_B (Jenkinson *et al*., 1995). In the cases of ICI-192,605 (1 nM) vs PGE_2 or misoprostol, antagonist affinities were obtained by 'single concentration' analysis (assuming a Schild's regression slope of 1) according to the equation: $pA_2 = -\log[\text{Antagonist}] + \log(r-1)$ (Lachnit *et al*., 1997; Rogines-Velo *et al*., 2002a). Statistical analysis was performed by means of paired or unpaired Student's t test. P-values lower than 0.05 were taken to indicate significant differences between means.

Terms and equations are as recommended by the IUPHAR Committee on Receptor Nomenclature and Drug Classification (Jenkinson *et al*., 1995).

Chemicals

5-HT creatinine sulphate complex was purchased from Research Biochemical Incorporated (Natick, MA, U.S.A.). ICI-192,605 was purchased from Tocris (Ballwin, MO, U.S.A.). U-46619 (9,11-dideoxy-9α, 11α-methanoepoxy prostaglandin $F_{2\alpha}$), indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-^{1}H-indole-3-acetic acid], SQ-29548, prostaglandin $F_{2\alpha}$, fluprostenol [16-(3-trifluoromethylphenoxy)-tetranor-prostaglandin $F_{2\alpha}$] and 17-phenyl-trinor-prostaglandin E_2 were purchased from Biomol Research Laboratories (Plymouth Meeting, PA, U.S.A.). AH 6809 (6-isopropoxy-9-oxoxanthere-2-carboxylic acid), prostaglandin E_2 [prosta-5,13-dien-1-oic acid, 11,15-dihydroxy-9-oxo-, (5Z, 11α, 13E, 15S)-] and misoprostol (9-oxo-11α, 16-dihydroxy-16-methyl-prost-13E-en-1-oic acid, methyl ester) were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.).

U-46619, indomethacin, SQ-29548, $PGF_{2\alpha}$, fluprostenol and 17-phenyl-trinor-PGE_2 stock solutions were made up with ethanol and subsequent dilutions were prepared in bidistilled water. 5-HT was dissolved in bidistilled water to give stock solutions, which was further diluted with bidistilled water directly before the experiments. ICI-192,605, AH 6809, PGE_2 and misoprostol stock solutions were made up with DMSO and subsequent dilutions were prepared in bidistilled water.

All stock solutions were stored frozen in aliquots, thawed and diluted daily. All concentrations of drugs are expressed as a final concentration in the organ bath. Control experiments in the presence of corresponding concentrations of ethanol and DMSO were preformed in order to rule out any possible nonspecific action of these solvents on tonus or contractility of the preparation.

Results

When the TP receptor selective antagonist SQ-29548 was evaluated in HUV rings, concentration–response curves to U-46619 on control tissues gave a pEC_{50} of 8.01 ± 0.03, with an n_H of 2.59 ± 0.40 and the maximum response was 13.56 ± 0.67 g (88.36% of 5-HT_{max}, $n = 11/11$; Figure 1a). Increasing concentrations of SQ-29548 produced a parallel rightward shift of the U-46619 concentration–response curve without affecting the maximum response indicative of competitive antagonism. Analysis of the data by Schild regression gave a slope (0.93 ± 0.17), which was not significantly different from unity, and a pA_2 value of 8.05 yielding a pK_B value from constrained Schild's plots of 7.96 ± 0.09 ($n = 19/11$, Figure 1b).

The effect of another TP receptor selective antagonist, ICI-192,605, on the contractile response to U-46619 was also evaluated. U-46619 on control tissues gave a pEC_{50} of 8.06 ± 0.03, with an n_H of 2.94 ± 0.48 and the maximum response was 13.36 ± 0.80 g (90.34% of 5-HT_{max}, $n = 12/12$; Figure 2a). Increasing concentrations of the antagonist produced a competitive rightward shift of U-46619 concentration–response curves. Data analysed by Schild's regression gave a slope (1.07 ± 0.14), which was not significantly different from unity, and a pA_2 value of 8.99 yielding a pK_B value from constrained Schild's plots of 9.07 ± 0.07 ($n = 21/12$, Figure 2b).

In other series of experiments, concentration–response curves to PGE_2 ($n = 8/8$; Figure 3a), misoprostol ($n = 7/7$; Figure 3b) and 17-phenyl-trinor-PGE_2 ($n = 9/9$; Figure 3c, d) were performed and pEC_{50} of 5.06 ± 0.05, 5.25 ± 0.03 and 5.32 ± 0.18 with maximum responses of 10.36 ± 1.11 g (76.20% of 5-HT_{max}), 12.37 ± 1.06 g (80.72% of 5-HT_{max}) and 9.92 ± 0.99 g (73.38% of 5-HT_{max}) were estimated, respectively. Concentration–response curves to both PGE_2 or 17-phenyl-trinor-PGE_2 were not modified in the presence of the DP/EP_1/EP_2 receptor antagonist AH 6809 1 μM (Figure 3a, d). ICI-192,605 1 nM produced a parallel rightward displacement of PGE_2 ($n = 4/4$) or misoprostol ($n = 4/4$) concentration–response curves without affecting the maximum responses giving a calculated pA_2 of 9.22 ± 0.16 and 8.91 ± 0.21, respectively (Figure 3a, b). A higher concentration of this TP receptor antagonist (10 nM) completely abolished the contractile response to both EP receptor agonists (Figure 3a, b). When 17-phenyl-trinor-PGE_2 concentration–response curves were pretreated with ICI-192,605 10 or 100 nM, only with the higher concentration a rightward shift of the curve with a marked reduction of the maximal effect of the agonist was observed (max: 3.45 ± 1.12 g, $n = 5/5$; Figure 3c).

When concentration–response curves to $PGF_{2\alpha}$ (endogenous agonist of FP receptor, $n = 10/10$; Figures 4a and 5) and fluprostenol (FP receptor selective agonist, $n = 4/4$; Figure 4b) were performed, pEC_{50} of 6.20 ± 0.06 and 5.82 ± 0.19 with maximum responses of 13.90 ± 0.69 g (91.15% of 5-HT_{max}) and 10.50 ± 2.30 g (61.94% of 5-HT_{max}) were obtained, respectively. ICI-192,605 100 nM was completely ineffective against $PGF_{2\alpha}$ or fluprostenol (Figure 4a, b). In addition, it was observed that there was a lack of antagonistic effect of AH 6809 1 μM against $PGF_{2\alpha}$ concentration–response (Figure 5).

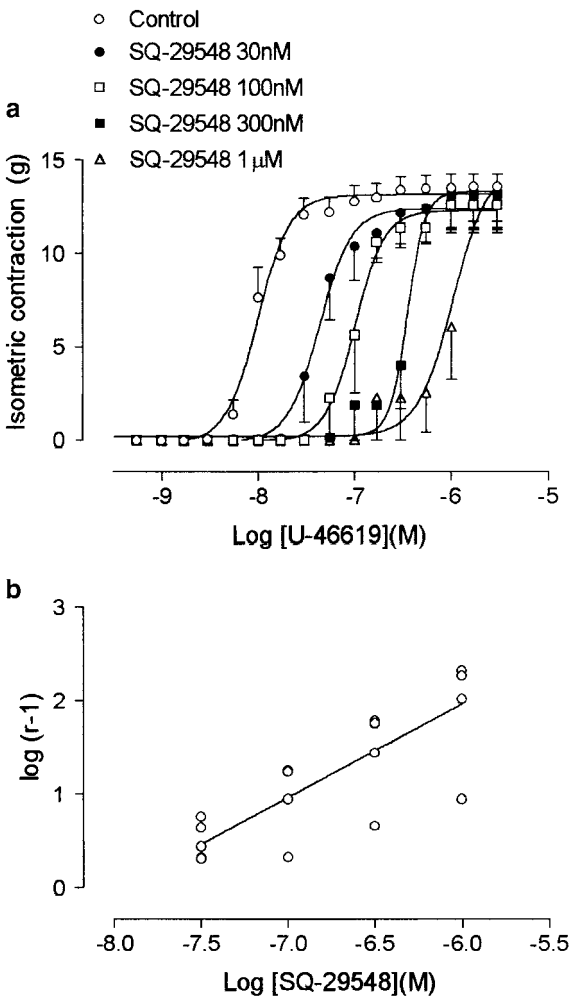

Figure 1 Antagonism of U-46619 by SQ-29548 in HUV. (a) Concentration–response curves to U-46619 on control rings ($n = 11/11$) and on tissues previously exposed to SQ-29548 (30 nM, $n = 6/6$; 100 nM, $n = 4/4$; 300 nM, $n = 5/5$; 1 μM, $n = 4/4$). Each symbol represents the mean and vertical lines represent s.e.mean. (b) Schild's plot for SQ-29548 vs U-46619 was constructed with concentration ratios from individual experiments. The slope parameter was found to be not significantly different from unity and it was subsequently constrained to unity to estimate a pK_B of 7.96 ± 0.09 ($n = 19/11$).

Discussion

The results of the present study show that U-46619 is a potent (pEC_{50}: 8.0) and full agonist (produces almost the same percentage of the maximal response to 5-HT) inducing vasoconstriction in HUV rings, suggesting that the TP receptors are involved in this effect. The pEC_{50} value estimated for U-46619 in HUV is in the same order as those estimated in other human smooth muscle tissues with functional TP receptors: 7.9 (bronchial smooth muscle, Coleman & Sheldrick, 1989); 8.1 (corpus cavernosum strips, Angulo *et al.*, 2002); 8.2 (penile resistance arteries, Angulo *et al.*, 2002);

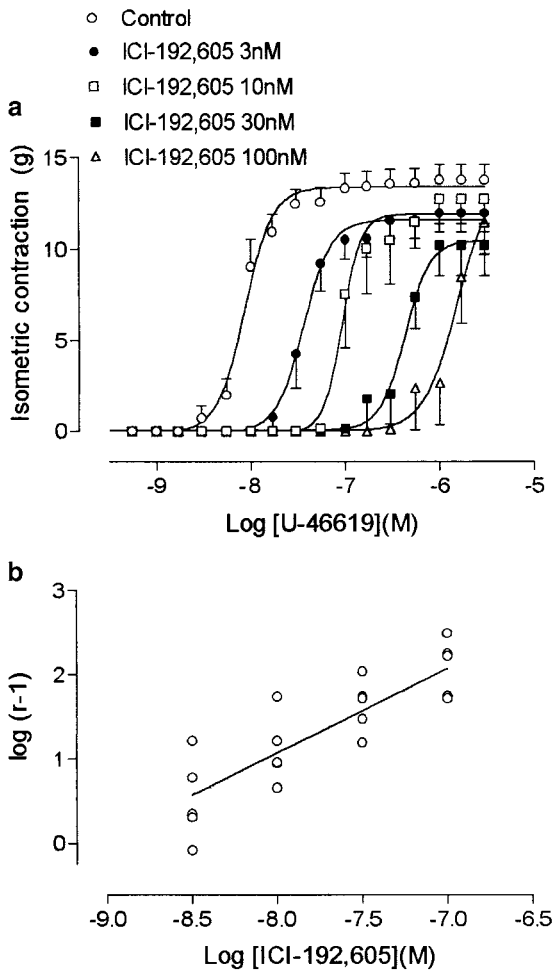

Figure 2 Antagonism of U-46619 by ICI-192,605 in HUV. (a) Concentration–response curves to U-46619 on control rings ($n=12/12$) and on tissues previously exposed to ICI-192,605 (3 nM, $n=6/6$; 10 nM, $n=5/5$; 30 nM, $n=5/5$; 100 nM, $n=5/5$). Each symbol represents the mean and vertical lines represent s.e.mean. (b) Schild's plot for ICI-192,605 vs U-46619 was constructed with concentration ratios from individual experiments. The slope parameter was found to be not significantly different from unity and it was subsequently constrained to unity to estimate a pK_B of 9.07 ± 0.07 ($n=21/12$).

8.4 (uterine artery, Baxter *et al*., 1995); 8.5 (intrapulmonary artery, Jino *et al*., 1996); 8.6 (pulmonary vein, Walch *et al*., 2001).

SQ-29548 and ICI-192,605 are recognized as competitive TP receptor antagonists (Ogletree *et al*., 1985; Jessup *et al*., 1988). In HUV, these compounds produced a rightward displacement of the concentration–response curves to U-46619 fulfilling the criteria of competitive antagonists. Furthermore, the Schild plot slope for both antagonists was not significantly different from unity, suggesting that the TP receptor selective agonist U-46619 is acting through a homogeneous TP receptor population in this tissue. In addition, the pK_B values for the

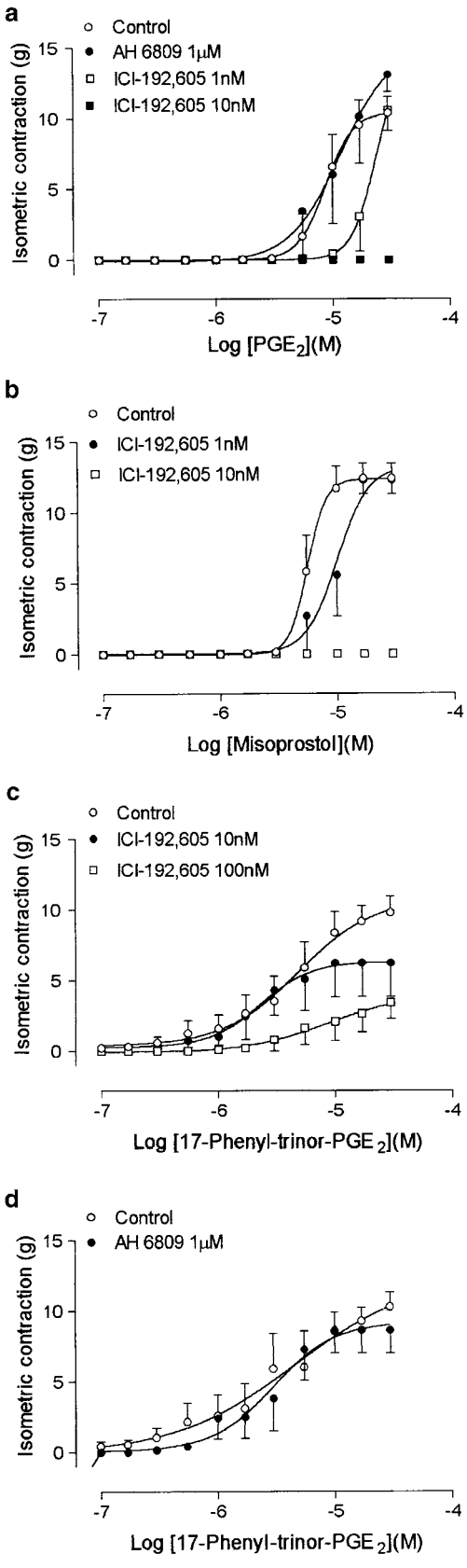

Figure 3 Antagonism of PGE_2, misoprostol and 17-phenyl-trinor-PGE_2 by ICI-192,605 and lack of effect of AH 6809 against PGE_2 or 17-phenyl-trinor-PGE_2 in HUV. (a) Concentration–response curves to PGE_2 in the presence of AH 6809 (1 μM, $n=4/4$) or ICI-192,605 (1 nM, $n=4/4$; 10 nM, $n=5/5$). (b) Concentration–response curves to misoprostol in the presence of ICI-192,605 (1 nM, $n=4/4$; 10 nM, $n=4/4$). (c) Concentration–response curves to 17-phenyl-trinor-PGE_2 in the presence of ICI-192,605 (10 nM, $n=4/4$; 100 nM, $n=5/5$). (d) Concentration–response curves to 17-phenyl-trinor-PGE_2 in the presence of AH 6809 (1 μM, $n=4/4$). Each symbol represents the mean and vertical lines represent s.e.mean.

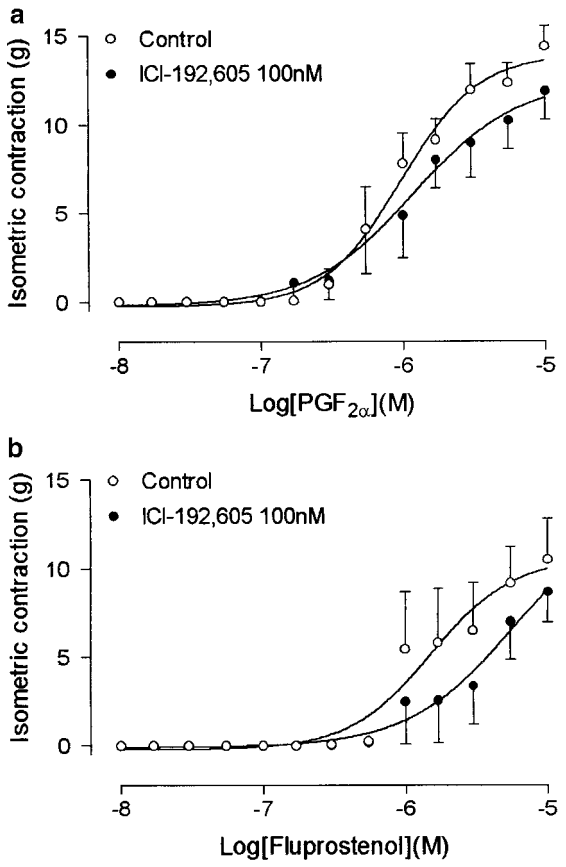

Figure 4 Lack of effect of ICI-192,605 against $PGF_{2\alpha}$ or fluprostenol in HUV. (a) Concentration–response curves to $PGF_{2\alpha}$ in the presence of ICI-192,605 (100 nM, $n=5/5$). (b) Concentration–response curves to fluprostenol in the presence of ICI-192,605 (100 nM, $n=4/4$). Each symbol represents the mean and vertical lines represent s.e.mean.

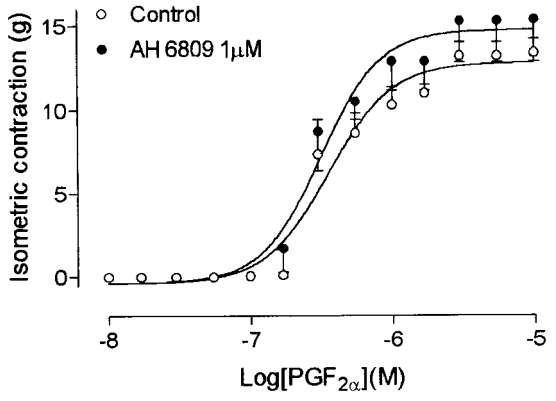

Figure 5 Lack of effect of AH 6809 against $PGF_{2\alpha}$ in HUV: concentration–response curves to $PGF_{2\alpha}$ in the presence of AH 6809 (1 μM, $n=5/5$). Each symbol represents the mean and vertical lines represent s.e.mean.

selective TP receptor antagonists SQ-29548 (8.0) and ICI-192,605 (9.1) were in the same range as those determined previously in other human smooth muscle preparations: 7.6–9.1 for SQ-29548 (umbilical artery (Boersma *et al*., 1999) and penile resistance artery (Angulo *et al*., 2002), respectively) and 8.1–9.2 for ICI-192,605 (umbilical artery (Boersma *et al*., 1999) and nonpregnant myometrium (Senchyna & Crankshaw, 1996), respectively). Moreover, the order of antagonist potency in our experimental conditions, ICI-192,605 > SQ-29548, agrees with those obtained in human myometrium (Senchyna & Crankshaw, 1996) and human umbilical artery (Boersma *et al*., 1999). Taken together, these results provide strong pharmacological evidence that TP receptors are involved in HUV vasoconstriction induced by U-46619.

To our knowledge, only Boura *et al*. (1986) have previously studied the vasoconstrictor response to U-46619 in HUV obtaining a potency (pEC_{50}: 7.7) similar to that observed in the present study. However, the study of Boura *et al*. (1986) was made using vein segments cut longitudinally into strips in order to perform the functional assays, in contrast to the present work where the tissue was cut into rings. Histologically, HUV contains two layers of smooth muscle, one longitudinal and the other one circular (Spivack, 1946). Thus, in functional study when using HUV ring preparations, the circular layer is being evaluated while the longitudinal layer is tested when using longitudinally strips. Altura *et al*. (1972) found significant differences in potency and efficacy of several vasoactive agents between helical and longitudinal HUV strip preparations. Recently, significative pharmacological differences were observed among our group employing rings and the group of Tuncer employing helical strips in the characterization of $5HT_{2A}$ receptor in HUV (Rogines-Velo *et al*., 2002b). Taking into account the pharmacological differences observed between both layers of HUV and the relevance of the circular layer in the *in vivo* vasoconstriction, reducing blood flow from the placenta to the foetus in different pathophysiological conditions, we consider important the pharmacological studies employing ring preparations.

U-46619 and TXA_2 are almost equipotent acting at the same TP receptor in different vascular preparations (Coleman *et al*., 1981). The plasma level of the stable metabolite of TXA_2 (TXB_2) in foetal cord blood, collected immediately after delivery of normal term pregnancies, reaches values higher than 1 nM (Liu *et al*., 1998). Taking into account these previous results, the pEC_{50} obtained with U-46619 in the present study is compatible with the hypothesis that TP receptors stimulation plays a key physiological role in the closure of umbilical vessels at birth (Coleman *et al*., 1994). On the other hand, in comparison with normal pregnancy, in pre-eclampsia it has been reported that placenta produces more thromboxane (Walsh, 1985) and that TXB_2 level in the foetal–placental circulation is higher (Liu *et al*., 1998). Therefore, TXA_2 could be involved in pre-eclampsia complicated with poor umbilical blood flow and foetal distress. The functional presence and pharmacological profile of TP receptors in HUV obtained from pre-eclampsia, remain to be determined.

When EP receptor agonists PGE_2, misoprostol and 17-phenyl-trinor-PGE_2 were evaluated, the vasoconstrictor potency obtained was very low for all the compounds used (pEC_{50}: 5.1, 5.2 and 5.3, respectively) and their efficacy was submaximal (approximately 75% of the maximal response to 5-HT for each one). EP_3 receptors are involved in vasoconstriction of guinea-pig aorta (Jones *et al*., 1998) and rat renal afferent arteriole (Tang *et al*., 2000). In human vascular tissues, functional expression of EP_3 receptors has been described only in the pulmonary artery (Qian *et al*., 1994). In this vessel, the EP_3 receptor agonist misoprostol was 145-fold more potent than in HUV, suggesting the absence of functional EP_3 receptors in our preparations. In animal

tissues, EP_1 receptor is involved in contractions of guinea-pig fundus (Coleman *et al*., 1987) and trachea (Lawrence *et al*., 1992). In human vascular tissues, functional EP_1 receptors have been described only in pulmonary vein (Walch *et al*., 2001). In this tissue, the EP_1 receptor agonist 17-phenyl-trinor-PGE_2 was 1740-fold more potent than in HUV. Furthermore, in the present study concentration–response curves to this agonist were not modified by the $DP/EP_1/EP_2$ receptor antagonist AH 6809 employed in a relatively high concentration, suggesting that EP_1 receptors are not involved in the contraction of this vessel.

On the other hand, the vasoconstriction elicited by EP receptor agonists at high concentrations seems to be mediated by a promiscuous interaction with TP receptors since pretreatment with ICI-192,605 produced a concentration-dependent antagonism of the responses to PGE_2, misoprostol and 17-phenyl-trinor-PGE_2 in HUV. This view is supported by the affinity values estimated in this tissue for ICI-192,605 against PGE_2 or misoprostol of 8.9 and 9.2, respectively, which were not significantly different from the pK_B of 9.1 obtained for ICI-192,605 against U-46619. Furthermore, PGE_2 promiscuous interaction with TP receptors has been also observed in other human vascular tissues: uterine artery (Baxter *et al*., 1995) and umbilical artery (Boersma *et al*., 1999).

Low concentrations of PGE_2 have been detected in human umbilical plasma after delivery at term, either from spontaneous labour and vaginal deliveries or elective caesarean (Mitchell *et al*., 1978; Bibby *et al*., 1979). In addition, PGE_2 was not detected (De Groot *et al*., 1998) or detected in amounts 10-fold lower than $PGF_{2\alpha}$ from cultured HUVEC (Watanabe *et al*., 1997). These data and the low potency value observed with PGE_2 in the present study suggest that this prostanoid is not involved in HUV vasoconstriction under physiological or pathophysiological conditions.

Functional FP receptors have been described in human nonvascular smooth muscle preparations like myometrium and urinary bladder (Senior *et al*., 1993; Palea *et al*., 1998). The reduced number of human tissues expressing functional FP receptors contrast with the large variety of human smooth muscle preparations on which the endogenous FP receptor agonist $PGF_{2\alpha}$ mediates contraction: bronchial smooth muscle, uterine artery, umbilical artery, and penile resistance artery and corpus cavernosum strips (Coleman & Sheldrick, 1989; Baxter *et al*., 1995; Boersma *et al*., 1999; Angulo *et al*., 2002, respectively). The contraction of these tissues in response to $PGF_{2\alpha}$ was abolished by TP receptor selective antagonists, showing the promiscuous activity of this natural prostanoid. Moreover, in human pulmonary vein AH 6809 abolished $PGF_{2\alpha}$-induced contraction, ascribing this response to the promiscuous interaction with EP_1 receptors (Walch *et al*., 2001). However, in human urinary bladder, $PGF_{2\alpha}$ induces vasoconstriction acting on FP receptors with a pEC_{50} of 6.36 (Palea *et al*., 1998), similar to the potency obtained in the present study (pEC_{50} of 6.20). In contrast to those several tissues in which $PGF_{2\alpha}$ has a promiscuous activity, in the present study $PGF_{2\alpha}$-mediated contractions of HUV rings are not antagonized either by a high concentration of ICI-192,605 or by a relatively high concentration of AH 6809. Taking together, the data obtained indicate that vasoconstriction mediated by FP receptors may occur in HUV. This view is supported by the effect of fluprostenol, which produces vasoconstriction on HUV with a pEC_{50} of 5.82 that is almost equipotent to the $PGF_{2\alpha}$, although less effective (60 vs 90% of the maximum response to 5-HT). Similar results have been published by Senior *et al*. (1993) on human myometrium at term pregnancy, in which $PGF_{2\alpha}$ and fluprostenol are equipotent and the former is more effective than the latter. Fluprostenol has been described by Coleman *et al*. (1994) to be at least as potent as $PGF_{2\alpha}$ and far more selective at the FP receptor. In relation to the high selectivity of this compound, it is relevant to mention the results obtained by Kiriyama *et al*. (1997) on eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells evaluating ligand-binding specificities and demonstrating that fluprostenol bound only to the FP receptors. Furthermore, fluprostenol-mediated contractions in HUV were not antagonized by a high concentration of the selective TP receptor antagonist ICI-192,605, indicating that TP receptor is not involved in this effect. In addition, in those tissues in which $PGF_{2\alpha}$ has a promiscuous activity fluprostenol has little or no effect (uterine artery (Baxter *et al*., 1995), umbilical artery (Boersma *et al*., 1999) and corpus cavernosum strips and penile resistance arteries (Angulo *et al*., 2002)). Recently, an analogue of $PGF_{2\alpha}$ (AL-8810) with properties of selective antagonist on FP receptors has been discovered (Griffin *et al*., 1999; Sharif *et al*., 2001; Kelly *et al*., 2003), but taking into account that this pharmacological tool is not freely available, it could not be used in the present study. Hence, the presence of FP receptors in HUV could only be examined by the employment of the selective agonist fluprostenol. In conclusion, the data obtained with $PGF_{2\alpha}$ and specially with fluprostenol suggest the presence of FP receptors in HUV.

Under control conditions, eicosanoids are released from cultured HUVEC and $PGF_{2\alpha}$ is secreted with values 10-fold higher compared to TXB_2 or PGE_2 (Watanabe *et al*., 1997). Consistent with these results, when HUVEC were exposed to plasma from women with normal pregnancies, a predominant production of $PGF_{2\alpha}$ was observed (De Groot *et al*., 1998). Furthermore, $PGF_{2\alpha}$ production was significantly greater in cultured HUVEC exposed to plasma from pre-eclamptic women than by identical cells exposed to plasma from normal pregnant patients (De Groot *et al*., 1998). Taken together, these results and our findings related to the putative FP receptors functionally present in HUV, suggest that FP receptor stimulation may be one of several possible mechanisms of the vasoconstriction observed in this vessel in physiological or pathological conditions.

In summary, the findings obtained with the TP-selective agonist and antagonists provide strong pharmacological evidence of the involvement of TP receptors promoting vasoconstriction in HUV. Furthermore, the action of the natural and synthetic EP receptor agonists appear to be mediated *via* TP receptors. On the other hand, the present results employing FP receptor agonists and antagonists of different prostanoid receptors suggest the functional presence of FP receptors mediating vasoconstriction in this vessel.

This research was supported by grants from the U.B.A. (Grant M-049). Rodolfo P. Rothlin is recipient of a research fellowship 'Ramón Carrillo-Arturo Oñativa', Ministerio de Salud, Secretaría de Investigación y Tecnología. Federico M. Daray is a research fellow of the U.B.A. We thank the Instituto Médico de Obstetricia (Buenos Aires) for their efforts in providing the umbilical tissues.

References

ALTURA, B.M., MALAVIYA, D., REICH, C.F. & ORKIN, L.R. (1972). Effects of vasoactive agents on isolated human umbilical arteries and veins. *Am. J. Physiol.,* **222,** 345–355.

ANGULO, J., CUEVAS, P., LA FUENTE, J.M., POMEROL, J.M., RUIZ-CASTANE, E., PUIGVERT, A., GABANCHO, S., FERNANDEZ, A., NEY, P. & SAENZ DE TEJADA, I. (2002). Regulation of human penile smooth muscle tone by prostanoid receptors. *Br. J. Pharmacol.,* **136,** 23–30.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.,* **14,** 48–52.

BAXTER, G.S., CLAYTON, J.K., COLEMAN, R.A., MARSHALL, K., SANGHA, R. & SENIOR, J. (1995). Characterization of the prostanoid receptors mediating constriction and relaxation of human isolated uterine artery. *Br. J. Pharmacol.,* **116,** 1692–1696.

BIBBY, J.G., BRUNT, J.D., HODGSON, H., MITCHELL, M.D., ANDERSON, A.B. & TURNBULL, A.C. (1979). Prostaglandins in umbilical plasma at elective caesarean section. *Br. J. Obstet. Gynaecol.,* **86,** 282–284.

BJORO, K., HOVIG, T., STOKKE, K.T. & STRAY-PEDERSEN, S. (1986). Formation of prostanoids in human umbilical vessels perfused *in vitro*. *Prostaglandins,* **31,** 683–698.

BOERSMA, J.I., JANZEN, K.M., OLIVEIRA, L. & CRANKSHAW, D.J. (1999). Characterization of excitatory prostanoid receptors in the human umbilical artery *in vitro*. *Br. J. Pharmacol.,* **128,** 1505–1512.

BOURA, A.L., GUDE, N.M., KING, R.G., MAK, K.K. & WALTERS, W.A. (1986). Characterization of thromboxane A2 receptors in the human fetal placental vessels and umbilical vein. *Clin. Exp. Pharmacol. Physiol.,* **13,** 83–86.

CAMACHO, M., LOPEZ-BELMONTE, J. & VILA, L. (1998). Rate of vasoconstrictor prostanoids released by endothelial cells depends on cyclooxygenase-2 expression and prostaglandin I synthase activity. *Circ. Res.,* **83,** 353–365.

COLEMAN, R.A., HUMPHREY, P.P., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H2-analogue, with those of prostaglandin H2 and thromboxane A2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.,* **73,** 773–778.

COLEMAN, R.A., KENNEDY, I. & SHELDRICK, R.L.G. (1987). Evidence for the existence of three subtypes of PGE_2 sensitive (EP) receptors in smooth muscle. *Br. J. Pharmacol.,* **91,** 323P.

COLEMAN, R.A. & SHELDRICK, R.L. (1989). Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors. *Br. J. Pharmacol.,* **96,** 688–692.

COLEMAN, R.A., SMITH, W.L. & NARUMIYA, S. (1994). VIII. International Union of Pharmacology Classification of Prostanoid Receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.,* **46,** 205–229.

DE GROOT, C.J., MURAI, J.T., VIGNE, J.L. & TAYLOR, R.N. (1998). Eicosanoid secretion by human endothelial cells exposed to normal pregnancy and preeclampsia plasma *in vitro*. *Prostaglandins Leukot. Essent. Fatty Acids,* **58,** 91–97.

DELEAN, A., MUNSON, P.J. & RODBARD, D. (1978). Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose–response curves. *Am. J. Physiol.,* **235,** E97–E102.

DUBIN, N.H., JOHNSON, J.W., CALHOUN, S., GHODGAONKAR, R.B. & BECK, J.C. (1981). Plasma prostaglandin in pregnant women with term and preterm deliveries. *Obstet. Gynecol.,* **57,** 203–206.

ERRASTI, A.E., ROGINES VELO, M.P., TORRES, R.M., SARDI, S.P. & ROTHLIN, R.P. (1999). Characterization of α_1-adrenoceptor subtypes mediating contraction in human umbilical vein. *Br. J. Pharmacol.,* **126,** 437–442.

FOX, S.B. & KHONG, T.Y. (1990). Lack of innervation of human umbilical cord. An immunohistological and histochemical study. *Placenta,* **11,** 59–62.

GRIFFIN, B.W., KLIMKO, P., CRIDER, J.Y. & SHARIF, N.A. (1999). AL-8810: a novel prostaglandin F2 alpha analog with selective antagonist effects at the prostaglandin F2 alpha (FP) receptor. *J. Pharmacol. Exp. Ther.,* **290,** 1278–1284.

JENKINSON, D.H., BARNARD, E.A., HOYER, D., HUMPHREY, P.P.A., LEFF, P. & SHANKLEY, N.P. (1995). International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. IX. Recommendations on terms and symbols in quantitative pharmacology. *Pharmacol. Rev.,* **47,** 255–266.

JESSUP, C.L., JESSUP, R. & WAYNE, M. (1988). The effects of ICI192,605, a selective thromboxane A_2 receptor antagonist, on platelets. *Br. J. Pharmacol.,* **95,** 676P.

JINO, H., KURAHASHI, K., USUI, H., NAKATA, Y., SHIMIZU, Y. & TEMMA, S. (1996). Pharmacological nature of TP receptor mediated contraction in human intrapulmonary artery. *Life Sci.,* **59,** 2059–2065.

JONES, R.L., QIAN, Y.M., CHAN, K.M. & YIM, A.P. (1998). Characterization of a prostanoid EP3-receptor in guinea-pig aorta: partial agonist action of the non-prostanoid ONO-AP-324. *Br. J. Pharmacol.,* **125,** 1288–1296.

KELLY, C.R., WILLIAMS, G.W. & SHARIF, N.A. (2003). Real-time intracellular Ca^{2+} mobilization by travoprost acid, bimatoprost, unoprostone, and other analogs *via* endogenous mouse, rat, and cloned human FP prostaglandin receptors. *J. Pharmacol. Exp. Ther.,* **304,** 238–245.

KIRIYAMA, M., USHIKUBI, F., KOBAYASHI, T., HIRATA, M., SUGIMOTO, Y. & NARUMIYA, S. (1997). Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.,* **122,** 217–224.

LACHNIT, W.G., TRAN, A.M., CLARKE, D.E. & FORD, A.P. (1997). Pharmacological characterization of an alpha 1A-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. *Br. J. Pharmacol.,* **120,** 819–826.

LAWRENCE, R.A., JONES, R.L. & WILSON, N.H. (1992). Characterization of receptors involved in the direct and indirect actions of prostaglandins E and I on the guinea-pig ileum. *Br. J. Pharmacol.,* **105,** 271–278.

LIU, H.S., CHU, T.Y., YU, M.H., CHANG, Y.K., KO, C.S. & CHAO, C.F. (1998). Thromboxane and prostacyclin in maternal and fetal circulation in pre-eclampsia. *Int. J. Gynaecol. Obstet.,* **63,** 1–6.

MITCHELL, M.D., BRUNT, J., BIBBY, J., FLINT, A.P., ANDERSON, A.B. & TURNBULL, A.C. (1978). Prostaglandins in the human umbilical circulation at birth. *Br. J. Obstet. Gynaecol.,* **85,** 114–118.

OGLETREE, M.L., HARRIS, D.N., GREENBERG, R., HASLANGER, M.F. & NAKANE, M. (1985). Pharmacological actions of SQ 29,548, a novel selective thromboxane antagonist. *J. Pharmacol. Exp. Ther.,* **234,** 435–441.

PALEA, S., TOSON, G., PIETRA, C., TRIST, D.G., ARTIBANI, W., ROMANO, O. & CORSI, M. (1998). Pharmacological characterization of thromboxane and prostanoid receptors in human isolated urinary bladder. *Br. J. Pharmacol.,* **124,** 865–872.

QIAN, Y.M., JONES, R.L., CHAN, K.M., STOCK, A.I. & HO, J.K. (1994). Potent contractile actions of prostanoid EP3-receptor agonists on human isolated pulmonary artery. *Br. J. Pharmacol.,* **113,** 369–374.

REILLY, F.D. & RUSSELL, P.T. (1977). Neurohistochemical evidence supporting an absence of adrenergic and cholinergic innervation in the human placenta and umbilical cord. *Anat. Rec.,* **188,** 277–286.

ROGINES-VELO, M.P., PELOROSSO, F.G., ZOLD, C.L., BRODSKY, P.T. & ROTHLIN, R.P. (2002a). Characterization of 5-HT receptor subtypes mediating contraction in human umbilical vein. 2. Evidence of involvement of 5-HT_{1B} receptors using functional studies. *Naunyn-Schmiedebergs Arch. Pharmacol.,* **366,** 596–604.

ROGINES-VELO, M.P., PELOROSSO, F.G., ZOLD, C.L., NOWAK, W., PESCE, G.O., SARDI, S.P., BRODSKY, P.T. & ROTHLIN, R.P. (2002b). Characterization of 5-HT receptor subtypes mediating contraction in human umbilical vein. 1. Evidence of involvement of 5-HT_{2A} receptors using functional and radioligand binding assays. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **366,** 587–595.

SARDI, S.P., PEREZ, H., ANTUNEZ, P. & ROTHLIN, R.P. (1997). Bradykinin B_1 receptors in human umbilical vein. *Eur. J. Pharmacol.,* **321,** 33–38.

SENCHYNA, M. & CRANKSHAW, D.J. (1996). Characterization of the prostanoid TP receptor population in human nonpregnant myometrium. *J. Pharmacol. Exp. Ther.,* **279,** 262–270.

SENIOR, J., MARSHALL, K., SANGHA, R. & CLAYTON, J.K. (1993). *In vitro* characterization of prostanoid receptors on human myometrium at term pregnancy. *Br. J. Pharmacol.,* **108,** 501–506.

SHARIF, N.A., WILLIAMS, G.W. & KELLY, C.R. (2001). Bimatoprost and its free acid are prostaglandin FP receptor agonists. *Eur. J. Pharmacol.,* **432,** 211–213.

SPIVACK, M. (1946). The anatomic peculiarities of the human umbilical cord and their clinical significance. *Am. J. Obstet. Gynecol.,* **52,** 387–401.

TANG, L., LOUTZENHISER, K. & LOUTZENHISER, R. (2000). Biphasic actions of prostaglandin E(2) on the renal afferent arteriole: role of EP(3) and EP(4) receptors. *Circ. Res.,* **86,** 663–670.

WALCH, L, DE MONTPREVILLE, V., BRINK, C. & NOREL, X. (2001). Prostanoid EP(1)- and TP-receptors involved in the contraction of human pulmonary veins. *Br. J. Pharmacol.,* **134,** 1671–1678.

WALSH, S.W. (1985). Preeclampsia: an imbalance in placental prostacyclin and thromboxane production. *Am. J. Obstet. Gynecol.,* **152,** 335–340.

WATANABE, K., NAGASAWA, N., WEN, F.Q., INOUE, K., KURODA, K. & YOSHIDA, M. (1997). Azelastine suppresses the release of arachidonic acid metabolites from cultured human umbilical vein endothelial cells. *Prostaglandins Leukot. Essent. Fatty Acids,* **56,** 209–211.

WRIGHT, D.H., ABRAN, D., BHATTACHARYA, M., HOU, X., BERNIER, S.G., BOUAYAD, A., FOURON, J.C., VAZQUEZ-TELLO, A., BEAUCHAMP, M.H., CLYMAN, R.I., PERI, K., VARMA, D.R. & CHEMTOB, S. (2001). Prostanoid receptors: ontogeny and implications in vascular physiology. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* **281,** 1343–1360.

(Received December 22, 2002
Revised April 14, 2003
Accepted May 12, 2003)