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Pharmacological characterization of thromboxane and prostanoid receptors in human isolated urinary bladder

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1 Cumulative concentration-response curves (CRC) to prostaglandin E_1 (PGE_1), PGE_2, PGD_2 and $PGF_{2\alpha}$ (0.01–30 μM) and to the thromboxane A_2 (TXA_2) receptor agonist U-46619 (0.01–30 μM) were constructed in human isolated detrusor muscle strips both in basal conditions and during electrical field stimulation.

2 All the agonists tested contracted the detrusor muscle. The rank order of agonist potency was: $PGF_{2\alpha} > U\text{-}46619 > PGE_2$ whereas weak contractile responses were obtained with PGD_2 and PGE_1. Any of the agonists tested was able to induce a clear plateau of response even at 30 μM.

3 The selective TXA_2 antagonist, GR 32191B (vapiprost), antagonized U-46619-induced contractions with an apparent pK_B value of 8.27 ± 0.12 ($n=4$ for each antagonist concentration). GR 32191B (0.3 μM) did not antagonize the contractile responses to $PGF_{2\alpha}$ and it was a non-surmountable antagonist of PGE_2 (apparent pK_B of 7.09 ± 0.04; $n=5$). The EP receptor antagonist AH 6809 at 10 μM shifted to the right the CRC to U-46619 (apparent pK_B value of 5.88 ± 0.04; $n=4$).

4 Electrical field stimulation (20 Hz, 70 V, pulse width 0.1 ms, trains of 5 s every 60 s) elicited contractions fully sensitive to TTX (0.3 μM) and atropine (1 μM). U-46619 (0.01–3 μM) potentiated the twitch contraction in a dose-dependent manner and this effect was competitively antagonized by GR 32191B with an estimated pK_B of 8.54 ± 0.14 ($n=4$ for each antagonist concentration). $PGF_{2\alpha}$ in the range 0.01–10 μM ($n=7$), but not PGE_2 and PGE_1 ($n=3$ for each), also potentiated the twitch contraction of detrusor muscle strips (23.5 ± 0.3% of KCl 100 mM-induced contraction) but this potentiation was unaffected by 0.3 μM GR 32191B ($n=5$).

5 Cumulative additions of U-46619 (0.01–30 μM) were without effect on contractions induced by direct smooth muscle excitation (20 Hz, 40 V, 6 ms pulse width, trains of 2 s every 60 s, in the presence of TTX 1 μM; $n=3$). Moreover, pretreatment of the tissue with 0.3 μM U-46619 did not potentiate the smooth muscle response to 7 μM bethanecol ($n=2$).

6 We concluded that TXA_2 can induce direct contraction of human isolated urinary bladder through the classical TXA_2 receptor. Prostanoid receptors, fully activated by PGE_2 and $PGF_{2\alpha}$ are also present. All these receptors are probably located post-junctionally. The rank order of agonist potency and the fact that GR32191B, but not AH6809, antagonized responses to PGE_2 seem to indicate the presence of a new EP receptor subtype. Moreover, we suggest the presence of prejuctional TXA_2 and FP receptors, potentiating acetylcholine release from cholinergic nerve terminals.

Keywords: Human urinary bladder; detrusor; smooth muscle contraction; TXA_2 receptors; prostanoid receptors; electrical field stimulation; acetylcholine release; vapiprost; U-46619; prostaglandin E_2; prostaglandin $F_{2\alpha}$

Introduction

It is generally accepted that prostaglandins have a role in the control of mammalian urinary bladder motility. It was originally shown that vesical distension and pelvic nerve stimulation evoke a release of prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ into the pelvic venous blood of anesthetized dogs (Gilmore & Vane, 1971; Khalaf *et al*., 1979). Later, it was found that intrarterial administration of PGE_2 and $PGF_{2\alpha}$ induced a significant decrease of the threshold micturition volume and that indomethacin induced a significant increase in the value of this parameter (Khalaf *et al*., 1981). Furthermore, a role for PGE_2 and $PGF_{2\alpha}$ in modulating cholinergic and purinergic contractions generated by electrical field stimulation was disclosed in rabbit isolated urinary bladder (Husted *et al*., 1980; Downye & Karmazyn, 1984). Human detrusor muscle *in vitro* relaxes in response to indomethacin and has been shown to produce PGE and PGF-like substances (Abrams *et al*., 1979), suggesting a role for endogenous prostanoids in the maintenance of normal human detrusor muscle tone. It has also been found that the human urinary bladder may synthesize thromboxane A_2 (TXA_2) and other prostanoids (Reyes & Klahr, 1990; Zwergel *et al*., 1991). TXA_2 produces potent contractions of vascular and visceral smooth muscle (see Moncada & Vane, 1979 for a review), but its activity on human detrusor muscle has never been assessed. Hence, we decided to investigate the effect of TXA_2 and some endogenous prostanoids ($PGF_{2\alpha}$, PGE_1, PGE_2 and PGD_2) on human unstimulated, isolated detrusor muscle and on contractile responses induced by trains of electrical pulses. Because TXA_2 is very unstable, a synthetic, metabolically stable analogue of it was used, namely U-46619 (Coleman *et al*., 1981). The effects of the selective TXA_2 antagonist GR 32191B, also known as vapiprost (Lumley *et al*., 1989) and the mixed EP_1-EP_2 receptor antagonist AH 6809, on contractions induced by U-46619, $PGF_{2\alpha}$ and PGE_2 were also examined.

A preliminary account of some of these data was given at the International Continence Society Annual Meeting (Palea *et al*., 1993).

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Methods

Preparation of human detrusor muscle strips

Specimens from the dome of the urinary bladder were obtained from 50 patients undergoing total cystectomy because of bladder malignancy (37 men and 8 women) or vesico-ureteral reflux (2 men and 3 women). The mean age of the patients was 57.0 ± 2.0 years (range between 20 and 75 years). Eight patients underwent chemotherapy before surgery. Immediately after surgical removal of the entire bladder or a portion of it, detrusor muscle strips of 2 cm in length were prepared from a large specimen and suspended in a 2 ml organ bath containing a Krebs solution at 37°C oxygenated with 5% CO_2 in O_2. The Krebs solution had the following composition (mM): NaCl 120, KCl 4.7, $MgSO_4$ $7.H_2O$ 0.6, KH_2PO_4 1.2, $NaHCO_3$ 2.5, $CaCl_2$ 2.0, glucose 10 and indomethacin 0.001. The presence of indomethacin was necessary because there is clear evidence that mechanical trauma to bladder epithelium liberates prostanoids in rabbit detrusor (Downie & Karmazyn, 1984) and that human urinary bladder *in vitro* is able to synthesize prostanoids (Abrams *et al*., 1979). Changes in tension were measured with a Grass Instrument FT03 isometric force displacement transducer connected to a Linseis model 7025 polygraph. When electrical stimulation was used, bladder strips were mounted vertically between two platinum electrodes. Detrusor strips were initially placed under a resting tension of 2 g and allowed to equilibrate for at least 1 h before the experiment was started. The contractile capacity of all preparations was tested by a single exposure to KCl 100 mM.

Characterization of the response to electrical stimulation

Bladder strips were electrically stimulated with a Grass S88 stimulator. Trains of electrical pulses of 5 s duration were delivered at intervals of 1 min, at pulse width of 0.1 ms and at supramaximal voltage (70 V). A frequency-response curve (1–60 Hz) was constructed, with the parameters indicated above, by using four strips obtained from four different patients, in order to find out a frequency value giving a submaximal response. For all the subsequent experiments the frequency of 20 Hz was used, producing approximately the 50% of the maximal contractile response.

Effect of U-46619 on direct electrical smooth muscle stimulation

Following a 30 min period of field stimulation with the parameters listed above, tissues were challenged with TTX (1 μM). When the twitch response was abolished, the pulse width was increased to 6 ms in order to stimulate the smooth muscle directly. Once these responses to stimulation stabilized, U-46619 was added cumulatively.

Measurement of the effects of the agonists and the antagonist GR32191B

Cumulative concentration-response curves (CRCs) to U-46619, $PGF_{2\alpha}$, PGE_1, PGE_2 or PGD_2 were constructed using 0.5 $\log_{10}$ increment in agonist concentration. Experiments were carried out on detrusor muscle strips at resting tension or during electrical field stimulation. Because the twitch response faded during the first 30 min of stimulation the CRC to the agonist was started only after stabilization of the electrically-induced contraction. A single CRC was performed on each strip in order to avoid fading of the responses due to desensitization. When the antagonists GR 32191B and AH 6809 were used, they were added to the organ bath 60 min before constructing the CRC to the agonist. On each day a parallel comparison between control strips (challenged only with agonists) and treated strips (incubated with different concentration of the antagonist) was performed.

In order to verify if the contractile effects of U-46619 and the natural prostanoids were mediated through a release of a neurotransmitter from nerve terminals, 0.3 μM TTX was incubated in the organ bath 30 min before the construction of the CRC to U-46619, PGE_2 and $PGF_{2\alpha}$.

In some other experiments atropine (0.03 μM) was added to the organ bath and after stabilization of the twitch contraction, a CRC to U-46619 was performed.

Data analysis

Responses to agonists in either stimulated or unstimulated tissues were transformed into a percentage of the contractile response to 100 mM KCl. Data are expressed as mean ± s.e.mean. CRCs to the agonists were analysed by a logistic curve fitting programme, ALLFIT (De Lean *et al*., 1978). The function used was:

$$\text{Response} = E_{max}\frac{[A]^{n_H}}{[A]^{n_H} + EC_{50}^{n_H}} \quad (1)$$

where E_{max} is the maximum response to the agonist [A]. EC_{50} is the mid point location parameter and n_H is the Hill slope parameter.

Antagonism was quantified by fitting the data with the following equation:

$$\text{Response} = E_{max}\frac{[A]^{n_H}}{([A]^{n_H} + (EC_{50})^{n_H}.(1 + (\frac{[B]^m}{K_B}))^{n_H})} \quad (2)$$

where [B] is the antagonist concentration, m the slope of the Schild plot and K_B the equilibrium dissociation constant for the antagonist-receptor complex when m is equal to one. In this case a second fit was performed by constraining m equal to 1. The other parameters are as stated before. Equation 2 was used to analyse the effect of GR32191B *versus* U-46619 in both unstimulated and electrically stimulated detrusor muscle. When only one concentration of antagonist was used, the pK_B value was estimated by using the following equation (Arunlakshana & Schild, 1959)

$$pK_B = -\log\frac{B}{DR - 1} \quad (3)$$

Where DR is the ratio between the EC_{50} of the agonist in the presence and absence of the antagonist. The other parameters are as stated before. Equation 3 was used to analyse the effect of AH6809 *versus* $PGF_{2\alpha}$.

The estimation of the equilibrium dissociation constant for a non-competitive antagonist (K_B) was derived by using the double-reciprocal plot analysis, as described by Kenakin, (1993). Assuming that equal fractional receptor occupancies lead to equal responses, equiactive concentrations of agonist can be related by equation 4:

$$\frac{[A]}{[A] + K_A} = \frac{[A']}{[A'] + K_A}.(1 - \gamma b) \quad (4)$$

where [A] and [A′] are the agonist concentrations in the absence and presence of the antagonist [B], K_A is the agonist dissociation constant and γb is the fraction of receptors bound by the antagonist. Equation 1 can be rearranged to equation 5:

$$\frac{1}{[A]} = \frac{1}{[A']} \cdot \frac{1}{1-\gamma b} + \frac{\gamma b}{(1-\gamma b).K_A} \quad (5)$$

The equilibrium dissociation constant for the antagonist-receptor complex (K_B) can be derived by the mass-action law (equation 6):

$$\gamma b = \frac{[B]}{[B] + K_B} \quad (6)$$

A regression of 1/[A] versus 1/[A′] yields a straight line of slope 1/1-γb and an intercept of γb/(1-γb)K_A. Therefore, the pK_B ($-\log K_B$) for a non-competitive antagonist can be derived as:

$$pK_B = -\log \frac{[B]}{\text{slope} - 1} \quad (7)$$

The double-reciprocal plot method has been utilized for the pK_B estimation of GR32191B versus PGE_2.

Drugs used

U-46619 (9, 11-dideoxy - 11α, 9α- epoxymethano- prostagladin H_2) was suppled by Cayman Chemical Company. $PGF_{2\alpha}$, PGE_1, PGE_2 and PGD_2, atropine, acetylcholine and tetrodotoxin (TTX) were all purchased from Sigma. GR 32191B ([1α(Z), 2β, 3β, 5α]-(+)-7-[5-[1,1′-biphenyl]-4-ylmethoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid) and AH 6809 (6-isopropoxy-9-oxaxanthene-2-carboxylic acid) were synthesized by the Medicinal Chemistry department of GlaxoWellcome (Medicines Research Centre, Stevenage, U.K.). U-46619, $PGF_{2\alpha}$ and PGE_2 were dissolved in 95% ethanol at a concentration of 1 mM, divided into aliquots and stored at −20°C until the day of the experiment when one aliquot was diluted to the required concentration in Krebs solution. GR 32191B was diluted in distilled water. The maximum concentration of ethanol in the organ bath was 0.01% (v/v). This concentration did not modify the response of the tissue (data not shown).

Results

Effect of U-46619, $PGF_{2\alpha}$, PGE_2, PGE_1 and PGD_2 on unstimulated human detrusor

Addition to the organ bath of 100 mM KCl induced a rapid and strong contraction in most of the strips tested (5.45 ± 0.26 g; $n = 134$). Strips developing a contraction less than 1.5 g were discarded.

U-46619 (0.01 – 30 μM) produced a concentration-dependent, slowly developing contraction of the detrusor muscle (Figure 1). However, a plateau of response was not achieved even with an agonist concentration of 30 μM; at this concentration the contractile response was equal to 56.0 ± 3.5% of KCl 100 mM-induced contraction. The estimated pEC_{50} for U-46619 (using equation 1) was 6.35 ± 0.10 ($n = 10$).

Incubation of the tissue with TTX 0.3 μM, added 30 min before starting the CRC, was without effect on the agonist-induced contraction, the estimated pEC_{50} for U-46619 in the presence of TTX (6.19 ± 0.15; $n = 4$) was not significantly different from the controls value ($P > 0.05$ by Allfit analysis).

$PGF_{2\alpha}$ (0.01 – 30 μM) and PGE_2 (0.01 – 30 μM) also produced a dose-dependent, slowly developing contraction of human detrusor muscle, with kinetics very similar to those exhibited by U-46619. Responses at the highest concentration tested (30 μM) were 56.6 ± 17.7% and 66.6 ± 14.3% of the KCl 100 mM-induced contraction, respectively (Figure 2). The pEC_{50} values (calculated by equation 1) were 6.37 ± 0.09 ($n = 7$) and 5.75 ± 0.035 ($n = 8$), for $PGF_{2\alpha}$ and PGE_2, respectively.

Pretreatment with TTX 0.3 μM ($n = 3$) was without effect on the cumulative CRC to PGE_2; estimated pEC_{50} value in the presence of TTX was 5.77 ± 0.11 ($P > 0.05$ with respect to

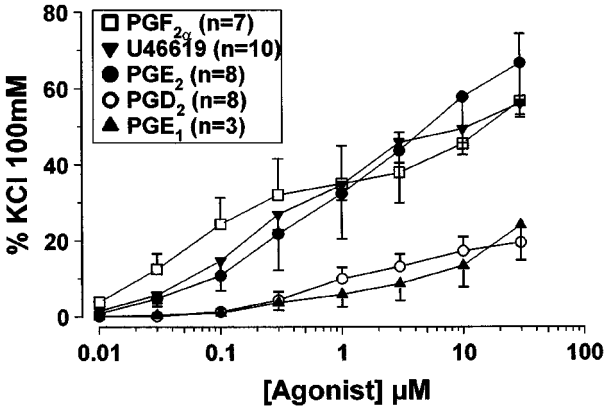

Figure 2 Cumulative concentration-response curves to $PGF_{2\alpha}$, U-46619, PGE_2, PGD_2 and PGE_1 in human isolated detrusor muscle at resting tension.

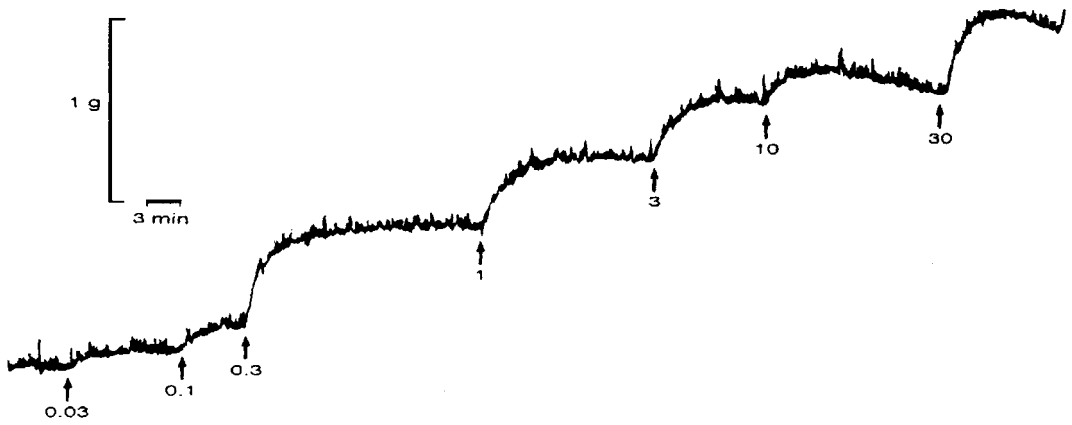

Figure 1 Experimental recording illustrating the direct contractile effect of the thromboxane A_2 agonist, U-46619, on human isolated detrusor muscle. Each arrow shows the point of addition of different agonist concentrations (expressed in μM).

controls by Allfit analysis) and the estimated maximal response was not significantly modified, being equal to 76.6±12.3% ($P>0.05$ by Allfit analysis).

The CRC to $PGF_{2\alpha}$ was clearly biphasic ($n=7$; see Figure 2). A plateau of the response was first achieved at 1 μM but on increasing the agonist concentrations to 10 and 30 μM a rising curve was still evident. However, the second plateau of the response was not reached even at the highest concentrations tested of the agonist. This observation was reinforced by the finding that incubation with 0.3 μM TTX ($n=6$; Figure 3) tended to shift to the right the first portion of the CRC to $PGF_{2\alpha}$, but was totally ineffective on the second one so that the maximal effect at 30 μM was not different from the control value (53.8±9.6%; $P>0.05$ by Allfit analysis).

PGE_1 and PGD_2 induced faint contractions, corresponding to 24.0±9.0% ($n=3$) and 20.7±4.6% ($n=8$), respectively, of the 100 mM KCl-induced contraction (Figure 2).

Effect of antagonists

GR 32191B (0.01–0.3 μM) antagonized U-46619-induced contractions in a concentration-dependent manner (Figure 4a). Since all the agonist CRC obtained in the presence of the antagonist converged to a similar maximum, a tentative estimation of the antagonist potency was carried out by applying equation 2. The analysis resulted in a Schild plot factor (m) equal to 1.12±0.12 which was not statistically different from one ($P>0.05$). Re-analysing the data with equation 2 and constraining m=1, a pK_B value of 8.27±0.12 was obtained ($n=4$ for each antagonist concentration).

Contractions induced by $PGF_{2\alpha}$ were not antagonized by 0.3 μM GR 32191B ($n=4$; Figure 4b). However, this compound antagonized, at 0.3 μM, the contractile responses induced by PGE_2 (Figure 4c). The Allfit analysis showed that the antagonism was non-competitive, so we decided to use the double-reciprocal plot to obtain a tentative estimation of the apparent affinity of this antagonist, even if the depression of the maximal response (45%) was slightly less than the value (50%) recommended for the use of this method (Kenakin, 1993). Using equation 7, we estimated an apparent pK_B value of 7.09 ($n=5$).

The selective EP receptor antagonist AH6809, tested at 10 μM ($n=4$), shifted to the right the CRC to U46619 in a competitive manner (Figure 5a). Using equation 3 we estimated an apparent pK_B value of 5.88.

AH6809, tested again at 10 μM ($n=4$; Figure 5b), induced a right shift of the CRC to $PGF_{2\alpha}$, but with a reduction of the maximal effect of the agonist. From the ratio of the agonist EC_{50}s obtained in the presence and absence of the antagonist and applying equation 3, an apparent pK_B value of 5.50±0.10 was obtained.

However, AH 6809 was completely ineffective against PGE_2-induced contractions, when tested at 10 μM ($n=4$; Figure 5c).

Effect of U-46619 and prostanoids on the contractile response of the electrically-stimulated human urinary bladder

Electrical field stimulation of human detrusor muscle strips caused contractile responses which were abolished by TTX

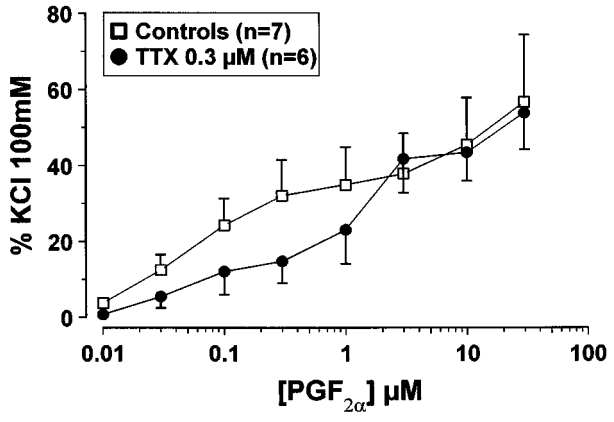

Figure 3 Cumulative concentration-response curves to $PGF_{2\alpha}$ in the absence and presence of 0.3 μM TTX (pre-incubated for 30 min) in human isolated detrusor muscle at resting tension.

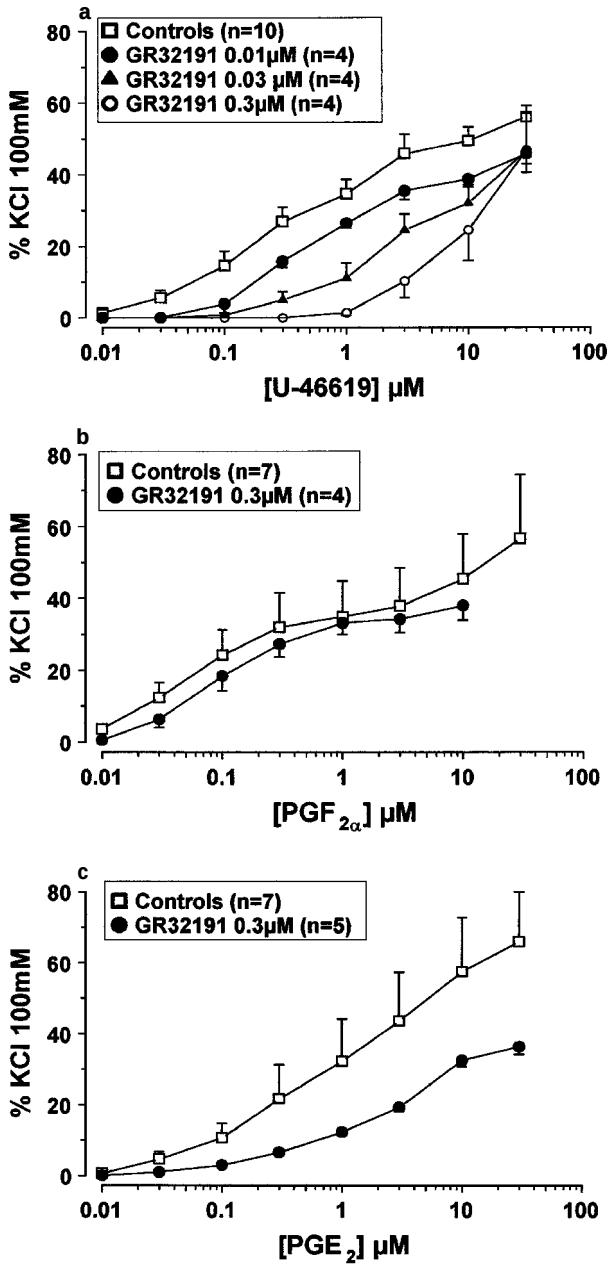

Figure 4 (a) Cumulative concentration-response curves to U-46619 in human detrusor muscle at resting tension in the absence and presence of the TP receptor antagonist GR32191B at different concentrations. (b) Lack of effect of GR32191B *versus* $PGF_{2\alpha}$. (c) Antagonistic effect of GR32191B *versus* PGE_2. The incubation time for all the experiments was 60 min.

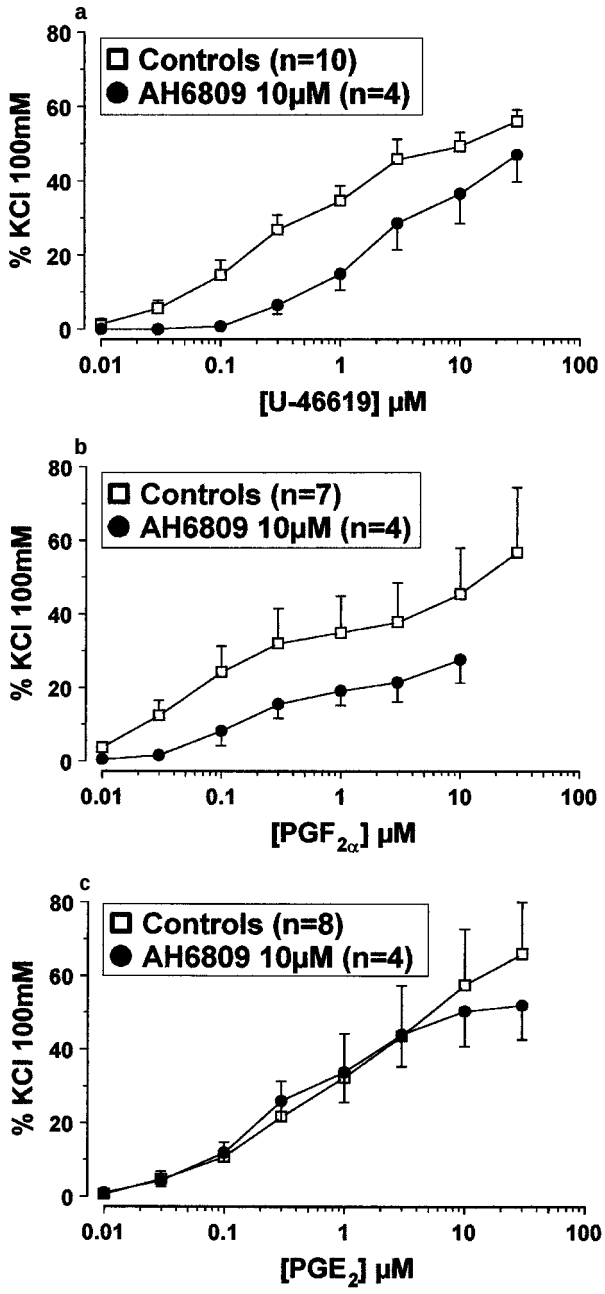

Figure 5 (a) Cumulative concentration-response curves to U-46619 in human detrusor muscle at resting tension in the absence and presence of the EP_1-EP_2 receptor antagonist AH6809. (b) Effect of AH6809 *versus* $PGF_{2\alpha}$. (c) Lack of effect of AH6809 *versus* PGE_2. The incubation time for all the experiments was 60 min.

(0.3 μM) consistent with their neurogenic origin. U-46619 (0.01–3 μM), in a concentration-dependent manner, potentiated the twitch contraction of the detrusor (Figure 6a). The pEC_{50} calculated from equation 1 was 7.12 ± 0.08 and the estimated maximal response was $23.2 \pm 1.1\%$ of KCl 100 mM-induced contraction ($n=4$). This effect was accompanied by a weak increase of the basal tone. This potentiation was completely abolished by preincubation with 0.03 μM atropine (Figure 6b).

GR 32191B (0.01–1 μM) antagonized the U-46619-induced potentiating effect in a concentration-dependent manner (Figure 7). The antagonism was competitive (Schild plot slope not different from unity; $P>0.05$) and a pK_B value of 8.54 ± 0.14 (7.94–9.14; 95% c.l.) was therefore calculated by using equation 2 ($n=4$ for each antagonist concentration). $PGF_{2\alpha}$, in the range 0.01–10 μM ($n=7$), but not PGE_2 or PGE_1 ($n=3$ for each in the same concentration range), potentiated the contraction induced by electrical stimulation of human isolated detrusor. The pEC_{50} calculated by equation 1 was 7.23 ± 0.01 and the estimated maximal response was $23.5 \pm 0.3\%$ of the KCl response. However, the $PGF_{2\alpha}$ effect was not antagonized by GR32191B at 0.3 μM ($n=5$; data not shown).

Effect of U-46619 on myogenic electrical field stimulation

Contractions of detrusor strips were obtained by direct excitation of the smooth muscle. In the presence of TTX 1 μM, contractions could be induced at a pulse width of 6 ms (20 Hz, for 2 s, every 60 s, 40 V). After stabilization of the myogenic response, cumulative addition of U-46619 (0.01–30 μM) was without effect on the amplitude of the smooth muscle contraction ($n=3$; data not shown).

Effect of U-46619 on the contractile response induced by bethanechol

In order to see whether U-46619-induced enhancement of the electrically-induced contractions was due to a pre- or a post-junctional action, two different strips of human detrusor were challenged four times with bethanechol 7 μM, a concentration corresponding to the EC_{50} for this agonist in this preparation, as determined by us in previous experiments. The mean amplitudes of the responses to bethanechol in the absence of U-46619 were, for the first and second strip, $74.5 \pm 1.7\%$ and $55.8 \pm 1.6\%$ of the KCl 100 mM-induced response, respectively. Then the same two strips were challenged with U-46619 (0.3 μM) two minutes before a new challenge with 7 μM bethanechol. The presence of U-46619 did not potentiate the smooth muscle response to bethanechol, contractions were 77.8% and 55.2% of the KCl 100 mM-induced response, respectively.

Discussion

A number of previous studies have described the effect of $PGF_{2\alpha}$, PGE_2 and PGE_1 on the human isolated detrusor (Abrams & Feneley, 1976; Anderson *et al*., 1977; Ueda *et al*., 1985) but the pharmacological effect of TXA_2 in this tissue has never been investigated. Therefore, the main objective of this study was to find out if human detrusor expresses functional TP receptors and to investigate the pharmacological effects of TP receptor activation. The selective TP receptor agonist, U-46619, produced potent concentration-dependent contractions of the isolated detrusor, suggesting the presence of a specific receptor. The estimated potency of the selective TP receptor antagonist, GR32191B ($pK_B=8.27$) is close to values found in other preparations known to express TP receptors, e.g. 8.50 in human isolated uterine artery (Baxter *et al*., 1995) and in some canine isolated blood vessels (values between 8.67 and 9.01; Matsuzaki *et al*., 1992). We also found that U-46619-induced contractions were insensitive to challenge with TTX. Taken together, these results strongly suggest the presence of a TP receptor in the human detrusor muscle. We also found that challenging the preparation with U-46619 during trains of neurogenic stimulation induced a potentiation of the evoked contractile response. GR32191B also antagonized this effect of U-46619 with a potency ($pK_B=8.54$) close to that found on the

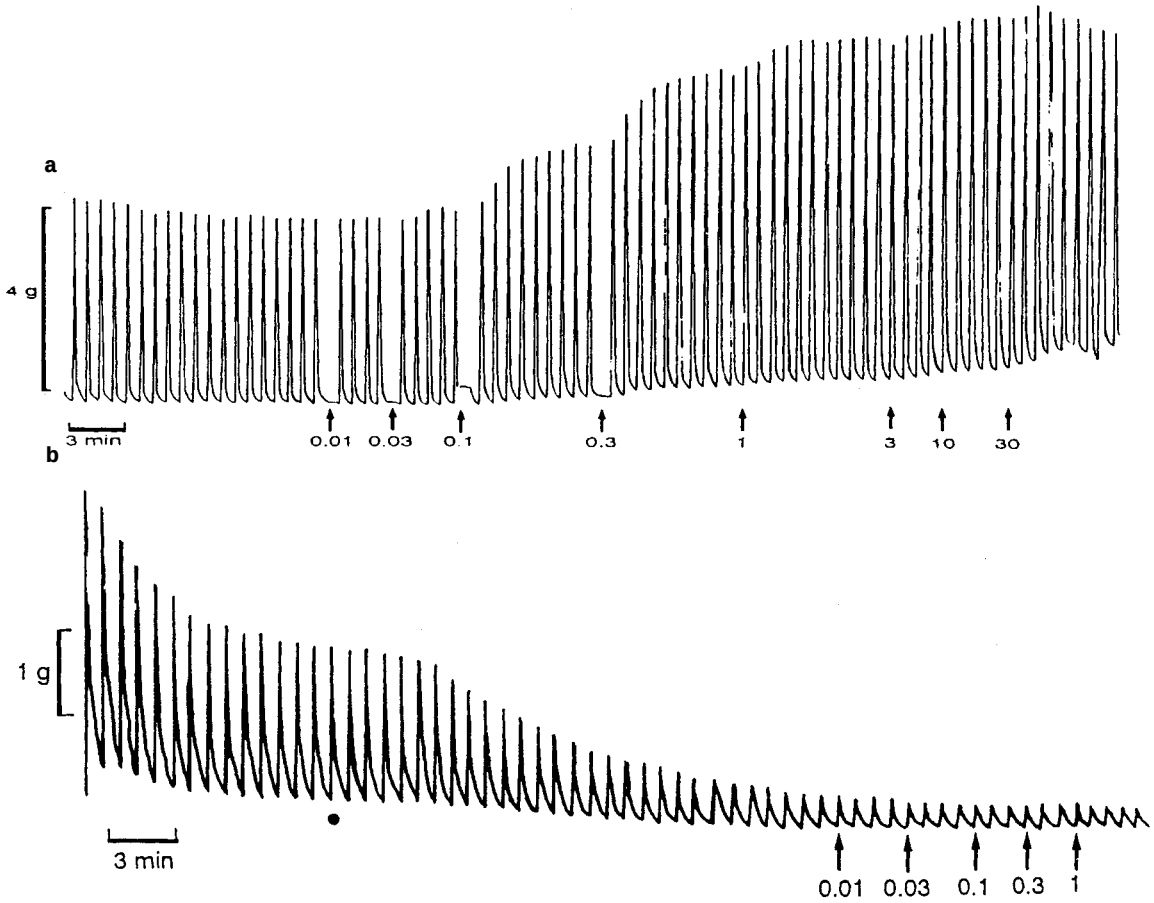

Figure 6 (a) Experimental recording illustrating the potentiating effect of U-46619 on the contractile response of human isolated detrusor muscle to neurogenic electrical stimulation. (b) Experimental recording illustrating the inhibitory effect of atropine 0.03 μM (•) on the potentiating effect of U-46619 on the contractile response of human isolated detrusor muscle to neurogenic electrical stimulation. Arrows represent the additions of cumulative concentrations of the agonist expressed in μM. Parameters of stimulations were: 50 V, 20 Hz, 0.1 ms pulse width, trains of 5 s every 60 s.

unstimulated strips, again suggesting the involvement of a TP receptor. Our finding that the potentiating effect was antagonized by low doses of atropine and the fact that incubation of tissues with U-46619 did not potentiate the smooth muscle response to bethanechol, suggest that the increase of the twitch contraction could be due to activation of a prejunctional mechanism controlling neurotransmitter release from nerve terminals, rather than to a direct action on the intracellular machinery controlling excitation-contraction coupling. This conclusion is supported by our observation that U-46619 was without effect on the response to electrical stimulation in the presence of TTX. It is well known that the presence of TTX does not entirely preclude neuronal activation, e.g. a residual release of [^{3}H]-noradrenaline by sympathetic nerve terminals in the presence of TTX has been demonstrated (Kirpekar & Prat, 1978). However, we do not think that contractions to U-46619 are mediated by ACh released from a TP receptor located prejunctionally, because the cholinergic-induced contraction in the detrusor muscle is very rapid, whereas that induced by U-46619 and the other prostanoids is typically slow.

So we hypothesize the existence of a prejunctional TP receptor on cholinergic nerve terminals of the human urinary bladder. Another group, studying the potentiating effect of $PGF_{2\alpha}$ and PGE_2 on the twitch response of the guinea-pig isolated urinary bladder (Poli *et al*., 1992) concluded that prejunctional prostanoid receptors were present. However, they did not characterize these receptors.

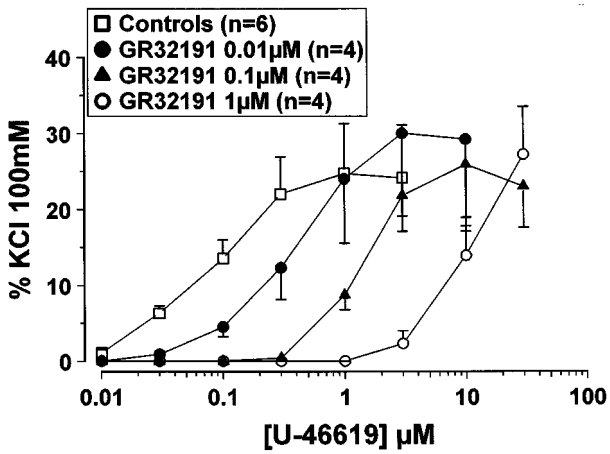

Figure 7 Cumulative concentration-response curves for the potentiating effect of U-46619 on the contractile response to neurogenic electrical stimulation in the absence and presence of GR3219B at different concentrations. Parameters of stimulation were: 60 V, 20 Hz, 0.1 ms pulse width, trains of 5 s every 60 s. The antagonist was incubated for 60 min.

The hypothesis for the presence of TP receptors located pre- and post-junctionally in the human urinary bladder could suggest the existence of TP receptors subtypes. However, we were not able to confirm this hypothesis by using GR32191B, since, in our paradigms, this antagonist displayed approxi-

mately the same affinity on the two response mediated by TP receptors. Interestingly, two putative TP receptor subtypes have been proposed to be present in human myometrium and human plateletes, respectively (Krauss *et al*., 1996). Further studies with other agonists and antagonist of the TP receptor are necessary to determine if the TXA_2 receptor in human detrusor is different from that present in platelets.

The fact that AH6809 antagonized U-46619-induced contractions with low affinity ($pK_B = 5.88$) is not surprising, because it was originally shown that this molecule had low affinity for both TP and DP receptors (Coleman *et al*., 1990). Interestingly, we found that human isolated detrusor contracted in the presence of $PGF_{2\alpha}$ and PGE_2. Since there is evidence that both $PGF_{2\alpha}$ and PGE_2 can activate TP receptors (Dorn *et al*., 1992; Sakanashi *et al*., 1994; He & Yang, 1995) we decided to test the effect of GR 32191B on the contractile response mediated by these two agonists. Surprisingly, we found that, although GR 32191B was without effect versus $PGF_{2\alpha}$, it antagonized quite well the response mediated by PGE_2 with a pK_B value (7.09) approximately 10–30 times lower than values originally obtained for TP receptors in various tissues (8.2–8.8; Lumley *et al*., 1989). These results do not support the interaction of $PGF_{2\alpha}$ and PGE_2 with the TP receptor in human detrusor muscle. On the other hand, the well known EP_1 receptor antagonist AH6809, at 10 μM, was ineffective in inhibiting PGE_2-induced contractions. This is quite surprising since this antagonist exhibits a pK_B of about 7.40 in other functional tests for the EP_1 receptor (Eglen & Whiting, 1988) and a K_i value of 1.3 μM in binding studies with the cloned rat EP_1 receptor (Boie *et al*., 1997). The rank order of potency of PGE_2 and PGE_1 and the inactivity of AH6809 are not consistent with the presence of EP_1 receptors in human detrusor. For the same reasons we can also discard the involvement of EP_2 receptors. In fact, AH6809 was found to be a ligand of the cloned rat EP_2 receptor ($K_i = 0.5$ μM) and a functional antagonist of the cloned human EP_2 receptor (approximate $pK_B = 6.5$, calculated by us from Figure 2B of Woodward *et al*., 1995). In a functional study the prostanoid receptors mediating inhibition of noradrenaline release was characterized as EP_3 but GR32191B exhibited a very limited potency (apparent $pA_2 = 4.50$; Exner & Schlicker, 1995) and this is in contrast with the relatively high pA_2 value we have found in human detrusor versus PGE_2. The EP_4 receptor was recently identified in piglet saphenous vein (Coleman *et al*., 1994) and in rabbit foetal ductus arteriosus (Smith *et al*., 1994) and in both tissues it mediates relaxation. Since it is known that AH6809 is inactive on this receptor (Smith *et al*., 1994; Boie *et al*., 1997), EP_4 could be the receptor subtype activated by PGE_2 in human detrusor muscle. However, this hypothesis does not fit with our results since: (a) PGE_1, on cloned and native EP_4 receptors, is equipotent or slightly less potent than PGE_2 (Marshall *et al*., 1997) and (b) this receptor is considered to mediate smooth muscle relaxation, not contraction (Coleman *et al*., 1994; Smith *et al*., 1994). In conclusion, we propose the existence of a new EP receptor subtype in human detrusor muscle which is more sensitive to PGE_2 than to PGE_1 and which is antagonized by GR32191B but not by AH6809.

With regard to the site of action of $PGF_{2\alpha}$, we noted that the CRC to this agonist was biphasic and that the first portion of the curve was inhibited by TTX. This seems to indicate the activation of a prejunctional FP receptor, which probably induces the release of ACh. Indeed, we found clear evidence for the presence of this receptor in a nerve-stimulated preparation, e.g. the potentiation of the cholinergic twitch by $PGF_{2\alpha}$ but not by PGE_2 and PGE_1 and the lack of antagonism by GR32191B. With regard to the direct contractile response to $PGF_{2\alpha}$, AH6809 was a non-competitive antagonist with low affinity ($pK_B = 5.55$). Unfortunately, there are no data in the literature regarding the affinity of this antagonist for FP receptors, so we can only speculate that $PGF_{2\alpha}$ is acting, postjunctionally, on its own receptor and, at higher concentrations, on another prostanoid receptor, possibly the subtype activated by PGE_2.

Our findings that U-46619 induced a potentiation of cholinergic neurotransmission suggest the possibility that the dysuria observed during bladder inflammation and bladder sensory urgency could be partly mediated by activation of a TP receptor. For this reason a TP receptor, antagonist, ideally having low affinity for the receptor isoform expressed in platelets, could be useful for treating some forms of micturition disorders.

Abbreviations

CRC, concentration-response curve; ACh, acetylcholine

References

ABRAMS, P.H. & FENELEY, R.C.L. (1976). The actions of prostaglandins on the smooth muscle of the human urinary tract *in vitro*. *Br. J. Urol.,* **47,** 909–913.

ABRAMS, P.H., SYKES, J.A.C., ROSE, A.J. & ROGERS, A.F. (1979). The synthesis and release of prostaglandins by human urinary bladder muscle *in vitro*. *Invest. Urol.,* **16,** 346–348.

ANDERSSON, K.E., EK, A. & PERSSON, C.G.A. (1977). Effects of prostaglandins on the isolated human bladder and urethra. *Acta Physiol. Scand.,* **100,** 165–171.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.,* **14,** 48–58.

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.

BOIE, Y., STOCCO, R., SAWYER, N., SLIPETZ, D.M., UNGRIN, M.D., NEUSCHAFER-RUBE, F., PUSCHEL, G.P., METTERS, K.M. & ABRAMOVITZ, M. (1997). Molecular cloning and characterization of the four rat prostaglandin E_2 prostanoid receptor subtypes. *Eur. J. Pharmacol.,* **340,** 227–241.

COLEMAN, R.A., GRIX, S.P., HEAD, S.A., LOUTTIT, J.B., MALLET, A. & SHELDRICK, R.L. (1994). A novel inhibitory prostanoid receptor in piglet saphenous vein. *Prostaglandins,* **47,** 151–168.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1981). Comparison of the actions of U-46619, a prostaglandin H_2-analogue, with those of protaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.,* **73,** 773–778.

COLEMAN, R.A., KENNEDY, I., HUMPHREY, P.P.A., BUNCE, K. & LUMLEY, P. (1990). Prostanoids and their receptors. In *Comprehensive Medicinal Chemistry* ed. Emmett, J.C. pp. 643–714. New York: Pergamon Press.

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,** 97–102.

DORN, G.W., II, BECKER, M.W. & DAVIS, M.G. (1992). Dissociation of the contractile and hypertrophic effects of vasoconstrictor prostanoids in vascular smooth muscle. *J. Biol. Chem.,* **267,** 24897–24905.

DOWNIE, J.W. & KARMAZYN, M. (1984). Mechanical trauma to bladder epithelium liberates prostanoids which modulate neurotransmission in rabbit detrusor muscle. *J. Pharmacol. Exp. Ther.,* **230,** 445–449.

EGLEN, R.M. & WHITING, R.L. (1988). The action of prostanoid receptor agonists and antagonists on smooth muscle and platelets. *Br. J. Pharmacol.,* **94,** 591–601.

EXNER, H.J. & SCHLICKER, E. (1995). Prostanoid receptors of the EP_3 subtype mediate the inhibitory effect of prostaglandin E_2 on noradrenaline release in the mouse brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **351,** 46–52.

GILMORE, N.J. & VANE, J.R. (1971). Hormones released into the circulation when the urinary bladder of the anaesthetized dog is distended. *Clin. Sci.,* **41,** 69–83.

HE, G. & YANG, C. (1995). Effect of thromboxane A2 antagonist GR32191B on prostanoid and nonprostanoid receptors in the human internal mammary artery. *J. Cardiovasc. Pharmacol.,* **26,** 13–19.

HUSTED, S., SJOGREN, C. & ANDERSSON, K.E. (1980). Role of prostaglandins in the responses of rabbit detrusor to non-cholinergic, non-adrenergic nerve stimulation and to ATP. *Arch. Int. Pharmacodyn.,* **246,** 84–97.

ICHIKAWA, A., SUGIMOTO, Y. & NEGISHI, M. (1996). Molecular aspects of the structures and functions of the prostaglandin E receptor. *J. Lipid Mediators Cell signalling,* **14,** 83–87.

KHALAF, I.M., GHONEIM, M.A. & ELHILALI, M.M. (1981). The effect of exogenous prostaglandins $F_{2\alpha}$ nd E_2 and indomethacin on micturition. *Br. J. Urol.,* **53,** 21–28.

KHALAF, I.M., LEHOUX, J.G., ELSHAWARBY, L.A. & ELHILALI, M.M. (1979). Release of prostaglandins into the pelvic venous blood of dogs in response to vesical distension and pelvic nerve stimulation. *Invest. Urol.,* **17,** 244–247.

KENAKIN, T.P. (1993). Allotopic, noncompetitive and irreversible antagonism. In *Pharmacological Analysis of Drug-Receptor Interaction,* 2nd Edition pp. 323–343. New York: Raven Press.

KIRPEKAR, S.M. & PRAT, J.C. (1978). Effect of tetraethylammonium on noradrenaline release rom cat spleen treated with tetrodotoxin. *Nature,* **276,** 623–624.

KRAUSS, A.H.P., WOODWARD, D.F., GIBSON, L.L., PROTZMAN, C.H., WILLIAMS, L.S., BURK, R.M., GAC, T.S., ROOF, M.B., ABBAS, F., MARSHALL, K. & SENIOR, J. (1996). Evidence for human thromboxane receptor heterogeneity using a novel series of 9,11-cyclic carbonate derivatives of prostaglandin $F_{2\alpha}$. *Br. J. Pharmacol.,* **117,** 1171–1180.

LUMLEY, P., WHITE, B.P. & HUMPHREY, P.P.A. (1989). GR32191, a highly potent and specific thromboxane A_2 receptor blocking drug on platelets and vascular and airways smooth muscle in vitro. *Br. J. Pharmacol.,* **97,** 783–794.

MARSHALL, F.H., PATEL, K., LUNDSTROM, K., CAMACHO, J., FOORD, S.M. & LEE, M.G. (1997). Characterization of [^{3}H]-prostaglandin E_2 binding to Prostaglandin EP_4 receptors expressed with Semliki Forest virus. *Br. J. Pharmacol.,* **121,** 1673–1678.

MATSUZAKI, T., NOGUCHI, K., NAKASONE, J., UEZU, K., HIGUCHI, M. & SAKANASHI, M. (1992). Effects of the new thromboxane A_2 antagonist vapiprost on isolated canine blood vessels. *Drug. Res.,* **42,** 1318–1322.

MONCADA, S. & VANE, J.R. (1979). Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.,* **30,** 293–331.

PALEA, S., CORSI, M., TOSON, G., PIETRA, C., ARTIBANI, W., ROMANO, O. & TRIST, D.G. (1993). Activity of the thromboxane A_2 receptor antagonist GR 32191 on human isolated detrusor muscle. *Neurourol. Urodyn.,* **12,** 355–356.

POLI, E., MACALUSO, G. & POZZOLI, C. (1992). Actions of two novel prostaglandin analogs, SC-29169 and SC-31391, on guinea-pig and human isolated urinary bladder. *Gen. Pharmacol.,* **23,** 805–809.

REYES, A.A. & KLAHR, S. (1990). Bladder contributes to eicosanoids excretes in urine. *Am. J. Physiol.,* **259,** F859–F861.

SAKANASHI, M., MATSUZAKI, T., NOGUCHI, K., NAKASONE, J., ITOMINE, T., UZA, M., TOYAMA, F. & HIGUCHI, M. (1994). Inhibitory effect of vapiprost on contractile responses of isolated dog renal arteries to thromboxane A_2 analogue, U46619. *Gen. Pharmacol.,* **25,** 617–622.

SMITH, G.C.S., COLEMAN, R.A. & MCGRATH, J.C. (1994). Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. *J. Pharmacol. Exp. Ther.,* **271,** 390–396.

UEDA, S., YOSHIDA, M., YANO, S., MUTOH, S., IKEGAMI, K. & SAKANASHI, M. (1985). Comparison of the effects of primary prostaglandins on isolated human urinary bladder. *J. Urol.,* **133,** 114–117.

WOODWARD, D.F., PEPPERL, D.J., BURKEY, T.H. & REGAN, J.W. (1995). 6-Isopropoxy-9-oxoxanthene-2-carboxylic acid (AH6809), a human EP2 receptor antagonist. *Biochem. Pharmacol.,* **50,** 1731–1733.

ZWERGEL, U., ZWERGEL, T., LEIS, H.J., GLEISPACH, H., ALEXANDRIDIS, T., BELLETZ, M. & KIRSTEN, R. (1991). Eicosanoid synthesis in the isolated human renal pelvis, ureter and bladder. *Br. J. Urol.,* **67,** 246–250.

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