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Prostanoid receptors mediating contraction in rat, macaque and human bladder smooth muscle in vitro

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ABSTRACT

Selective prostaglandin EP_1 antagonists have been suggested for the treatment of bladder dysfunction. This study assessed the contractile prostanoid receptor subtypes in human and non-human bladder in vitro. Classical tissue bath studies were conducted using bladder strips exposed to prostanoid agonists and antagonists. Prostaglandin E_2 (PGE_2) contracted rat, macaque and human bladder smooth muscle strips (pEC_{50} 7.91 ± 0.06 (n=7), 6.40 ± 0.13 (n=7), and 6.07 ± 0.11 (n=5), respectively). The EP_1 receptor antagonist, PF2907617 (300 nM), caused a rightward shift of the PGE_2 concentration–response curve in the rat bladder only (pK_B 8.40 ± 0.15, n=3). PGE_2 responses in rat and macaque bladders, but not human, were antagonised by the EP_3 antagonist CJ24979 (1 μM). Sulprostone, a mixed EP_1/EP_3/FP receptor agonist, induced potent contractions of rat bladder muscle (pEC_{50} 7.94 ± 0.31, n=6). The FP receptor agonist, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), induced bladder contraction in all species tested, but with a lower potency in rat. The selective FP receptor agonist latanoprost caused potent contractions of macaque and human bladder strips only. SQ29548, a selective TP antagonist, and GW848687X, a mixed EP_1/TP antagonist caused rightward shifts of the concentration–response curves to the selective TP agonist, U46619 (pK_B estimates 8.53 ± 0.07 and 7.56 ± 0.06, n=3, respectively). Responses to U46619 were absent in rat preparations. These data suggest significant species differences exist in bladder contractile prostanoid receptor subtypes. We conclude that the EP_1 subtype does not represent the best approach to the clinical treatment of bladder disorders targeting inhibition of smooth muscle contraction.

1. Introduction

Prostaglandins are synthesized from arachidonic acid and mediate a wide array of biological responses (Coleman et al., 1994), including modulation of bladder smooth muscle tone (Coleman et al., 1994; Palea et al., 1998; Schröder et al., 2004; Su et al., 2008). Prostaglandins are synthesized by the urinary bladder (Jeremy et al., 1987; Zwergel et al., 1991) and their effects on bladder function have been reported in several species (Palea et al., 1998; Larsson, 1980). Reductions in bladder capacity have been attributed to one or more of the contractile prostanoid receptor subtypes EP_1, EP_3, FP and TP (Schröder et al., 2004; Wang et al., 2008; Su et al., 2008; Palea et al., 1998). Furthermore, inhibition of the EP_1 prostanoid receptor has been shown to increase bladder capacity in rats in vivo by Lee et al. (2007).

In patients with disorders of the bladder such as overactive bladder, urinary PGE_2 and $PGF_{2\alpha}$ levels are increased compared with control subjects (Kim et al., 2006). Urinary excretion of PGE_2 is also increased in patients with interstitial cystitis (Lynes et al., 1987). In addition, nonsteroidal anti-inflammatory drugs, such tiaprofenic acid, that inhibit prostanoid synthesis are reported to cause cystitis (Ahmed and Davison., 1991). Prostaglandin biology is thus of great interest in bladder disorder research. The characterization of these receptors in various tissues has been hampered by the lack of agonists and antagonists with good selectivity for the different prostanoid receptors. Furthermore, to our knowledge, neither detailed cross-species investigations, nor studies that examine the role of the urothelium in the in vitro smooth muscle response to prostanoids have been reported. Our aim was to characterize the prostanoid receptor subtypes responsible for contraction of bladder smooth muscle in rat and cynomolgus macaque, two species commonly used in preclinical bladder research, and compare with the receptors in the human bladder. To assess the role of the urothelium and sub-mucosal neurones in prostanoid-induced contractions we also conducted studies in intact bladder muscle preparations.

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2. Methods

2.1. Tissue preparation

Urinary bladders from female Sprague Dawley CD rats (225–300 g, $n=16$) and male cynomolgus macaque monkeys (*macaca fascicularis*) from placebo-treated unrelated studies ($n=8$) were acquired in accordance with UK Home Office regulations. Human bladder specimens (12 donors; 4 female, 8 male; age range 47–77 years) were acquired with informed consent and in accordance with The Human Tissue Act. After transport to the laboratory in Krebs solution (NaCl 118.2 mM, KCl 4.69 mM, $MgSO_4 \cdot 7H_2O$ 0.5 mM, KH_2PO_4 1.19 mM, glucose 11.1 mM, $NaHCO_3$ 25.0 mM), bladder muscle from non-trigone regions was cut into strips approximately 2 mm wide by 5 mm long, and where required the urothelium was removed. Histological confirmation of this method to remove the urothelium was performed in previous (unpublished) studies. Strips were mounted in 5 ml tissue baths containing modified Krebs solution (NaCl 118.2 mM, KCl 4.69 mM, $MgSO_4 \cdot 7H_2O$ 0.5 mM, KH_2PO_4 1.19 mM, glucose 11.1 mM, $NaHCO_3$ 25.0 mM, $CaCl_2 \cdot 6H_2O$ 2.5 mM, naproxen 0.01 mM) pH 7.4 at 37 °C and gassed with 95% CO_2 and 5% O_2. Tissues were attached to isometric force-displacement transducers by silk sutures and placed under 1.5 g tension. During a minimum of 60 min, the bladder strips were perfused continuously with modified Krebs solution at 5 ml/min and tension adjusted if required. Perfusion was stopped and tissues left to equilibrate for 10 min, followed by addition of 80 mM KCl to assess viability. Tissues that contracted less than 0.5 g were discounted from the study. After 50 min continuous perfusion with modified Krebs solution and 10 min equilibration without perfusion, antagonists or vehicle were added to each bath 30 min prior to challenging the tissues with cumulatively increasing concentrations of prostanoid agonists. Each concentration of agonist was left in contact for 5 min or until response plateau. Neuronal involvement in the PGE_2 response was assessed by a 30 min pre-incubation of tissues with 1 μM tetrodotoxin (TTX) prior to PGE_2 challenge.

2.2. Data analysis

Measurements were recorded using an amplifier connected to Notocord acquisition software (v4.2). The average tension for a 30 s interval upon plateau of response was calculated as a percentage of the maximal response to 80 mM KCl. Unconstrained concentration response curves were fitted using an in house Microsoft Excel add-in package by non-linear, 4 parameter, logistical regression analysis. From these curves pEC_{50}, E_{max} and slope values were estimated. pK_B values were estimated using the Gaddum-Schild equation (apparent pK_B=log[concentration ratio-1]-log[antagonist]). Statistical analysis was conducted where appropriate using one-way ANOVA, and $P<0.05$ was considered significant. All values are mean ± S.E.M., n values equate to the number of individual bladders used in a study.

2.3. Chemicals

The following compounds were used: SQ29548 (IDS, U.K.), PGE_2, $PGF_{2\alpha}$, latanoprost, naproxen, sulprostone, tetrodotoxin, U-46619 (Sigma Aldrich, U.K.). KCl (Sigma Aldrich, U.K.) was dissolved in distilled water. CJ-24979 (5-bromo-2-methoxy-N-[3-(naphthalen-2-yl-methylphenyl)-acryloyl]-benzenesulphonamide, Juteau et al., 2001), GW848687X (6-[2-(5-chloro-2-{[(2,4-difluorophenyl)methyl]oxy}phenyl)-1-cyclopenten-1-yl]-2-pyridinecarboxylic acid, Giblin et al., 2007) and PF-2907617 (Lee et al., 2007) were synthesized in–house (purities >95%). Compounds were dissolved in 100% dimethylsulphoxide (DMSO) and diluted in modified Krebs solution. Volumes added to the baths for each concentration point were 3.5–10 μl, totalling a maximum DMSO concentration of 0.3% at the highest concentration assessed.

3. Results

3.1. Effect of PGE_2

Application of PGE_2 to urothelium-free rat, cynomolgus macaque and human bladder detrusor muscle strips caused concentration-dependent contractions, with estimated pEC_{50} values of 7.91 ± 0.06, 6.40 ± 0.13, and 6.07 ± 0.11, respectively ($n=7$, 7 and 5 respectively; Fig. 1A, Table 1). The nature of the PGE_2-induced contractions differed between species; with an increase in spontaneous activity predominant in the rat, but an increase in tone accompanied by a small degree of increasing spontaneous activity in the macaque and human. Relative to the response to 80 mM KCl, maximum responses to PGE_2 were smaller in rat bladder strips (18.0 ± 0.8%, $n=5$, Fig. 1A), than those of macaque and human strips (85.8 ± 9.4% and 111.8 ± 23.9% respectively, $P<0.05$, Fig. 1A).

3.2. Effect of agonists

In the absence of urothelium, the mixed EP_1/EP_3/FP agonist, sulprostone >300 nM, caused weak contractions in macaque and human bladder strips, however, no response plateau was obtained (Fig. 1C). In rat bladder strips without urothelium, sulprostone induced potent concentration-dependent contractions (pEC_{50} 7.95 ± 0.32, $n=6$, Fig. 1C).

$PGF_{2\alpha}$ was more potent in macaque and human bladder strips than rat bladder strips (pEC_{50} values of 7.63 ± 0.14 ($n=4$), 7.03 ± 0.25 ($n=5$), and 6.29 ± 0.15 ($n=6$) respectively (Fig. 1D); with significance ($P<0.01$) only between macaque and rat). The selective FP agonist, latanoprost, did induce small contractions in rat detrusor strips (Fig. 1E, $n=4$), but only at concentrations above 1 μM). In contrast, latanoprost was a potent agonist in the macaque and human bladder, producing complete concentration response curves with pEC_{50} values of 8.05 ± 0.12 and 7.50 ± 0.09 respectively ($n=4$, Fig. 1E).

The selective TP agonist, U46619, induced substantial contractions in macaque detrusor strips with a mean pEC_{50} of 7.74 ± 0.13 (E_{max} 78 ± 9% 80 mM KCl, $n=3$, Fig. 1F). However, similar to latanoprost, U46619 induced small contractions in rat detrusor muscle strips only at high concentrations (10 μM, 11 ± 1% 80 mM KCl, Fig. 1F).

No differences in any agonist response was noted when data from human strips were analysed by sex.

3.3. Effect of antagonists

Pre-incubation of rat bladder strips without urothelium with 300 nM PF-2907617 or 1 μM CJ24979 produced rightward parallel shifts of the PGE_2 concentration response curves with apparent pK_B values of 8.45 ± 0.07 and 6.33 ± 0.08 respectively ($n=3$–4, Fig. 2A). The EP_1 antagonist PF-2907617 (300 nM) did not inhibit the PGE2-induced contractions in macaque and human bladder strips (Fig. 2C and D respectively). However, the EP_3 antagonist CJ24979 (1 μM) induced rightward shifts of the PGE_2 concentration response curves in macaque bladder smooth muscle, yielding an apparent pK_B of 5.96 ± 0.22, $n=4$, Fig. 2C). CJ24979 (1 μM) had no effect on the PGE_2-induced contractions in human bladder strips (Fig. 2D).

Additional studies with U46619 were completed only in macaque bladder due to availability of human samples and the lack of potency in rat. Both the selective TP antagonist SQ29548 (100 nM)