

## ORIGINAL ARTICLE

EP<sub>4</sub> receptor as a new target for bronchodilator therapyJames Buckley,<sup>1</sup> Mark A Birrell,<sup>1</sup> Sarah A Maher,<sup>1</sup> Anthony T Nials,<sup>2</sup> Deborah L Clarke,<sup>1</sup> Maria G Belvisi<sup>1</sup>

► Additional materials are published online only. To view these files please visit the journal online (<http://thorax.bmj.com>).

<sup>1</sup>Respiratory Pharmacology, Pharmacology and Toxicology Section, Imperial College London, Faculty of Medicine, National Heart and Lung Institute, London, UK  
<sup>2</sup>Respiratory CEDD, GlaxoSmithKline Research and Development, Medicines Research Centre, Stevenage, Hertfordshire, UK

**Correspondence to**

Professor Maria G Belvisi, Respiratory Pharmacology, Pharmacology and Toxicology Section, Imperial College London, Faculty of Medicine, National Heart and Lung Institute, Sir Alexander Fleming Building, London SW7 2AZ, UK; [m.belvisi@imperial.ac.uk](mailto:m.belvisi@imperial.ac.uk)

Received 30 December 2010  
 Accepted 6 April 2011  
 Published Online First  
 23 May 2011

**ABSTRACT**

**Background** Asthma and chronic obstructive pulmonary disease are airway inflammatory diseases characterised by airflow obstruction. Currently approved bronchodilators such as long-acting  $\beta_2$  adrenoceptor agonists are the mainstay treatments but often fail to relieve symptoms of chronic obstructive pulmonary disease and severe asthma and safety concerns have been raised over long-term use. The aim of the study was to identify the receptor involved in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-induced relaxation in guinea pig, murine, monkey, rat and human airways *in vitro*.

**Methods** Using an extensive range of pharmacological tools, the relaxant potential of PGE<sub>2</sub> and selective agonists for the EP<sub>1–4</sub> receptors in the presence and absence of selective antagonists in guinea pig, murine, monkey, rat and human isolated airways was investigated.

**Results** In agreement with previous studies, it was found that the EP<sub>2</sub> receptor mediates PGE<sub>2</sub>-induced relaxation of guinea pig, murine and monkey trachea and that the EP<sub>4</sub> receptor mediates PGE<sub>2</sub>-induced relaxation of the rat trachea. These data have been confirmed in murine airways from EP<sub>2</sub> receptor-deficient mice (*Ptger2*). In contrast to previous publications, a role for the EP<sub>4</sub> receptor in relaxant responses in human airways *in vitro* was found. Relaxant activity of AH13205 (EP<sub>2</sub> agonist) was also demonstrated in guinea pig but not human airway tissue, which may explain its failure in clinical studies.

**Conclusion** Identification of the receptor mediating PGE<sub>2</sub>-induced relaxation represents a key step in developing a novel bronchodilator therapy. These data explain the lack of bronchodilator activity observed with selective EP<sub>2</sub> receptor agonists in clinical studies.

**INTRODUCTION**

Asthma and chronic obstructive pulmonary disease (COPD) are inflammatory diseases of the airway characterised by airflow limitation. The Global Burden of Disease Study<sup>1</sup> has projected that COPD, which ranked sixth as the cause of death in 1990, will become the third leading cause of death worldwide by 2020. Globally, approximately 300 million people have asthma, which is the most common chronic disease in children.<sup>2,3</sup>

Currently, the majority of patients with inflammatory diseases of the airway are treated with a combination of long-acting  $\beta$  agonists (LABAs) and corticosteroids, but significant issues exist with these therapies, particularly LABAs. While long- and short-acting  $\beta$  agonists help to provide patients with short-term relief from airflow limitation, they

**Key messages****What is the key question?**

New bronchodilator therapies are needed, given that currently approved bronchodilators such as long-acting  $\beta_2$  adrenoceptor agonists often fail to relieve symptoms of COPD and severe asthma and safety concerns have been raised over long-term use.

**What is the bottom line?**

A number of clinical studies have reported beneficial effects of inhaled PGE<sub>2</sub> on airway calibre in normal subjects and patients with asthma and COPD, and we have identified a role for the EP<sub>4</sub> receptor in these relaxant responses.

**Why read on?**

Identification of the receptor mediating PGE<sub>2</sub>-induced relaxation represents a key step in developing a novel bronchodilator therapy.

do little to treat the underlying pathology. Furthermore, since the introduction of LABAs, there has been increasing concern that their use may be associated with an increased risk of death from asthma.<sup>4</sup>

A number of clinical studies have reported beneficial effects of inhaled prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) on airway calibre in normal subjects<sup>5–8</sup> and in patients with chronic bronchitis and asthma.<sup>5,9,10</sup> In addition, PGE<sub>2</sub> has anti-inflammatory properties in patients with asthma,<sup>9,11</sup> providing an ideal dual therapy for the treatment of these diseases. Despite the benefits of inhaled PGE<sub>2</sub>, the development of prostanoid agonists for the treatment of airway inflammatory diseases has been hindered as prostanoids induce irritancy of the upper airway resulting in a reflex cough.<sup>11</sup> PGE<sub>2</sub> can act on a number of receptors, so it was hoped that the beneficial bronchodilator and anti-inflammatory effects could be dissociated from the airway irritancy to provide a novel therapeutic target.

There are currently nine known prostanoid receptors. PGE<sub>2</sub> has relatively low affinity for the FP, IP, TP, DP and CRTh2 receptors and acts predominantly via the EP receptors. The EP receptors have been subclassified into EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub> and EP<sub>4</sub> (encoded by *Ptger1–Ptger4* genes),<sup>12–14</sup> and distinct signalling pathways initiate diverse and opposing downstream effects in different



This paper is freely available online under the BMJ Journals unlocked scheme, see <http://thorax.bmj.com/site/about/unlocked.xhtml>

tissues.<sup>13–15</sup> Several investigators have documented bronchodilator activity *in vitro* and *in vivo* in a wide range of species including guinea pigs, mice and humans, and it has been suggested that EP<sub>2</sub> receptor activation is responsible for PGE<sub>2</sub> activity.<sup>16–19</sup> However, a selective EP<sub>2</sub> receptor agonist (AH13205) with a promising preclinical profile did not appear to have any bronchodilator activity in man.<sup>16</sup> Previous studies have been hampered by the lack of selective pharmacological tools. We now have a wide range of tool compounds and, by adopting a pharmacological approach and prostanoid receptor-deficient mice, we provide substantial evidence that, although the EP<sub>2</sub> receptor mediates PGE<sub>2</sub>-induced relaxation in several species, it is in fact the EP<sub>4</sub> receptor that mediates relaxation of human airways. This is an important finding which could lead to the development of a new class of bronchodilator therapy.

## METHODS

Male C57BL/6 mice (18–20 g), Dunkin-Hartley guinea pigs (300–500 g) and Cynomolgus (Cyno) monkey (*Macaca fascicularis*) tissue were purchased from Harlan (Bicester, Oxon, UK) and male Sprague Dawley rats (250–275 g) were purchased from Charles River (Margate, UK). EP receptor gene-deleted mice were originally provided by Dr Shuh Narumiya, Kyoto University and breeding colonies were maintained at Imperial College, London. Human airway samples (trachea, major bronchus, secondary bronchi) were obtained from donor patients (n=8, 3 male) or recipients (emphysema, n=5, 2 male; cystic fibrosis, n=3, 1 male) for lung transplants performed at the Royal Brompton or Harefield Hospital. Approval was obtained from the Royal Brompton and Harefield ethics committee after receiving the relevant consent from patients and relatives.

The trachea (apart from murine) was opened longitudinally by cutting through the cartilage directly opposite to the smooth muscle layer. Unless stated otherwise, the epithelium was left intact and transverse segments were prepared and sutured in order to be suspended from steel hook transducers in 10 ml baths containing Krebs–Henseleit (KH) solution warmed to 37°C and bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Indomethacin (10 µM), a non-selective COX inhibitor, was present in KH solution in all experiments to prevent the production and release of endogenous prostanoids. Changes in force were measured isometrically using force displacement transducers connected to a data acquisition system as previously described.<sup>20</sup>

Carbachol (CCh; 1 µM) was used to induce increased tension in tissues over a 30 min period before the addition of any antagonists or their appropriate vehicles which were incubated for a further 30 min. This concentration of CCh was required to produce approximately 80% of the supramaximal response to 1 mM ACh in all species. Cumulative concentration–response curves were then constructed using the relevant drugs and subsequently, at the end of the experiment, the non-specific phosphodiesterase inhibitor papaverine (100 µM) was used to assess the maximum capacity for relaxation of each tissue. For experiments on smaller airways, third to fourth generation bronchi (1–2 mm diameter) were dissected and sutured as described. For these experiments, a higher concentration of CCh (10 µM) was necessary to induce the required level of increased tension.

Full details are given in the online supplement.

## Data analysis and statistics

Data are expressed as mean±SEM of n independent observations. Concentration–response curves were analysed by non-linear regression using the PRISM curve-fitting program to

produce curves of best fit, from which EC<sub>50</sub> values were subsequently derived. Estimates of antagonist affinity were calculated using the equation  $pK_B = \log(CR - 1) - \log[B]$  as described by Gaddum,<sup>21</sup> where CR is the concentration ratio calculated from the EC<sub>50</sub> of agonist in the presence of the antagonist divided by the EC<sub>50</sub> of the agonist alone, K<sub>B</sub> is the equilibrium dissociation constant and [B] is the concentration of antagonist. In the experiments described here, the term pA<sub>2</sub> is substituted for pK<sub>B</sub> as antagonists were used at one concentration only, which precludes assumptions being made about the nature of the antagonism.

## RESULTS

### Effect of PGE<sub>2</sub> on guinea pig tracheal strips

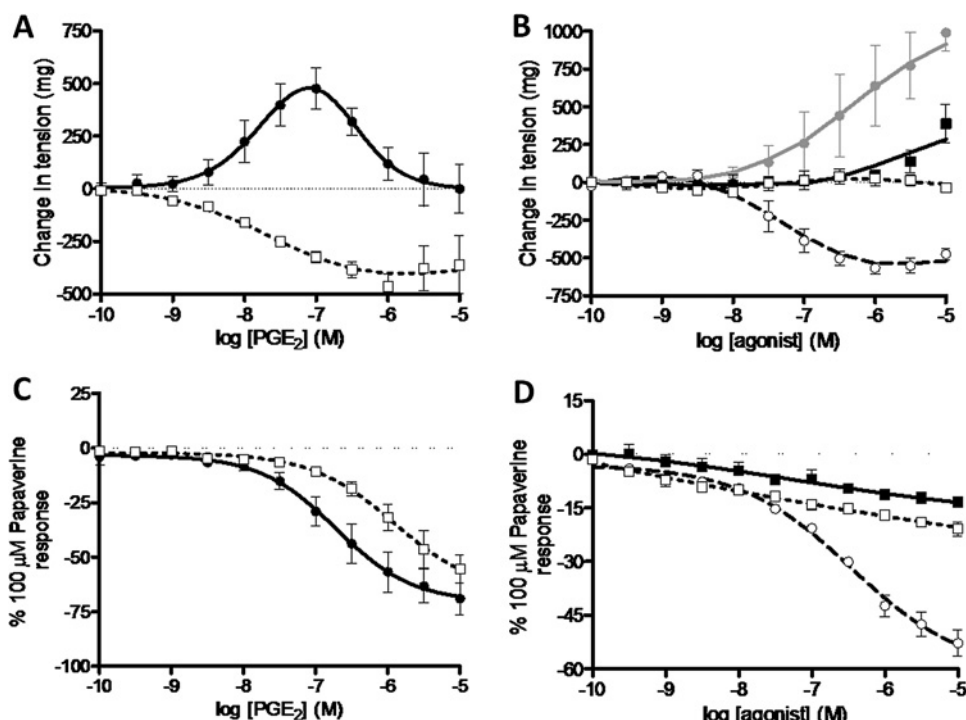
Under basal levels of tension (basal tone), increasing concentrations of PGE<sub>2</sub> (0.1 nM–10 µM) resulted in a biphasic response producing a pronounced contraction at lower concentrations (35.4±4.0% of the maximum contraction to ACh, 10 mM) that was later attenuated by increasing the concentration of PGE<sub>2</sub> in the bath (figure 1A). This contractile response was completely absent in the presence of the EP<sub>1</sub> antagonist GW848687X (1 µM). This was then confirmed using selective EP receptor agonists, with the EP<sub>1</sub> selective agonist ONO-D1-004 being the most potent and effective at inducing contraction of the guinea pig trachea (figure 1B).

When tension was induced in the guinea pig trachea using CCh (1 µM) (induced tone), increasing concentrations of PGE<sub>2</sub> (0.1 nM–10 µM) produced substantial relaxation (69.1±7.3% of the maximum relaxation to 100 µM papaverine). Due to the apparent contractile actions of PGE<sub>2</sub> via the EP<sub>1</sub> receptor, PGE<sub>2</sub>-induced relaxation in guinea pig trachea was performed in the presence of GW848687X (1 µM). Under these conditions, compared to its vehicle control, addition of AH6809 (10 µM) to the bath before the administration of PGE<sub>2</sub> antagonised the relaxation produced (figure 1C), resulting in a rightward shift in the curve (pA<sub>2</sub>=5.7) commensurate with an action at the EP<sub>2</sub> receptor (previous studies report antagonist affinities of approximately 5.9–6.3).<sup>22–24</sup> Subsequently, the response to PGE<sub>2</sub> was also assessed in the presence of selective antagonists for EP<sub>3</sub>, EP<sub>4</sub>, IP and FP receptors (L-826266, 1 µM; GW627368X, 1 µM; RO3244794, 10 µM; and AL-8810, 10 µM, respectively). Compared with their vehicle controls, addition of these antagonists did not produce any effect on PGE<sub>2</sub>-induced relaxation in guinea pig tracheal strips (figures not shown). When increasing concentrations of each EP receptor agonist (0.1 nM–1 µM) was applied to guinea pig tissue, only the EP<sub>2</sub> agonist ONO-AE1-259 induced substantial relaxation (52.8±3.62% of the maximum relaxation to 100 µM papaverine) (figure 1D). These data, alongside the AH6809 data, indicate that the EP<sub>2</sub> receptor mediates PGE<sub>2</sub>-induced relaxation in guinea pig trachea consistent with previous data.<sup>16</sup>

### Effect of PGE<sub>2</sub> on murine tracheal segments

In contrast to the guinea pig, the addition of increasing concentrations of PGE<sub>2</sub> to mouse tracheal tissue under basal tone failed to produce a contractile response (data not shown). However, when applied to murine tracheal segments under induced tone, PGE<sub>2</sub> induced a substantial relaxation response in the tissue (92.6±3.1% of the maximum response to 100 µM papaverine) (figure 2A). This response to PGE<sub>2</sub> observed in tissue taken from C57BL/6 wild type mice was then compared with that produced in tissue from prostanoid receptor gene-deficient mice. Of the gene-deleted tissues tested (deficient for EP<sub>1</sub>, EP<sub>2</sub>,

**Figure 1** Involvement of prostanoid EP receptors in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-induced contraction and relaxation of guinea pig tracheal strips. (A) Inhibition of PGE<sub>2</sub>-induced contraction under basal tone by the EP<sub>1</sub> antagonist (GW848687X, 1 μM) (PGE<sub>2</sub> + the appropriate vehicle (filled circle) or GW848687X in the presence of PGE<sub>2</sub> (square), n=4–6). (B) Response to selective EP receptor agonists under basal tone (ONO-D1-004 (filled circle), ONO-AE1-259 (circle), ONO-AE-248 (filled square) and ONO-AE1-329 (square)=EP<sub>1–4</sub> respectively, n=4). (C) Inhibition of PGE<sub>2</sub>-induced relaxation under induced tone by AH6809 (10 μM, EP<sub>1</sub>/EP<sub>2</sub>/DP antagonist), (PGE<sub>2</sub> + vehicle (filled circle), PGE<sub>2</sub> + AH6809 (square), n=4). (D) Response to selective EP receptor agonists under induced tone (ONO-AE1-259 (circle), ONO-AE-248 (filled square) and ONO-AE1-329 (square)=EP<sub>2–4</sub> respectively, n=4). Data represent mean±SEM.



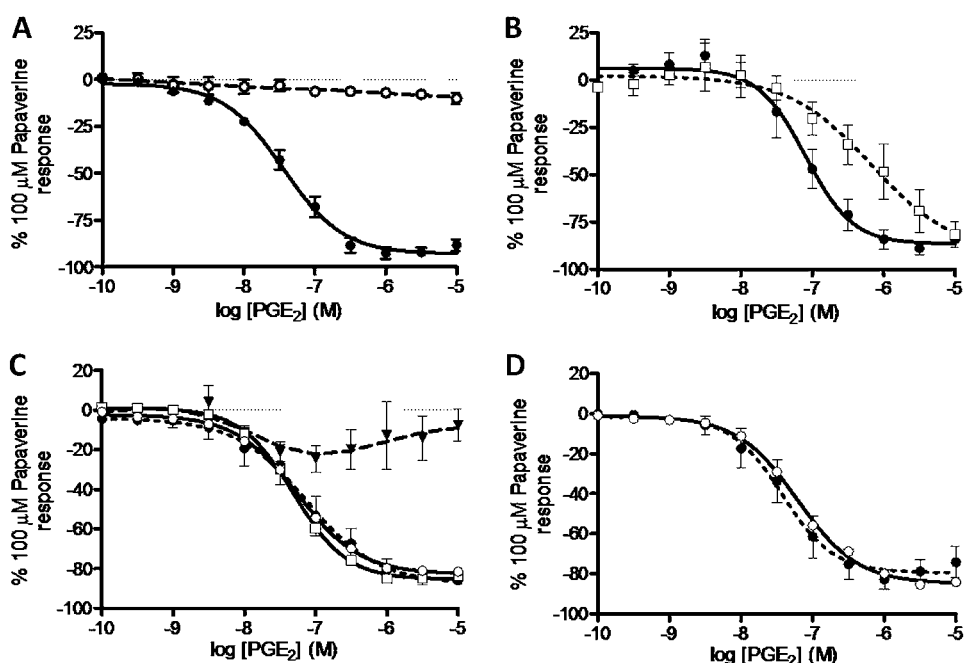
EP<sub>3</sub>, EP<sub>4</sub>, IP, FP, DP<sub>1</sub> or TP receptors), only the response of tracheal segments taken from *Ptger2* (EP<sub>2</sub>)-deficient mice differed substantially from tissue taken from wild type equivalents (figure 2C,D). The relaxation response produced by PGE<sub>2</sub> in *Ptger2*<sup>-/-</sup> tracheal tissue was substantially reduced (24.5±6.6% of the maximum response to 100 μM papaverine), indicating that PGE<sub>2</sub>-induced relaxation in mouse tracheal tissue is largely dependent on EP<sub>2</sub> receptors. Furthermore, AH6809 antagonised PGE<sub>2</sub>-induced relaxation in mouse tracheal segments under induced tone, producing a rightward shift in the curve (pA<sub>2</sub>=5.9) commensurate with affinity at the EP<sub>2</sub> receptor (figure 2B). Interestingly, there was a small reduction in the relaxation

produced by PGE<sub>2</sub> in IP receptor-deficient tissues (72.0±5.8% of the maximum response to 100 μM papaverine vs 85.9±1.8% for the wild type control), possibly indicating a minor role for the IP receptor in PGE<sub>2</sub>-induced relaxation in mouse tracheal segments. The maximum contractile responses to ACh and the maximum relaxation responses to papaverine were not changed in the prostanoid receptor deficient animals.

#### Effect of PGE<sub>2</sub> on rat tracheal strips

PGE<sub>2</sub> (1 nM–10 μM) did not induce a contractile response in rat tracheal strips under basal tone. However, under induced tone, PGE<sub>2</sub> produced a concentration-dependent relaxation although

**Figure 2** Involvement of the prostanoid EP receptors in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-induced relaxation of murine tracheal segments under induced tone. (A) PGE<sub>2</sub>-induced relaxation (PGE<sub>2</sub> (filled circle), ethanol vehicle control (circle), n=5). (B) Inhibition of PGE<sub>2</sub>-induced relaxation by AH6809 (10 μM, EP<sub>1</sub>/EP<sub>2</sub>/DP<sub>1</sub> receptor antagonist). (PGE<sub>2</sub> + vehicle (filled circle), PGE<sub>2</sub> + AH6809 (square), n=4). (C) Effect of specific individual EP receptor gene deficiency on PGE<sub>2</sub>-induced relaxation compared with the respective response elicited in wild-type tissues (C57BL/6 wild type (filled circle), *Ptger1*<sup>-/-</sup> (square), *Ptger2*<sup>-/-</sup> (filled inverted triangle), *Ptger3*<sup>-/-</sup> (circle), n=4). (D) Effect of specific individual EP receptor gene deficiency on PGE<sub>2</sub>-induced relaxation compared with the respective response elicited in wild-type tissues (129/Ola × C57BL/6 wild type (filled circle), *Ptger4*<sup>-/-</sup> (circle), n=4). Data represent mean±SEM.



smaller in magnitude than in other species ( $31.9 \pm 1.5\%$  of the maximum response to  $100 \mu\text{M}$  papaverine). EP receptor selective agonists ( $1 \text{ nM}$ – $10 \mu\text{M}$ ) were evaluated and only the EP<sub>4</sub> agonist ONO-AE1-329 caused substantial relaxation ( $36.7 \pm 4.0\%$  of maximum relaxation to  $100 \mu\text{M}$  papaverine) (figure 3A). Furthermore, two structurally distinct EP<sub>4</sub> antagonists inhibited PGE<sub>2</sub>-induced relaxation compared with their vehicle control ( $0.1\%$  DMSO) (figure 3B). Both GW627368X ( $1 \mu\text{M}$ ) and ONO-AE3-208 ( $100 \text{ nM}$ ) produced a substantial rightward shift in the curve ( $pA_2=6.5 \pm 0.1$  and  $8.9 \pm 0.1$ , respectively) commensurate with an action at the EP<sub>4</sub> receptor (previous studies report antagonist affinities of approximately 7 and 8.9 for GW627368X and ONO-AE3-208, respectively<sup>25, 26</sup>). Finally, the effect of removing the epithelium on the response of rat trachea to ONO-AE1-329 was investigated. When the responses of tracheal strips with the epithelium intact were compared with tracheal strips with epithelial cells removed, there was no substantial difference in relaxation to ONO-AE1-329 (epithelium intact:  $\log EC_{50}=-7.5$ , maximum relaxation= $338.3 \pm 99.7 \text{ mg}$ ; epithelium removed:  $\log EC_{50}=-7.6$ , maximum relaxation= $310.5 \pm 74.2 \text{ mg}$ ).

### Effect of PGE<sub>2</sub> on Cyno monkey tissue

PGE<sub>2</sub> produced a concentration-dependent relaxation of induced tone ( $53.4 \pm 5.9\%$  of the maximum relaxation response to  $100 \mu\text{M}$  papaverine) (figure 3C). Of the selective agonists, only the EP<sub>2</sub> selective agonist ONO-AE1-259 caused relaxation ( $36.5 \pm 8.0\%$  of the maximum relaxation response to  $100 \mu\text{M}$  papaverine), therefore indicating that, like guinea pig and mouse, PGE<sub>2</sub>-induced relaxation of Cyno monkey trachea appears to be mediated by the EP<sub>2</sub> receptor.

### Effect of PGE<sub>2</sub> on human airways

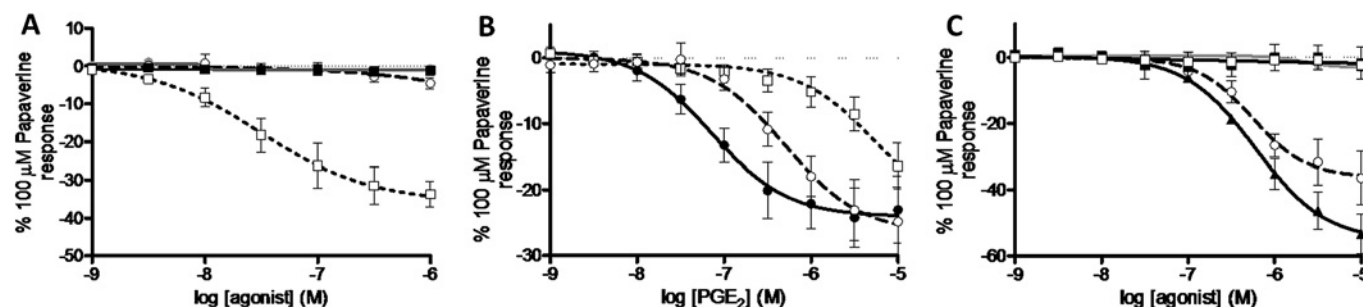
PGE<sub>2</sub> ( $0.1 \text{ nM}$ – $1 \mu\text{M}$ ) failed to produce a contractile response under conditions of basal tone but produced significant relaxation under conditions of induced tone ( $62.3\%$  of the maximum relaxation to  $100 \mu\text{M}$  papaverine; data not shown). For comparison, the clinically used LABA formoterol produced  $73.8 \pm 7.1\%$  of maximum relaxation to papaverine with  $\log EC_{50}=-8.9 \pm 0.2$ , consistent with values in previously published data.<sup>27</sup> Of the selective agonists tested, only the EP<sub>4</sub> selective agonist ONO-AE1-329 produced a substantial degree of relaxation ( $76.2 \pm 8.6\%$  of maximum relaxation to  $100 \mu\text{M}$  papaverine) (figure 4A). AH6809 failed to antagonise PGE<sub>2</sub>-

induced relaxation in human airway samples (figure 4B). Furthermore, increasing concentrations of two EP<sub>2</sub> agonists—AH13205 ( $10^{-10}$ – $10^{-5} \text{ M}$ ) and ONO-AE1-259 ( $1 \text{ nM}$ – $10 \mu\text{M}$ )—failed to produce any significant relaxation (figures 4A and 5B). In experiments on human tissue we used the tool compound with the most optimal profile with regard to antagonist affinity and selectivity (ONO-AE3-208).<sup>26</sup> The EP<sub>4</sub> antagonist ONO-AE3-208 ( $100 \text{ nM}$ ) inhibited both PGE<sub>2</sub>- and ONO-AE1-329-induced relaxation ( $pA_2=8.0 \pm 0.2$  and  $8.7 \pm 0.3$ , respectively; figure 4C,D). Experiments comparing PGE<sub>2</sub> with ONO-AE3-208 in human tissue were conducted in the presence of SQ29548 (TP antagonist,  $1 \mu\text{M}$ ) to prevent PGE<sub>2</sub>-induced contraction via TP receptors. The relaxation response to ONO-AE1-329 was also tested against an alternative non-muscarinic spasmogen. In the presence of  $10 \mu\text{M}$  histamine-induced tone, ONO-AE1-329 still produced substantial relaxation ( $63.4 \pm 19.6\%$  of maximum relaxation to papaverine,  $\log EC_{50}=-7.6 \pm 0.5$ ). To ensure the EP<sub>4</sub>-induced relaxation was not specific to larger airways and not subject to regional differences in the airways, the effect of PGE<sub>2</sub>, ONO-AE1-259 and ONO-AE1-329 was measured in smaller calibre airways (third to fourth generation bronchi,  $1$ – $2 \text{ mm}$  diameter). Responses in these airways were similar to those seen in trachea/primary bronchi with PGE<sub>2</sub> and ONO-AE1-329 producing relaxation (PGE<sub>2</sub>:  $43.1 \pm 5.4\%$  of maximum relaxation to papaverine,  $\log EC_{50}=-7.2$  ( $n=2$ ); ONO-AE1-329:  $32.1 \pm 5.0\%$  of maximum relaxation to papaverine,  $\log EC_{50}=-7.6 \pm 0.1$  ( $n=3$ )), with ONO-AE1-259 having no substantial effect.

Finally, in order to confirm that the biological activity of AH13205 was similar to that observed in previous studies, we demonstrated its relaxant activity in guinea pig trachea studies (figure 5A).<sup>16</sup> However, this relaxant activity was not paralleled in human airway tissue, which may explain its failure in clinical studies (figure 5B).

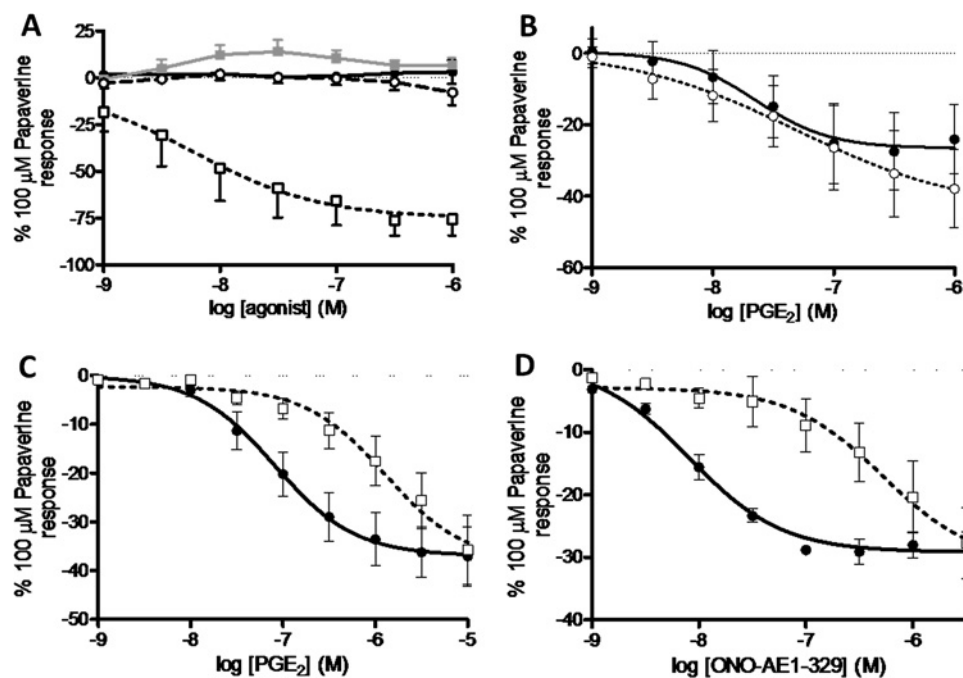
## DISCUSSION

Inhaled  $\beta_2$  adrenoceptor agonists are currently the gold standard bronchodilators prescribed to patients with respiratory diseases such as asthma and COPD. While long- and short-acting  $\beta$  agonists help to provide patients with short-term relief from airflow limitation, they do little to treat the underlying pathology. Furthermore, since the introduction of LABAs, there has been increasing concern about their safety, particularly when used as monotherapy.<sup>4</sup> For these reasons, the search is on for



**Figure 3** Involvement of the prostanoid EP receptors in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-induced relaxation of rat tracheal strips and monkey airway samples under induced tone. (A) Response of rat tracheal strips under induced tone to selective EP receptor agonists (ONO-D1-004 (filled circle), ONO-AE1-259 (circle), ONO-AE-248 (filled square) and ONO-AE1-329 (square)=EP<sub>1–4</sub> respectively,  $n=3$ ). (B) Inhibition of PGE<sub>2</sub>-induced relaxation of rat tracheal strips by ONO-AE3-208 ( $100 \text{ nM}$ ) and GW627368X ( $1 \mu\text{M}$ ) (PGE<sub>2</sub> in the presence of the appropriate vehicle (filled circle), PGE<sub>2</sub> in the presence of ONO-AE3-208 (square) or GW627368X (circle),  $n=4$ ). (C) Response of Cyno monkey airway samples under induced tone to selective EP receptor agonists (ONO-D1-004 (filled circle), ONO-AE1-259 (circle), ONO-AE-248 (filled square) and ONO-AE1-329 (square)=EP<sub>1–4</sub>, respectively, and PGE<sub>2</sub> (filled triangle),  $n=3$ ). Data represent mean  $\pm$  SEM.

**Figure 4** Involvement of EP prostanoid receptors in prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)-induced relaxation of human airway smooth muscle under induced tone. (A) Comparative effects of selective EP receptor agonists (ONO-D1-004 (filled circle), ONO-AE1-259 (circle), ONO-AE-248 (filled square) and ONO-AE1-329 (square))=EP<sub>1-4</sub>, respectively, n=3–4). (B) PGE<sub>2</sub>-induced relaxation was not inhibited in the presence of AH6809 (EP<sub>1</sub>/EP<sub>2</sub>/DP antagonist, 10 μM) (PGE<sub>2</sub> + vehicle (filled circle), PGE<sub>2</sub> + AH6809 (circle), n=8). (C) Inhibition of PGE<sub>2</sub>-induced relaxation by ONO-AE3-208 (EP<sub>4</sub> antagonist, 100 nM) in the presence of SQ29548 (TP antagonist, 1 μM) (PGE<sub>2</sub> + vehicle and SQ29548 (filled circle), PGE<sub>2</sub> + ONO-AE3-208 and SQ29548 (square), n=4). (D) Inhibition of ONO-AE1-329 (EP<sub>4</sub> agonist)-induced relaxation by ONO-AE3-208 (EP<sub>4</sub> antagonist, 100 nM) (ONO-AE1-329 + vehicle (filled circle), ONO-AE1-329 + ONO-AE3-208 (square), n=4). Data represent mean ± SEM.



alternative bronchodilator therapies which have an increased safety profile. Identification of a safe therapeutic agent which also possesses anti-inflammatory activity would make for an extremely attractive alternative to LABAs.

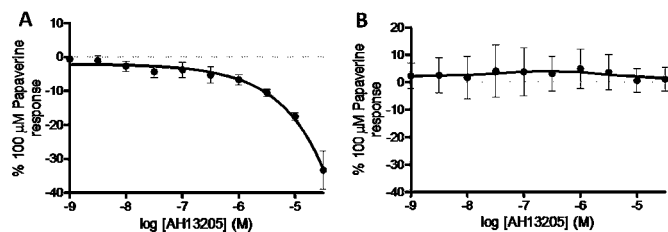
PGE<sub>2</sub> causes relaxation of animal<sup>28</sup> and human<sup>17, 29</sup> isolated airway smooth muscle. Inhaled PGE<sub>2</sub> is a bronchodilator in animals,<sup>18, 29, 30</sup> normal subjects<sup>5–8</sup> and in patients with chronic bronchitis and asthma.<sup>5, 9, 10</sup> In addition, PGE<sub>2</sub> has anti-inflammatory properties in patients with asthma,<sup>9, 11</sup> providing an ideal dual therapy for the treatment of these diseases. Despite the benefits of inhaled PGE<sub>2</sub>, the development of prostanoid agonists for the treatment of airway inflammatory diseases has been hindered as prostanoids induce irritancy of the upper airway resulting in a reflex cough.<sup>11</sup>

We recently identified the EP<sub>3</sub> receptor as being responsible for PGE<sub>2</sub>-induced airway irritancy (in human, guinea pig and murine *in vitro* assays) and in a guinea pig cough model, so an opportunity exists to find a therapeutic agent devoid of this sensory irritant side effect if alternative receptors are identified as being responsible for the bronchodilator activity.<sup>31</sup> Several investigators have documented bronchodilator activity both *in vitro* and *in vivo* in a wide range of species including guinea pigs, mice and humans, and it has been suggested that EP<sub>2</sub> receptor activation is responsible for this activity.<sup>16–19</sup> However, a selec-

tive EP<sub>2</sub> receptor agonist (AH13205) with a promising preclinical profile did not appear to have any bronchodilator activity in man.<sup>16</sup> Recently, however, improved tools have become available (selective ligands and prostanoid receptor-deficient mice) so, in the light of the issues with current bronchodilators, it seemed pertinent to revisit this area of research.

To investigate which receptor PGE<sub>2</sub> acts to cause airway smooth muscle relaxation, a range of selective tools were tested on constricted isolated trachea tissue from guinea pigs, mice, monkeys, rats and humans. In all species PGE<sub>2</sub> caused a concentration-dependent relaxation of airway smooth muscle. Experiments using selective ligands on guinea pig, monkey and mouse tissue identified the EP<sub>2</sub> receptor as mediating this response, which is consistent with previous studies.<sup>16–19</sup> This was further confirmed using mice deficient in individual prostanoid receptors, in that the response to PGE<sub>2</sub> present in wild-type animals was substantially diminished in *Pter2*<sup>-/-</sup> mice, again confirming previous data.<sup>18, 19, 30</sup> In contrast, but consistent with previous data, PGE<sub>2</sub>-induced relaxation of rat isolated tracheal smooth muscle was mediated via activation of the EP<sub>4</sub> receptor.<sup>32</sup>

In contrast, the data from human tissue studies clearly showed that PGE<sub>2</sub>-induced relaxation is mediated via the EP<sub>4</sub> and not the EP<sub>2</sub> receptor. This conclusion is based on the *in vitro* relaxant activity of the selective EP<sub>4</sub> receptor agonist (ONO-AE1-329) and the lack of activity of the selective EP<sub>2</sub> receptor agonists (ONO-AE1-259, AH13205) in human airways. A role for the EP<sub>4</sub> compared with the EP<sub>2</sub> receptor was also substantiated by the lack of effect of AH6809 (EP<sub>1/2</sub>/DP receptor antagonist) and the effectiveness of the EP<sub>4</sub> antagonist (ONO-AE3-208) in inhibiting PGE<sub>2</sub>-induced relaxation of human airway smooth muscle. These data are in contrast to a previous publication describing a role for the EP<sub>2</sub> receptor in PGE<sub>2</sub>-induced relaxation of human airway smooth muscle.<sup>17</sup> However, there are some differences between our studies and previous ones which, in the main, can be explained by the lack of selectivity of the tools available at the time the previous studies were carried out. Compared with the study by Norel *et al*,<sup>17</sup> our study has benefited from the use of optimised, more potent and selective agonists (EP<sub>2</sub> receptor



**Figure 5** Species differences in the response of airway smooth muscle under induced tone to the EP<sub>2</sub> selective agonist AH13205. (A) AH13205-induced relaxation of guinea pig tracheal strips (n=6). (B) Response of human airway smooth muscle to AH13205 (n=3). Data represent mean ± SEM.

**Table 1** Prostanoid EP receptor agonist and antagonist affinities

|                   | Ligand      | Action       | Affinity        | Units | References |
|-------------------|-------------|--------------|-----------------|-------|------------|
| <b>Agonist</b>    |             |              |                 |       |            |
| EP <sub>1</sub>   | ONO-D1-004  | Full agonist | 6.8 (mouse)     | pKi   | 34         |
| EP <sub>2</sub>   | AH 13205    | Full agonist | 6 (human)       | pKi   | 35         |
|                   | ONO-AE1-259 | Full agonist | 8.5 (mouse)     | pKi   | 34         |
|                   | Butaprost   | Full agonist | 5.9–7.0 (human) | pKi   | 24         |
|                   | Misoprostol | Full agonist | 5.0 (human)     | pKi   | 24         |
|                   | ONO-AE-248  | Full agonist | 5.4 (mouse)     | pKi   | 34         |
|                   | ONO-AE1-329 | Full agonist | 5.7 (mouse)     | pKi   | 34         |
| EP <sub>3</sub>   | Misoprostol | Full agonist | 6.5 (human)     | pKi   | 24         |
|                   | Butaprost   | Full agonist | 5.8 (human)     | pKi   | 24         |
|                   | ONO-AE-248  | Full agonist | 8.1 (mouse)     | pKi   | 34         |
| EP <sub>4</sub>   | ONO-AE1-329 | Full agonist | 8.0 (mouse)     | pKi   | 24         |
|                   | Misoprostol | Full agonist | 5.3 (human)     | pKi   | 24         |
|                   | Butaprost   | Full agonist | 4.7 (human)     | pKi   | 24         |
| <b>Antagonist</b> |             |              |                 |       |            |
| EP <sub>1</sub>   | AH6809      | Antagonist   | 5.9 (human)     | pKi   | 24         |
| EP <sub>2</sub>   | AH6809      | Antagonist   | 5.9 (human)     | pKi   | 24         |
| EP <sub>4</sub>   | AH23848     | Antagonist   | 4.9–5.6 (human) | pKi   | 24         |
|                   | ONO-AE3-208 | Antagonist   | 8.9 (mouse)     | pKi   | 26         |
|                   | GW627568    | Antagonist   | 7.0–7.1 (human) | pKi   | 25         |

agonist ONO-AE1-259; EP<sub>4</sub> receptor agonist ONO-AE1-329) compared with misoprostol which is a non-selective agonist at EP<sub>2–4</sub> receptors<sup>33</sup> (table 1). Furthermore, we had the opportunity to use a highly selective EP<sub>4</sub> antagonist with greater affinity and selectivity for the receptor than AH23848B, which was used in the previous studies and is a weak antagonist at both the EP<sub>4</sub> and TP receptor (pKi 4.9–5.6 and 6.2, respectively), and AH6809, which was used as an EP<sub>2</sub> antagonist but also has affinity at the EP<sub>1</sub> and DP<sub>1</sub> receptors (table 1).<sup>33</sup> In the paper by Nials *et al.*,<sup>16</sup> the EP<sub>2</sub> receptor dependence of the relaxant response (mostly investigated in non-human species) was suggested by the activity of misoprostol, AH13205 and butaprost which all have a relatively weak affinity for the EP<sub>2</sub> receptor.<sup>16</sup> The relaxant activity of AH13205 in guinea pig trachea described in the study by Nials *et al.* was reproduced in our studies, suggesting that the lack of effect on human airway relaxation was a real phenomenon. The absence of relaxant activity on human airway smooth muscle preparations also provides a likely explanation for the poor efficacy of the EP<sub>2</sub> receptor agonist in clinical trials as a bronchodilator in humans.<sup>16</sup>

Interestingly, it would appear that, of all the animal species tested, PGE<sub>2</sub>-induced relaxation of human airway smooth muscle and the role of the EP<sub>4</sub> receptor was most accurately represented in the rat. The responses in non-human primate tissue were not representative of those in human tissue, given the EP<sub>2</sub> receptor dependence of the relaxant response to PGE<sub>2</sub>. This would imply that bioassay of rat tissue would be amenable for medium throughput screening if drug discovery programmes were initiated in this area, thus limiting the use of valuable human airway specimens.

In conclusion, we believe that these data show that the EP<sub>4</sub> receptor is responsible for PGE<sub>2</sub>-induced relaxation of human airway smooth muscle. We recently identified the EP<sub>3</sub> receptor as being responsible for PGE<sub>2</sub>-induced airway irritation and cough,<sup>31</sup> so an opportunity exists to find a therapeutic agent devoid of this sensory irritant side effect liability. However, as PGE<sub>2</sub> also enhances plasma leakage in numerous anatomical sites via enhanced blood flow,<sup>13</sup> it would be prudent to investigate the receptor responsible as an ideal bronchodilator should not worsen inflammatory exudation or oedema. In summary, EP<sub>4</sub>

selective compounds could potentially be the next generation of bronchodilators identified since the introduction of β<sub>2</sub> adrenoceptors and anticholinergics more than half a century ago.

**Acknowledgements** We would like to thank Nicole Dale for her assistance with some of the Cynomolgus (Cyno) monkey tissue experiments.

**Funding** The research project and SAM were funded by a project grant from the Medical Research Council (MRC) UK (G0800195). MAB was funded by a grant from the MRC (G0800196). DLC was funded by an MRC project grant Experimental Medicine Grant (G0502019). The human tissue experiments in this study were undertaken with the support of the NIHR Biomedical Research Unit in Advanced Lung Disease at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College London and partly funded by the NIHR Biomedical Research Unit funding scheme. JB was funded by a Studentship from a Capacity Building Award in Integrative Mammalian Biology funded by BBSRC, BPS, HEFCE KTN and MRC.

**Competing interests** None.

**Patient consent** Obtained.

**Ethics approval** This study was conducted with the approval of the Royal Brompton and Harefield ethics committee.

**Provenance and peer review** Not commissioned; externally peer reviewed.

## REFERENCES

- Rabe KF, Hurd S, Anzueto A, *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;**176**:532–55.
- Lemanske RF Jr, Busse WW. 6. Asthma. *J Allergy Clin Immunol* 2003;**111**: S502–9.
- Bateman ED, Hurd SS, Barnes PJ, *et al.* Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J* 2008;**31**:143–78.
- Nelson HS, Weiss ST, Bleecker ER, *et al.* The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. *Chest* 2006;**129**:15–26.
- Kawakami Y, Uchiyama K, Irie T, *et al.* Evaluation of aerosols of prostaglandins E1 and E2 as bronchodilators. *Eur J Clin Pharmacol* 1973;**6**:127–32.
- Seth RV, Clarke VS, Lewis RA, *et al.* Effect of propranolol on the airway response to prostaglandin E<sub>2</sub> in normal man. *Br J Clin Pharmacol* 1981;**12**:731–5.
- Walters EH, Bevan M, Davies BH. Interactions between response to inhaled prostaglandin E<sub>2</sub> and chronic beta-adrenergic agonist treatment. *Thorax* 1982;**37**:430–7.
- Costello JF, Dunlop LS, Gardiner PJ. Characteristics of prostaglandin induced cough in man. *Br J Clin Pharmacol* 1985;**20**:355–9.
- Pavord ID, Wong CS, Williams J, *et al.* Effect of inhaled prostaglandin E<sub>2</sub> on allergen-induced asthma. *Am Rev Respir Dis* 1993;**148**:87–90.
- Melillo E, Woolley KL, Manning PJ, *et al.* Effect of inhaled PGE<sub>2</sub> on exercise-induced bronchoconstriction in asthmatic subjects. *Am J Respir Crit Care Med* 1994;**149**:1138–41.
- Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE<sub>2</sub> on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1999;**159**:31–6.
- Coleman RA, Smith WL, Narumiya S. International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol Rev* 1994;**46**:205–29.
- Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: structures, properties and functions. *Physiol Rev* 1999;**79**:1193–226.
- Breyer RM, Bagdassarian CK, Myers SA, *et al.* Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol* 2001;**41**:661–90.
- Tsuboi K, Sugimoto Y, Ichikawa A. Prostanoid receptor subtypes. *Prostaglandins Other Lipid Mediat* 2002;**68–69**:535–56.
- Nials AT, Vardey CJ, Denyer LH, *et al.* AH13205, A selective prostanoid EP<sub>2</sub>-receptor agonist. *Cardiovasc Drug Rev* 1993;**11**:165–79.
- Norel X, Walch L, Labat C, *et al.* Prostanoid receptors involved in the relaxation of human bronchial preparations. *Br J Pharmacol* 1999;**126**:867–72.
- Sheller JR, Mitchell D, Meyrick B, *et al.* EP(2) receptor mediates bronchodilation by PGE(2) in mice. *J Appl Physiol* 2000;**88**:2214–18.
- Fortner CN, Breyer RM, Paul RJ. EP<sub>2</sub> receptors mediate airway relaxation to substance P, ATP, and PGE<sub>2</sub>. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**: L469–74.
- Birrell MA, De Alba J, Catley MC, *et al.* Liver X receptor agonists increase airway reactivity in a model of asthma via increasing airway smooth muscle growth. *J Immunol* 2008;**181**:4265–71.
- Gaddum JH. Theories of drug antagonism. *Pharmacol Rev* 1957;**9**:211–18.
- Kiryama M, Ushikubi F, Kobayashi T, *et al.* Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br J Pharmacol* 1997;**122**:217–24.

23. **Boie Y**, Stocco R, Sawyer N, *et al.* Molecular cloning and characterisation of the four rat prostaglandin E<sub>2</sub> prostanoid receptor subtypes. *Eur J Pharmacol* 1997;**304**:227–41.
24. **Abramovitz M**, Adam M, Boie Y, *et al.* The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim Biophys Acta* 2000;**1483**:285–93.
25. **Wilson RJ**, Giblin GM, Roomans S, *et al.* GW627368X ((N-{2-[4-(4, 9-dithoxy-1-oxo-1, 3-dihydro-2H-benzo [f] isoindol-2-yl) phenyl] acetyl} benzene sulphonamide): a novel, potent and selective prostanoid EP<sub>4</sub> receptor antagonist. *Br J Pharmacol* 2006;**148**:326–9.
26. **Kabashima K**, Saji T, Murata T, *et al.* The prostaglandin receptor EP<sub>4</sub> suppresses colitis, mucosal damage and CD4 cell activation in the gut. *J Clin Invest* 2002;**109**:883–93.
27. **Bouyssou T**, Casarosa P, Naline E, *et al.* Pharmacological characterization of olodaterol, a novel inhaled beta<sub>2</sub>-adrenoceptor agonist exerting a 24-hour-long duration of action in preclinical models. *J Pharmacol Exp Ther* 2010;**334**:53–62.
28. **Main IH**. The inhibitory actions of prostaglandins on respiratory smooth muscle. *Br J Pharmacol Chemother* 1964;**22**:511–19.
29. **Sweatman WJ**, Collier HO. Effect of prostaglandins on human bronchial smooth muscle. *Nature* 1968;**217**:69.
30. **Tilley SL**, Hartney JM, Erikson CJ, *et al.* Receptors and pathways mediating the effects of prostaglandin E<sub>2</sub> on airway tone. *Am J Physiol Lung Cell Mol Physiol* 2000;**284**:L599–606.
31. **Maier SA**, Birrell MA, Belvisi MG. Prostaglandin E<sub>2</sub> mediates cough via the EP<sub>3</sub> receptor: implications for future disease therapy. *Am J Respir Crit Care Med* 2009;**180**:923–8.
32. **Lydford SJ**, McKechnie K. Characterization of the prostaglandin E<sub>2</sub> sensitive (EP)-receptor in the rat isolated trachea. *Br J Pharmacol* 1994;**112**:133–6.
33. **Harmar AJ**, Hills RA, Rosser EM, *et al.* IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels. *Nucl Acids Res* 2009;**37**(Database issue):D680–5.
34. **Suzawa T**, Miyaura C, Inada M, *et al.* The role of prostaglandin E receptor subtypes (EP<sub>1</sub>, EP<sub>2</sub>, EP<sub>3</sub>, and EP<sub>4</sub>) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology* 2000;**141**:1554–9.
35. **Stillman BA**, Breyer MD, Breyer RM. Importance of the extracellular domain for prostaglandin EP<sub>2</sub> receptor function. *Mol Pharmacol* 1999;**56**:545–51.



British Thoracic Society

## BTS SHORT COURSE PROGRAMME FOR 2012 NOW AVAILABLE

Course fees held at 2011 prices for BTS members

Short courses in  
**Thoracic Ultrasound,**  
**Lung Transplantation,**  
**Interventional Bronchoscopy**  
 and many others including some new for next year.

Limited places only available on some BTS courses,  
 so early booking is advised.

For more information and to book your place on a  
 BTS Short Course in 2012, visit:  
[www.brit-thoracic.org.uk](http://www.brit-thoracic.org.uk)

*Working for healthier lungs*

