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The role of prostanoid TP- and DP-receptors in the bronchoconstrictor effect of inhaled PGD_2 in anaesthetized guinea-pigs: effect of the DP-antagonist BW A868C

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1 In anaesthetized, pump-ventilated guinea-pigs, bolus intravenous injection of prostaglandin D_2 (PGD_2 5–160 $\mu g\,kg^{-1}$) caused a dose-dependent rise in both heart rate and systemic mean arterial blood pressure with only a small monophasic rise in pulmonary inflation pressure (PIP).

2 In contrast, inhaled PGD_2 (0.1–1 $mg\,ml^{-1}$, 30 s) provoked a substantial concentration-dependent biphasic rise in PIP. The bronchoconstrictor action of inhaled PGD_2 was accompanied by minimal cardiovascular effects.

3 The 3-benzyl substituted hydantoin BW A868C (0.1–1 $mg\,kg^{-1}$ i.v.) a novel prostanoid DP-receptor antagonist, had no significant effect on the cardiovascular or bronchoconstrictor effects of intravenously administered or inhaled PGD_2.

4 However, BW A868C (0.1–1 $mg\,kg^{-1}$ i.v.) did inhibit the hypotensive actions of the DP-receptor agonist, BW 245C (1–3 $\mu g\,kg^{-1}$ i.v.).

5 The prostanoid TP-receptor antagonist BM 13.177 (2.5 $mg\,kg^{-1}$ i.v.) strongly inhibited the bronchoconstrictor effect of inhaled PGD_2, abolishing the first phase of this response and reducing the peak increase in PIP provoked by PGD_2 (0.1 or 1 $mg\,ml^{-1}$ for 30 s), by 67 ± 16% and 44 ± 5% respectively.

6 A combination of BW A868C (0.1 or 1 $mg\,kg^{-1}$ i.v.) with BM 13.177 (2.5 $mg\,kg^{-1}$ i.v.) produced no greater inhibition of the bronchoconstrictor effect of inhaled PGD_2 than that seen with BM 13.177 (2.5 $mg\,kg^{-1}$ i.v.) alone.

7 Neither bilateral vagotomy, nor selective inhibition of arachidonate cyclo-oxygenase with indomethacin or 5-lipoxygenase with the novel acetohydroxamic acid BW A4C, significantly reduced the bronchoconstrictor effect of inhaled PGD_2.

8 These findings indicate that the bronchoconstrictor effect of inhaled PGD_2 in guinea-pigs *in vivo* is mediated primarily through direct TP-receptor activation and not through actions on DP-receptors.

Introduction

Prostaglandin D_2 (PGD_2) is the principal prostanoid released from human lung fragments and purified lung mast cells after antigen challenge *in vitro* (Holgate *et al.*, 1984). In studies *in vivo*, a 150 fold increase in the level of PGD_2 in bronchoalveolar lavage fluid can be detected following local antigen challenge in asthmatics (Murray *et al.*, 1986). PGD_2 contracts human isolated airways (Finney *et al.*, 1985; Black *et al.*, 1986) and when inhaled, is a potent bronchoconstrictor agent in asthmatics (Hardy *et al.*, 1984). In conjunction with other inflammatory mediators, PGD_2 may also be involved in the pathogenesis of airway hyperreactivity (Fuller *et al.*, 1986). These observations thus suggest that PGD_2 may be a causative mediator of bronchial asthma.

Like many other prostaglandins, PGD_2 is known to interact with several different types of prostanoid receptor (see review by Giles & Leff, 1988), including those defined as TP- (the receptor for thromboxane A_2) and DP-receptors using the nomenclature of Kennedy *et al.* (1982). In both human and guinea-pig airway smooth muscle, the contractile effect of PGD_2 *in vitro* has been suggested to be mediated largely through an interaction at TP-receptors (Jones *et al.*, 1982; Eglen & Whiting, 1988; McKenniff *et al.*, 1988; Beasley *et al.*, 1989; Coleman & Sheldrick, 1989). It is nevertheless possible that DP-receptor activation could also play either a direct or modulator role in this process *in vivo*. Furthermore, it has been postulated that the bronchoconstrictor effects of PGD_2 *in vivo* may result from both direct and indirect actions (Beasley *et al.*, 1987). However, the lack of a selective DP-receptor antagonist has precluded the direct investigation of the role of DP receptors in bronchoconstriction *in vivo*.

Recently, some aspects of the *in vitro* and *in vivo* pharmacology of the novel, selective and competitive DP-receptor antagonist, BW A868C have been described (Giles *et al.*, 1989; Hamid-Bloomfield & Whittle, 1989; Trist *et al.*, 1989). In the present study, we have used BW A868C and the previously described TP-receptor antagonist BM 13.177 (Stegmeier *et al.*, 1984) to investigate the relative contributions of DP- and TP-receptors to the bronchoconstrictor effect of inhaled PGD_2 in anaesthetized guinea-pigs. In order to differentiate between direct bronchoconstrictor actions and possible indirect actions of PGD_2, mediated through either vagal mechanisms or the secondary release of other broncho-active eicosanoids, we have also investigated the effect of bilateral vagotomy and selective inhibition of either the arachidonate cyclo-oxygenase or 5-lipoxygenase pathways by use of indomethacin or the selective 5-lipoxygenase inhibitor BW A4C (Jackson *et al.*, 1988) respectively.

A preliminary account of this work has been communicated to the British Pharmacological Society (Hamid-Bloomfield *et al.*, 1989).

Methods

Male Dunkin-Hartley guinea-pigs (450–500 g body wt.) were anaesthetized with sodium pentobarbitone (60 $mg\,kg^{-1}$ i.p.) and mechanically ventilated (54 × 1 ml 100 g^{-1} body wt min^{-1}) through a mid-cervical tracheostomy. Pulmonary inflation pressure (PIP), an index of intrathoracic airway calibre, was measured from a lateral port in the ventilator circuit with a pressure transducer (Elcomatic EM759 or Statham P23 ID). Systemic arterial blood pressure was measured with a second pressure transducer (Elcomatic EM750 or Statham Pb21AA) from a cannula inserted into a carotid

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artery. Both pressure transducers were connected to pre-amplifiers (Buxco Electronics Inc., Sharon, Conn., U.S.A.) the outputs of which were connected to a Buxco Cardiovascular Analyzer for final amplification and derivation of heart rate (HR) and mean arterial blood pressure (MBP).

All measured primary and derived physiological indices were displayed and recorded on-line by an IBM AT microcomputer via a Buxco DL-12A Data Logger connected to the Cardiovascular Analyzer. Acquisition, display and subsequent handling of data together with final statistical analyses were facilitated by a commercially available custom programmed software package (CV-STAT, Branch Technology, Dexter, MI., U.S.A.). In addition, parallel analogue hard copy of PIP, MBP and HR was generated in real time by means of a Grass Model 7D polygraph interfaced with the Cardiovascular Analyzer. Typical resting values were: PIP 8–12 cmH_2O; MBP 43 mmHg; HR 270 beats min^{-1}.

Rectal temperature was maintained at 37°C by heat from a thermostatically controlled infra-red lamp. A jugular vein was cannulated for bolus intravenous administration of drugs (dose volume kept constant at 1 ml kg^{-1}).

Aerosol administration of PGD_2

Aqueous aerosols were generated from stock solutions of PGD_2 at concentrations of 0.1–1 mg ml^{-1} in sodium bicarbonate (1.25% w/v) and administered by direct inhalation for 30 s by use of an in-line ultrasonic nebuliser (Devilbiss Pulmosonic, Feltham, Middx., U.K.) adapted and incorporated into the ventilator circuit as described previously (Payne & Nucci, 1987). The concentrations of PGD_2 used and the time of aerosol administration were selected on the basis of results from preliminary experiments (data not shown). By use of gravimetric analysis, the amounts of PGD_2 administered during the 30 s nebulisation of the 0.1 and 1 mg ml^{-1} solutions were estimated to be approximately 2.5 and 25 μg respectively, of which probably less than 10% was retained in the lung, the remainder being exhaled.

Experimental design

In initial studies with the intravenous route, increasing incremental doses of PGD_2 (5–160 $\mu g\,kg^{-1}$) were administered sequentially as bolus injections at 10–15 min intervals and the peak instantaneous effects on PIP, MBP and HR calculated. In subsequent aerosol studies, a single aerosol of PGD_2 (0.1 or 1 mg ml^{-1}) was administered following pretreatment with either one or a combination of the following: BW A868C (0.1–1 mg kg^{-1} i.v.), BM 13.177 (2.5 mg kg^{-1}); BW A868C and BM 13.177; indomethacin (10 mg kg^{-1} i.v.); BW A4C (50 mg kg^{-1} p.o.). BW A4C was administered 30 min before induction of anaesthesia and subsequent surgical preparation, the total pretreatment time being 1 h. In these animals, food was withdrawn 12 h before dosing but drinking water was allowed *ad libitum*. The pretreatment time for all other drugs was 10 min before the PGD_2 aerosol. Following PGD_2 aerosol, mean values for subsequent changes in PIP at fixed time points (0.5, 1.5, 2–10 min post PGD_2 inhalation) were calculated, as were the peak changes in this index. None of the pretreatment regimes themselves caused a statistically significant change in PIP. Control animals (vehicle alone) for the different pretreatment regimes were incorporated concurrently in each individual series of experiments. None of the vehicles used for drug pretreatment studies had any significant effect on the bronchoconstriction induced by inhaled PGD_2.

To demonstrate the antagonism of DP-receptors in the guinea-pig, the effects of BW A868C (0.1–1 mg kg^{-1} i.v.) on the hypotensive actions of BW 245C (1–3 $\mu g\,kg^{-1}$ i.v.), a DP-receptor agonist (Whittle *et al.*, 1983; Giles *et al.*, 1989; Hamid-Bloomfield & Whittle, 1989), were investigated. Since resting MBP in these anaesthetized preparations was too low (40–45 mmHg) to demonstrate consistent hypotensive actions, resting MBP was elevated to 80–90 mmHg by the continuous intravenous infusion of phenylephrine (10 $\mu g\,kg^{-1}\,min^{-1}$).

Drugs

Sodium pentobarbitone (Sagatal) was supplied by May and Baker, Dagenham, Essex. (−)-Phenylephrine hydrochloride (doses quoted as base), PGD_2 and indomethacin were obtained from Sigma Chemical Co., Poole, Dorset. BW A868C [(±)-3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)hydantoin); BW 245C (5-(6-carboxyhexyl)-1-(3-cyclohexyl-3-hydroxypropyl)hydantoin]; BM 13.177 (4-[2-(benzyene sulphonamido)ethyl]-phenoxyacetic acid) and BW A4C (N-(3-phenoxycinnamyl)-acetohydroxamic acid) were synthesized in the Department of Medicinal Chemistry, Wellcome Research Laboratories, Beckenham.

Sagatal was diluted to 10 mg ml^{-1} with 0.9% w/v sodium chloride solution (saline) before use. PGD_2, BW A868C, BW 245C and BM 13.177 were each dissolved as stock solutions (10 mg ml^{-1}; 4°C) in absolute ethanol (BDH) which was evaporated off under N_2 before being diluted with 1.25% w/v $NaHCO_3$ solution when required. Solutions of indomethacin (10 mg ml^{-1}) were prepared daily in 1 M Tris buffer pH 8.5. BW A4C was administered p.o. as a ball-milled (12 h) suspension (20 mg ml^{-1}) in celacol 0.25% w/v. Doses of antagonists and inhibitors used were chosen from literature precedent (Stegmeier *et al.*, 1984; Payne *et al.*, 1988; Hamid-Bloomfield & Whittle, 1989).

Statistical analysis

Results are presented as the mean ± s.e.mean, where n is the number of values. The statistical difference between groups was evaluated by Student's t test for paired or unpaired data as appropriate. $P < 0.05$ was taken to be statistically significant.

Results

Effect of intravenous PGD_2

Bolus i.v. injection of PGD_2 (5–160 $\mu g\,kg^{-1}$) induced a dose-dependent rise in MBP and HR (Figure 1). These effects were accompanied by a dose-related, but transient small monophasic increase in PIP up to a maximum of 5.5 ± 3.1 cmH_2O ($n = 5$) as shown in Figure 1.

Effect of PGD_2 aerosol

In contrast to the small bronchoconstrictor effect of i.v. PGD_2, inhaled PGD_2 (0.1–1 mg ml^{-1}, 30 s) provoked a

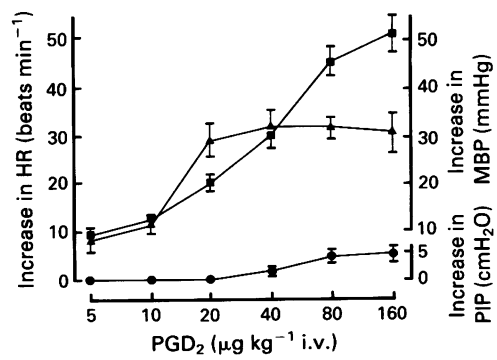

Figure 1 Effect of bolus i.v. administration of prostaglandin D_2 (PGD_2, 5–160 $\mu g\,kg^{-1}$) on systemic mean arterial blood pressure (MBP, ■), heart rate (HR, ▲) and pulmonary inflation pressure (PIP, ●) in anaesthetized guinea-pigs. Each point represents the mean result from 5 animals, each of which received all doses of PGD_2 in sequentially increasing increments, vertical lines indicate s.e.mean.

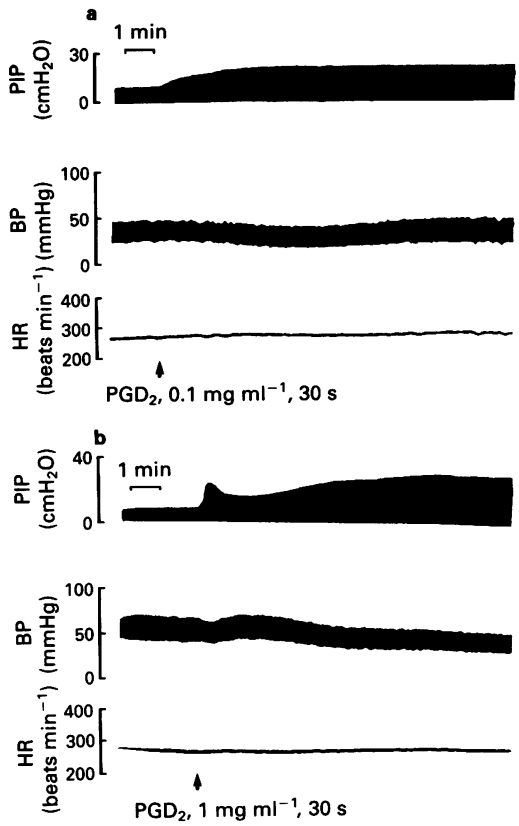

Figure 2 Tracing of original records showing representative effect in separate experiments of inhaled prostaglandin D_2 (PGD_2) 0.1 mg ml^{-1} (a) and 1 mg ml^{-1} (b), administered for 30 s, on pulmonary inflation pressure (PIP), systemic arterial blood pressure (BP) and heart rate (HR) in anaesthetized guinea-pigs.

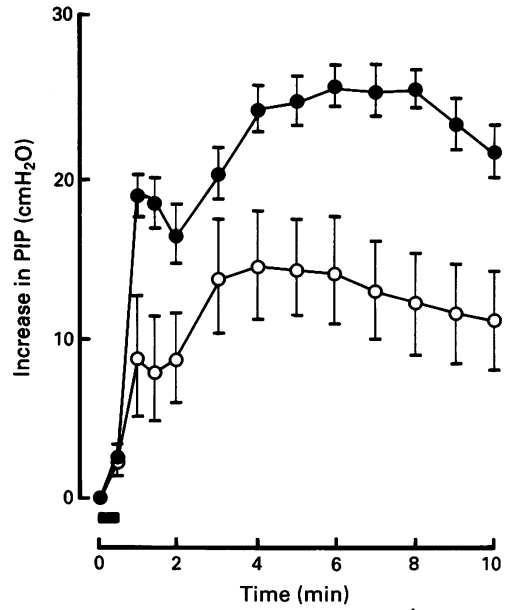

Figure 3 Time course of increase in pulmonary inflation pressure (PIP) provoked by inhaled prostaglandin D_2 (PGD_2) 0.1 mg ml^{-1} (○, $n = 5$) and 1 mg ml^{-1} (●, $n = 5$) in anaesthetized guinea-pigs. The 30 s duration of the PGD_2 inhalation is represented by the solid horizontal bar adjacent to the abscissa scale. Each point represents the mean result at each time interval after the commencement of the administration of PGD_2 aerosol, vertical lines indicate s.e.mean.

marked concentration-related rise in PIP (Figure 2) with minor cardiovascular effects (rise in MBP of 1.6 ± 1 mmHg and HR of 10 ± 5 beats min^{-1}, $n = 5$). Invariably with the higher and in some instances with the lower PGD_2 concentration, this rise in PIP was biphasic. As shown in Figure 3, there was a rapid transient initial increase in PIP of up to 19.3 $\pm$ 1.0 cmH_2O ($n = 5$), which peaked approximately 1 min after the administration of the PGD_2 aerosol had begun. This was followed by a more-slowly developing secondary and thereafter sustained increase which peaked approximately 5 min later, resulting in a total rise in PIP of up to 26.4 $\pm$ 1.4 cmH_2O from the initial baseline.

In view of the more marked airway effects of PGD_2 by the inhaled route as compared to the i.v. route, inhaled prostanoid was employed in further studies to evaluate the pharmacological mechanism of this bronchoconstriction.

Effects of the DP-receptor antagonist, BW A868C

Pretreatment with BW A868C (0.1 mg kg^{-1} i.v.) had only a marginal effect on the bronchoconstrictor effect of inhaled PGD_2 (1 mg ml^{-1}, 30 s) over the 10 min observation period, there being no significant reduction in the peak increase in PIP during either the first or second phase of the response to PGD_2 (Figure 4).

As the dose of inhaled PGD_2 used in the above studies was near-maximal, further experiments were conducted with a lower concentration of inhaled PGD_2 (0.1 mg ml^{-1}). However, BW A868C (0.1 mg kg^{-1} i.v.) also had no significant effect on the bronchoconstrictor response to this concentration of inhaled PGD_2 (Table 1). In further experiments, a higher dose of BW A868C (1 mg kg^{-1}) was also used, yet it did not significantly inhibit the bronchoconstriction induced by the low concentration of inhaled PGD_2 (Figure 5).

To exclude the possibility that the route of administration of PGD_2 could obscure the actions of BW A868C, its effect on the small bronchoconstriction induced by intravenously-administered PGD_2 was also evaluated. However, BW A868C (0.1 mg kg^{-1} i.v.) failed to reduce significantly the increase in PIP induced by i.v. PGD_2 (40 μg kg^{-1}) in 3 experiments (data

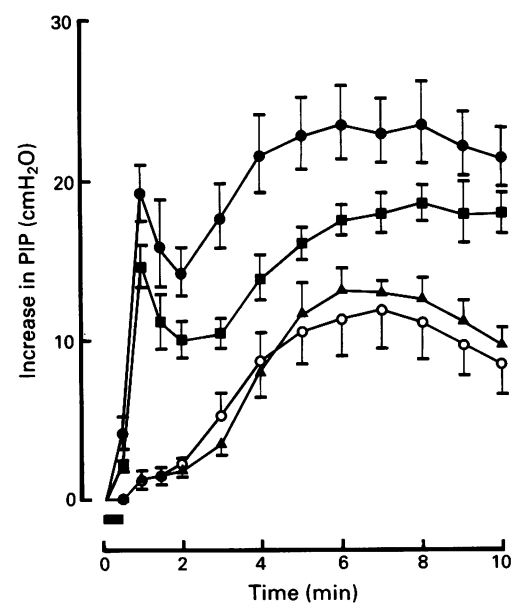

Figure 4 Effect of i.v. pretreatment with either BW A868C or BM 13.177, alone and in combination, on the rise in pulmonary inflation pressure (PIP) provoked by inhaled prostaglandin D_2 (PGD_2, 1 mg ml^{-1}) in anaesthetized guinea-pigs. Control PGD_2 responses alone (●, $n = 5$), following BW A868C 0.1 mg kg^{-1} (■, $n = 5$), BM 13.177 2.5 mg kg^{-1} (▲, $n = 5$) and BW A868C 0.1 mg kg^{-1} plus BM 13.177 2.5 mg kg^{-1} (○, $n = 5$). The 30 s duration of the PGD_2 inhalation is represented by the solid horizontal bar adjacent to the abscissa scale. Each point represents the mean result at each time interval after commencement of the administration of PGD_2 aerosol, vertical lines indicate s.e.mean.

Table 1 Effect of BW A868C and BM 13.177 (alone or in combination) on bronchoconstriction induced by inhaled prostaglandin D_2 (PGD_2, 0.1 mg ml^{-1}, 30 s) in anaesthetized guinea pigs

	PIP (% control)	(n)
PGD_2 (1 mg ml^{-1}) + BW A868C (0.1 mg kg^{-1})	80.0 ± 5.6	(5)
+ BM 13.177 (2.5 mg kg^{-1})	56.1 ± 4.7*	(5)
+ BM 13.177 + BW A868C (2.5 mg kg^{-1} + 0.1 mg kg^{-1})	53.1 ± 5.0*	(5)
PGD_2 (0.1 mg ml^{-1}) + BW A868C (0.1 mg kg^{-1})	135.4 ± 16.1	(5)
+ BW A868C (1 mg kg^{-1})	113.0 ± 24.8	(5)
+BM 13.177 (2.5 mg kg^{-1})	33.5 ± 15.5*	(5)
+BM 13.177 + BW A868C (2.5 mg kg^{-1} + 1 mg kg^{-1})	31.0 ± 10.6*	(5)

Values for peak changes in pulmonary inflation pressure (PIP) in each pretreatment group are expressed as mean ± s.e.mean percentages of the respective control response to inhaled PGD_2 in (*n*) experiments, where * $P < 0.01$ versus control for untransformed data.

not shown). Furthermore, in the same experiments BW A868C also failed to reduce significantly the rise in MBP provoked by i.v. PGD_2.

Effects of BW A868C on the hypotensive actions of BW 245C

In order to confirm that DP-receptor antagonism could be achieved following administration of these doses of BW A868C, its effects on the hypotensive actions of the DP-receptor agonist, BW 245C were studied. However, to demonstrate vasodilatation in the anaesthetized guinea-pig, it was necessary to induce a stable elevated level of MBP (80–90 mmHg) by the continuous i.v. infusion of phenylephrine (10 μg kg^{-1} min^{-1}). Under these conditions, bolus i.v. administration of BW 245C (1–3 μg kg^{-1}) induced a dose-related fall in MBP, yet had no significant action on PIP ($n = 3$). The fall in MBP (14 ± 4 mmHg, $n = 3$) induced by BW 245C (3 μg kg^{-1} i.v.) was significantly ($P < 0.05$) reduced to 1.3 ± 1.6 mmHg ($n = 3$) and 0.3 ± 0.1 mmHg ($n = 3$) by pretreatment with BW A868C 0.1 and 1 mg kg^{-1} i.v., respectively. Neither dose of BW A868C itself significantly altered the phenylephrine-elevated MBP.

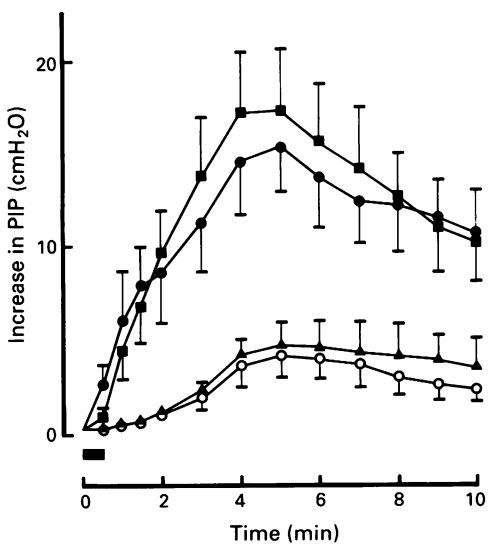

Figure 5 Effect of i.v. pretreatment with either BW A868C or BM 13.177, alone and in combination, on the rise in pulmonary inflation pressure (PIP) provoked by inhaled prostaglandin D_2 (PGD_2, 0.1 mg ml^{-1}) in anaesthetized guinea-pigs. Control PGD_2 responses alone (●, $n = 5$), following BW A868C 1 mg kg^{-1} (■, $n = 5$), BM 13.177 2.5 mg kg^{-1} (▲, $n = 5$) and BW A868C 1 mg kg^{-1} plus BM 13.177 2.5 mg kg^{-1} (○, $n = 5$). The 30 s duration of the PGD_2 inhalation is represented by the solid horizontal bar adjacent to the abscissa scale. Each point represents the mean result at each time interval after commencement of the administration of PGD_2 aerosol, vertical lines indicate s.e.mean.

Effects of the TP-receptor antagonist, BM 13.177

Pretreatment with BM 13.177 (2.5 mg kg^{-1} i.v.) abolished the first phase of the bronchoconstrictor effect of inhaled PGD_2 (1 mg ml^{-1}) and caused a significant reduction (44 ± 5%, $n = 5$) of the second phase (Figure 4). Intravenous administration of a higher dose of BM 13.177 (5 mg kg^{-1} i.v.) induced no greater degree of inhibition (48 ± 7%, $n = 4$) of the increase in PIP induced by PGD_2. In further studies, BM 13.177 (2.5 mg kg^{-1} i.v.) induced a significant reduction (67 ± 16%, $n = 5$) in the bronchoconstrictor response to the lower concentration of inhaled PGD_2 (0.1 mg ml^{-1}), as shown in Figure 5.

Effects of BW A868C and BM 13.177 in combination

Administration of a combination of BW A868C (0.1 mg kg^{-1} i.v.) and BM 13.177 (2.5 mg kg^{-1} i.v.) caused no further attenuation of the bronchoconstriction induced by PGD_2 (1 mg ml^{-1}) than did BM 13.177 (2.5 mg kg^{-1} i.v.) alone (Figure 4). Similarly, when administered in combination, a higher dose of BW A868C (1 mg kg^{-1} i.v.) and BM 13.177 (2.5 mg kg^{-1} i.v.) caused no further attenuation of PGD_2-induced bronchoconstriction than did BM 13.177 (2.5 mg kg^{-1} i.v.) alone. The effects of BW A868C and BM 13.177, administered either alone or in combination, on the peak rise in PIP produced by PGD_2 (0.1–1 mg ml^{-1}, 30 s) aerosol are summarized in Table 1.

Effect of bilateral vagotomy

Transection of both cervical vagi had no significant effect on the bronchoconstriction provoked by inhaled PGD_2 (0.1 mg ml^{-1}, 30 s) in five experiments, as shown in Table 2.

Effect of indomethacin or BW A4C

To investigate the possibility that the secondary release of other eicosanoids contributed to the bronchoconstrictor action of inhaled PGD_2, the effects of pretreatment with either the cyclo-oxygenase inhibitor indomethacin (10 mg kg^{-1} i.v.) or the selective 5-lipoxygenase inhibitor BW

Table 2 Effect of bilateral vagotomy, indomethacin or BW A4C on bronchoconstriction induced by inhaled PGD_2 (0.1 mg ml^{-1}) in anaesthetized guinea-pigs

	PIP (% control)	(n)
Bilateral vagotomy	110.7 ± 27.2	(5)
Indomethacin (10 mg kg^{-1} i.v.)	118.4 ± 28.8	(5)
BW A4C (50 mg kg^{-1} p.o.)	77.4 ± 29.1	(5)

Values for peak changes in pulmonary inflation pressure (PIP) in each pretreatment group are expressed as mean ± s.e.mean percentages of the respective control response to inhaled PGD_2 in (*n*) experiments. There was no significant difference from control values in any of these groups.

A4C (50 mg kg^{-1} p.o.) were investigated ($n = 5$ for each). At these doses, neither indomethacin nor BW A4C had a significant effect on the bronchoconstriction induced by PGD_2 (0.1 mg ml^{-1}) as shown in Table 2.

Discussion

There is increasing interest in the putative role of PGD_2 in asthma. However, the relative importance of this prostanoid compared with the many other potential mediators of asthma such as leukotrienes or platelet-activating factor, and hence the possible therapeutic value of selective antagonists of the actions of PGD_2, is still uncertain. This uncertainty is compounded by the ability of PGD_2 to interact with several types of prostaglandin receptors including the postulated DP- and TP-receptors (Jones *et al.*, 1982; Hamid-Bloomfield & Whittle, 1986; Giles & Leff, 1988). Thus, the airway effects of PGD_2 *in vitro* and *in vivo* may result from multiple prostanoid-receptor interactions (Eglen & Whiting, 1988; McKeniff *et al.*, 1988; Beasley *et al.*, 1989; Coleman & Sheldrick, 1989). In this respect, selective receptor antagonists such as the novel DP-receptor antagonist BW A868C are valuable tools in defining the contribution of different prostanoid receptors to the bronchial actions of PGD_2. The primary aim of the present study was therefore to investigate the effect of BW A868C, alone or in combination with the TP-receptor antagonist, BM 13.177, on PGD_2-induced bronchoconstriction *in vivo* in anaesthetized guinea-pigs.

In common with many other bronchoactive agents, the effects of prostanoids on pulmonary mechanics are known to differ depending on the route of administration (Spannhake *et al.*, 1981). For this reason, we initially compared the bronchoconstrictor effects of intravenous and inhaled PGD_2. By the intravenous route, PGD_2 at doses of up to 160 μg kg^{-1} provoked only a small rise in PIP, with more prominent actions being exerted on the cardiovascular system with increased arterial BP and heart rate. These findings confirm preliminary results on the intravenous potency of PGD_2 as a bronchoconstrictor in the guinea-pig (Coleman *et al.*, 1981). The weak bronchoconstrictor action of intravenous PGD_2 in the guinea-pig contrasts to its relatively potent action by this route in the anaesthetized dog (Wasserman *et al.*, 1977; Spannhake *et al.*, 1978), but parallels the low activity of intravenous PGD_2 on lung function in man (Heavey *et al.*, 1984).

In the present study, inhaled PGD_2 had a marked bronchoconstrictor effect in guinea-pigs, as previously found in man (Hardy *et al.*, 1984) and in the rhesus monkey (Patterson *et al.*, 1980). Furthermore, the bronchoconstrictor action of inhaled PGD_2 was accompanied by minimal cardiovascular effects. The difference in potency between inhaled and intravenous PGD_2 may reflect access to different prostanoid receptors, different mechanisms of disposition or metabolism or simply the higher local concentration of PGD_2 within the airways achieved by the inhaled route. The bronchoconstrictor response to inhaled PGD_2 was clearly biphasic at the higher (1 mg ml^{-1}) and less so at the lower (0.1 mg ml^{-1}) concentration of this prostanoid. This phenomenon may represent a temporal dissociation between spasmogenic (airway smooth muscle contraction) and non-spasmogenic (inflammatory) effects of inhaled PGD_2. Non-spasmogenic effects such as lung oedema would also contribute to reduced airway calibre, and thus to an increased PIP, whilst reinforcing a direct spasmogenic action of PGD_2.

Intravenous administration of the selective DP receptor antagonist BW A868C was without significant effect on either phase of bronchoconstriction induced by inhaled PGD_2. Furthermore, BW A868C failed to antagonise the bronchoconstrictor actions of PGD_2 administered by the intravenous route, suggesting that this is not simply a consequence of a problem of distribution or pharmacokinetics with this agent. The maximum dose of BW A868C (1 mg kg^{-1} i.v.) used in the present study was ten fold in excess of that causing a substantial inhibition of the vasodepressor actions of PGD_2 and the DP-receptor agonist, BW 245C in anaesthetized rats (Hamid-Bloomfield & Whittle, 1989). Furthermore, in the present studies in the guinea-pig, these doses of BW A868C abolished the hypotensive actions of BW 245C that could be demonstrated under conditions of elevated resting BP, indicating that adequate systemic levels of BW A868C to antagonize DP-receptors, at least in the vasculature, could be achieved. The lack of effect of BW A868C against PGD_2-induced bronchoconstriction *in vivo* is consistent with the previously demonstrated failure of this antagonist to modify PGD_2-induced contraction of guinea-pig trachea *in vitro* (Giles *et al.*, 1989). It has also been found that the mixed EP_1/DP-receptor antagonist AH 6809 does not reduce the contractile effect of PGD_2 in this tissue *in vitro* (Eglen & Whiting, 1988).

The near-maximal inhibition of the first phase of the bronchoconstrictor effect of inhaled PGD_2 by the intravenous administration of BM 13.177 suggests strongly that this phase is mediated almost entirely through TP-receptor activation. BM 13.177 also substantially but not completely suppressed the second phase of PGD_2-induced bronchoconstriction, suggesting that this phase is also mediated predominantly through TP-receptor activation. Furthermore, co-administration of BW A86AC with BM 13.177 did not increase the degree of inhibition of either phase, suggesting that there was no interaction between TP and DP receptors in this response to PGD_2. However, the possibility cannot be totally excluded that DP-receptor subtypes may exist and if so, a DP-receptor may be involved in PGD_2-induced bronchoconstriction that is not susceptible to BW A868C-blockade. Such a receptor may be of the type tentatively identified as mediating the vasoconstrictor effect of PGD_2 in the sheep (Jones, 1976), contrasting with that mediating the inhibition of platelet aggregation or relaxation of vascular smooth muscle by this prostanoid. However, it is clear from the present study, that any such DP receptors could only play a minor role in the response to inhaled PGD_2.

Apart from prolonged non-spasmogenic actions, the extended duration of the secondary phase of bronchoconstriction to inhaled PGD_2 may also result from the conversion of PGD_2 to 9α,11β-PGF_2, the principal metabolite of PGD_2 in guinea-pigs and man, that has similar bronchoconstrictor potency to PGD_2 (Bacon *et al.*, 1987; Beasley *et al.*, 1987; Robinson *et al.*, 1985). Indeed in human isolated airways, the bronchoconstrictor effects of 9α,11β-PGF_2 have been demonstrated to be mediated predominantly through TP-receptors (Coleman & Sheldrick, 1989).

Whilst the TP-receptor antagonist BM 13.177, alone or in combination with BW A868C, substantially reduced the bronchoconstrictor effect of inhaled PGD_2, it did not produce complete blockade, leaving a residual component that did not appear to be mediated either by TP- or DP-receptors. Likewise, the contractile effect of PGD_2 in guinea-pig isolated trachea is only partly inhibited by the TP-receptor antagonist EP-045 (Jones *et al.*, 1982). Consequently, we have also investigated the possible contribution of indirect mechanisms to PGD_2-induced bronchoconstriction, especially since the bronchoconstrictor effect of inhaled PGD_2 in asthmatics has been suggested to be partially mediated by cholinergic mechanisms (Beasley *et al.*, 1987). However, we found no evidence for such a mechanism in the present study in the guinea-pig since bilateral vagotomy did not reduce PGD_2-induced bronchoconstriction. Similarly, we obtained no evidence for a role of secondary release of cyclo-oxygenase or lipoxygenase metabolites of arachidonic acid as neither indomethacin nor the selective 5-lipoxygenase inhibitor BW A4C inhibited the bronchoconstrictor effect of inhaled PGD_2. This negative effect of indomethacin does, however, illustrate that the TP-receptor mediated component of the bronchoconstrictor action of PGD_2 resulted from a direct interaction of PGD_2 with this receptor rather than via an indirect action through the release of endogenous TXA_2.

The results of the present *in vivo* study with BW A868C and BM 13.177 thus indicate that the bronchoconstrictor effect of inhaled PGD_2 in guinea-pigs does not involve DP-receptors, but is mediated primarily through direct TP-receptor activation by this prostanoid. Recent studies on human isolated bronchial smooth muscle and also in asthmatic subjects suggest that it is also unlikely that DP-receptors contribute substantially to the bronchoconstrictor effect of PGD_2 in man since these responses were attenuated by the specific TP-receptor antagonists, AH 23848 and GR32191 respectively (Coleman & Sheldrick, 1989; Beasley *et al.*, 1989), although this awaits direct confirmation with selective DP receptor antagonists. However, the possibility remains that DP-receptors could mediate other airway actions of PGD_2, such as those that may contribute to airway hyperreactivity (Fuller *et al.*, 1986).

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