Br. J. Pharmacol. (1989), **96**, 688–692

Prostanoid-induced contraction of human bronchial smooth muscle is mediated by TP-receptors

[1] R.A. Coleman & R.L.G. Sheldrick

Peripheral Pharmacology Department, Glaxo Group Research Ltd, Ware, Herts SG12 0DJ

1 A range of naturally-occurring prostaglandins sulprostone, 16,16-dimethyl prostaglandin E_2 (DME_2) and the thromboxane A_2 (TXA_2)-mimetic, 11α,9α-epoxymethano prostaglandin H_2 (U-46619) have been tested for contractile agonist activity on human isolated bronchial smooth muscle.

2 Prostaglandin D_2 (PGD_2), $PGF_{2\alpha}$, 9α,11β-PGF_2 (11β-PGF_2) and U-46619 all caused concentration-related contractions. U46619 was at least 300 fold more potent than the other prostanoids with a mean EC_{50} of 12 nM. Sulprostone caused contraction only at the highest concentration tested (30 μM). PGE_2 and PGI_2 caused relaxations at low concentrations, and only caused contractile responses at high concentrations ($\geqslant$ 10 μM). In contrast, DME_2 caused small contractions at low concentrations but relaxation at the highest concentration tested (30 μM).

3 The rank order of contractile agonist potency was: U-46619 $\gg$ 11β-PGF_2 $\simeq$ $PGF_{2\alpha}$ > PGD_2 > PGE_2 > PGI_2 $\simeq$ sulprostone $\simeq$ DME_2.

4 The TP-receptor blocking drug, AH23848 (1 μM) antagonized the contractile effects of U-46619, PGD_2, $PGF_{2\alpha}$ and 11β-PGF_2, but had no effect against contractions to carbachol. In a single experiment, a pA_2 of 8.3 (slope = 1.2) was obtained for AH23848 against U-46619.

5 In most preparations, administration of AH23848 (1 μM) to human bronchus resulted in small, transient contractile responses.

6 The results obtained with both the agonists and the antagonist, AH23848 are therefore consistent with prostanoid-induced contractions of human bronchial smooth muscle being mediated by TP-receptors.

Introduction

Since Mathé and his colleagues (Mathe' *et al.*, 1973) demonstrated that asthmatics may be up to 8,000 times more sensitive than non-asthmatics to the bronchoconstrictor effects of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), there has been an interest in the possible role of prostanoids as causative factors in bronchial asthma. It is now well known that human isolated bronchial smooth muscle can contract in response to prostanoids (Strandberg & Hedqvist, 1977; Karim *et al.*, 1980; Black *et al.*, 1986; Seibert *et al.*, 1987), and that not only $PGF_{2\alpha}$, but also PGD_2 and the principle metabolite of PGD_2, 9α,11β-PGF_2(11β-PGF_2), all cause bronchoconstriction in man (Robinson *et al.*, 1987). A preliminary investigation into the nature of the receptors mediating this effect has recently been reported by McKenniff *et al.* (1988), who speculated that contraction of human isolated bronchus may be mediated by a novel prostanoid receptor.

In the present study, therefore, we have evaluated the constrictor effects of a range of prostanoids on human isolated bronchial smooth muscle. We have also investigated the interaction of some of these agonists with the TP-receptor blocking drug, AH23848 (Brittain *et al.*, 1985). From the results of these studies, some conclusions have been drawn as to the nature of the receptors mediating prostanoid-induced bronchoconstriction in man.

Methods

Tissue preparation

Samples of human bronchus were obtained from patients undergoing surgical resection of the lung. Bronchial tissue was dissected clear of lung par-

[1] Author for correspondence.

enchyma and vascular tissue, and stored overnight in modified Krebs solution at 20°C. The composition of the Krebs solution was as described by Apperley *et al.* (1976). Bronchus of lumen diameter 3–4 mm was cut into rings of 3–4 mm width, which were then opened to form strips. These strips were mounted in organ baths and bathed in Krebs solution at 37°C, containing indomethacin (2.8 μM) and bubbled with 5% CO_2 in O_2. A resting tension of 1 g was applied to each preparation.

Experimental design and evaluation of agonist and antagonist potency

In all agonist studies, control cumulative concentration-effect curves to the standard, the thromboxane A_2 mimetic U-46619 (Coleman *et al.*, 1981), were repeated at approximately 90 min intervals until constant responses were obtained, after which a similar curve was constructed to another agonist. Agonist potency is expressed as equipotent molar concentration (EPC, U-46619 = 1), defined as:

$$\frac{EC_{50} \text{ test agonist}}{EC_{50} \text{ U-46619}}$$

In antagonist studies, agonist cumulative concentration-effect curves were repeated at approximately 90 min intervals until constant responses were obtained, and then a single concentration of AH23848 (1 μM) was added to the bathing solution before a further agonist concentration-effect curve was constructed. An antagonist contact time of between 30 and 45 min was used. Antagonist potency is expressed as concentration-ratio (CR), defined as:

$$\frac{\text{agonist } EC_{50} \text{ in presence of antagonist}}{\text{agonist } EC_{50} \text{ in absence of antagonist}}$$

In a single experiment, a pA_2 value for AH23848 against U-46619 was determined by the method of Arunlakshana & Schild (1959).

Drugs used

The following drugs were used: [1α(Z),2β,5α]-(±)-7-[5-[[(1,1'-biphenyl)-4-yl]methoxy]-2-(4-morpholinyl)-3-oxocyclopentyl]-4-heptenoic acid, calcium salt (AH23848, Glaxo Group Research); carbamylcholine chloride (carbachol, BDH); indomethacin (Sigma); PGD_2 (Glaxo Group Research); PGE_2 (Upjohn); $PGF_{2\alpha}$ tromethamine (Upjohn); PGI_2 Na salt (Glaxo Group Research); 11β-PGF_2 (Glaxo Group Research); 11α,9α-epoxymethano PGH_2 (U-46619, Glaxo Group Research); sulprostone (Glaxo Group Research) and 16,16-dimethyl PGE_2 (DME_2, Glaxo Group Research). Carbachol was dissolved in 0.9% w/v NaCl solution (saline), indomethacin, PGD_2, PGE_2, 11β-PGF_2, DME_2 and U-46619 were all dissolved in 1.0% $NaHCO_3$ in saline, sulprostone was dissolved in 3% EtOH/0.01% Tween 80 in saline, AH23848 was dissolved in 6% EtOH/0.01% Tween 80 in saline. All dilutions were made in saline. PGI_2 was dissolved in Tris/HCl buffer pH 9.0 on the day of the experiment, and dilutions made in Tris/HCl buffer pH 8.0 immediately before use.

Results

Agonist studies

PGD_2 (0.1–30 μM), $PGF_{2\alpha}$ (0.1–30 μM), 11β-PGF_2 (0.1–30 μM) and U-46619 (0.001–0.3 μM) all caused concentration-related contractions of human bronchial strips, while sulprostone caused a contraction at the highest concentration tested (30 μM) only. In contrast, low concentrations of PGE_2 (0.01–1.0 μM) and PGI_2 (0.1–10 μM) caused concentration-related relaxation, higher concentrations (PGE_2, 10–30 μM and PGI_2, 30 μM) causing contractions. In a single experiment, DME_2 caused small contractions at 1.0–10 μM and relaxation at 30 μM. U-46619 was the most potent agonist, with a mean EC_{50} of 12 nM (95% confidence limits = 7–17, $n = 7$), determined from those experiments in which a maximum response was clearly established. Mean concentration-effect curves to the agonists tested are shown in Figure 1 and the contractile agonist potencies of the prostanoids, relative to U-46619, are summarised in Table 1.

Due to their relatively low potency, full contractile concentration-effect curves were only obtained in a proportion of experiments with 11β-PGF_2 ($n = 3/4$), $PGF_{2\alpha}$ ($n = 2/4$) and PGD_2 ($n = 2/4$), in the concentration-range tested. Thus the maximum

Table 1 Mean equipotent molar concentration (EPC) values (U-46619 = 1) for prostanoids in contracting human isolated bronchial smooth muscle

Agonist	*EPC*	(95% CL)	n
U-46619	1	–	21
11β-PGF_2	319	(47–2176)	4
$PGF_{2\alpha}$	383	(177–830)	4
PGD_2	628	(58–6804)	4
PGE_2	1260	(540–2930)	4
PGI_2	>2000	–	2
Sulprostone	9400–15000[a]	–	2
DME_2	>1000	–	1

[a] Range of EPC values.

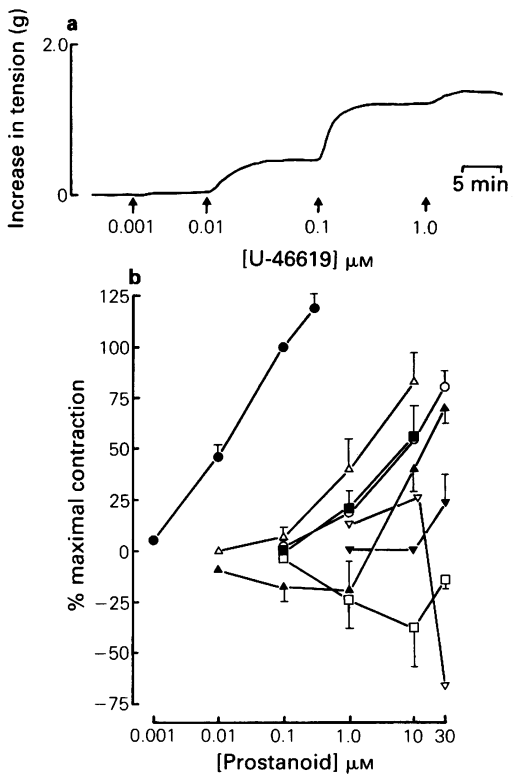

Figure 1 (a) Typical concentration-effect curve to U-46619 on human bronchus. (b) Mean concentration-effect curves to U-46619 (●), 11β-PGF_2 (△), PGD_2 (■), $PGF_{2\alpha}$ (○), PGE_2 (▲), PGI_2 (□), sulprostone (▼) and DME_2 (▽) on human bronchus. All responses were calculated as a percentage of the response obtained to U-46619 (0.1 μM) in the final control curve. Each point is the mean of 4 experiments except for U-46619 ($n = 21$), PGI_2 and sulprostone ($n = 2$) and DME_2 ($n = 1$). Vertical bars represent s.e.mean except for PGI_2 and sulprostone, where they represent range.

obtainable responses to $PGF_{2\alpha}$ and PGD_2 were consistently (7–25%) less than the corresponding maximum responses obtained to U-46619. In two of those experiments in which 11β-PGF_2 produced full concentration-effect curves, response maxima were identical to those to U-46619, whereas in the other experiment, the maximum was only 58% of the U-46619 maximum.

Antagonist studies

AH23848 (1 μM) was tested for its ability to inhibit contractile responses to U-46619, PGD_2, $PGF_{2\alpha}$, 11β-PGF_2 and carbachol. In some experiments (15/20), addition of AH23848 (1 μM) caused modest, transient contractile responses. The mean amplitude of these contractions was 53 (±13) mg as compared with 791 (±181) mg obtained with U-46619 (0.1 μM). In all cases, the AH23848-induced contraction had disappeared within 3–8 min, despite the continued presence of the drug. AH23848 caused parallel rightward shifts of curves to U-46619, with a mean concentration-ratio (CR) (95% confidence limits) of 313 (109–897, $n = 8$), consistent with a pA_2 value of 8.5 as determined by the Gaddum equation (Gaddum, 1957). In a single experiment, AH23848 at concentrations of 0.03, 0.3 and 3.0 μM caused parallel, rightward shifts of concentration-effect curves to U-46619 resulting in CRs of 11, 150 and 2600 respectively. From these data a pA_2 value of 8.3 (slope = 1.2) was calculated. In all experiments in the presence of AH23848, contractions to $PGF_{2\alpha}$ ($n = 2$), PGD_2 ($n = 3$) and 11β-PGF_2 ($n = 3$) were small or absent even at the highest concentration tested, such that accurate CR values could not be determined. In the presence of AH23848, $PGF_{2\alpha}$ at concentrations of 10–100 μM actually caused small relaxations. In contrast, curves to carbachol were unaffected by AH23848 (1 μM), giving a mean CR of 1.5 (0.4–6.3, $n = 4$). Mean concentration-effect curves to U-46619, $PGF_{2\alpha}$, 11β-PGF_2 and carbachol in the absence and presence of AH23848 (1 μM) are illustrated in Figure 2.

Discussion

In the present study, we have confirmed the findings of Seibert *et al.* (1987) who showed that 11β-PGF_2 contracts human isolated bronchial smooth muscle, and that its potency is similar to that observed with $PGF_{2\alpha}$ and PGD_2. We have also demonstrated that the rank order of prostanoid contractile agonist potency on human bronchial smooth muscle is similar to that previously reported in preparations containing TP-receptors, such as guinea-pig lung and rat aorta (Kennedy *et al.*, 1982), with U-46619 being at least two orders of magnitude more potent than PGD_2, PGE_2, $PGF_{2\alpha}$ and PGI_2. In support of this, we have shown that AH23848 specifically inhibits the contractile agonist actions of U-46619, PGD_2, $PGF_{2\alpha}$ and 11β-PGF_2, suggesting not only that human bronchial smooth muscle contains TP-receptors, but that all of these prostanoids exert their contractile activities through this receptor. Furthermore, the similarity in the pA_2 value determined for AH23848 against U-46619 on human bronchus with those previously obtained on blood platelets and vascular smooth muscle from a range of species (Brittain *et al.*, 1985), suggests that the TP-receptors in all of these tissues are of the same type. The weak contractile actions of PGE_2, DME_2, sulprostone

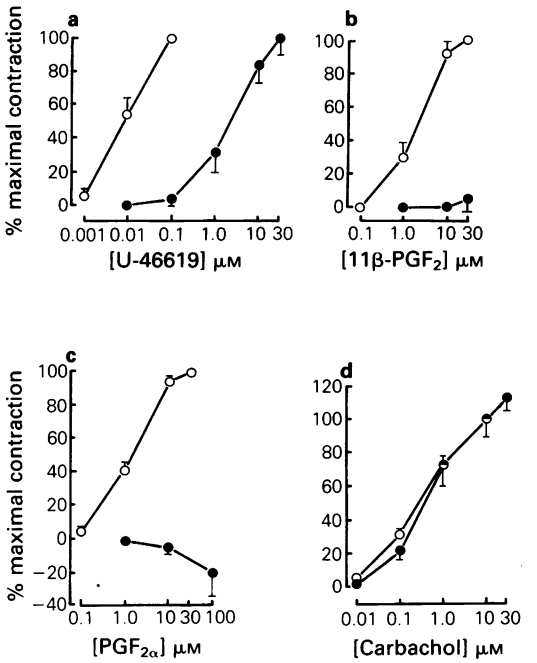

Figure 2 Mean concentration-effect curve to (a) U-46619, (b) 11β-PGF_2, (c) $PGF_{2\alpha}$, and (d) carbachol in the absence (○) and presence (●) of AH23848 (1 μM) on human bronchus. The ordinate scale shows % contraction, 100% being defined as the response in the absence of AH23848 to (a) U-46619 (0.1 μM), (b) 11β-PGF_2 (30 μM), (c) $PGF_{2\alpha}$ (30 μM) and (d) carbachol (10 μM). All responses are expressed as a percentage of this 100% response. Each point is the mean of 6–7 (a), 3 (b), 2 (c), 4 (d) experiments. Vertical bars represent s.e.mean (U-46619 and carbachol) or range (11β-PGF_2 and $PGF_{2\alpha}$).

and PGI_2 are presumably also mediated by TP-receptors, but to date AH23848 has not been tested against any of these prostanoids. Transient contractile agonist actions of AH23848, like those observed in the present study, have previously been reported in other TP-receptor containing tissues, such as human pulmonary vascular preparations and dog saphenous vein (Brittain *et al.*, 1985). It is believed that this activity results from a partial agonist action of AH23848 at TP-receptors (Humphrey *et al.*, 1986).

The results in this study differ from those obtained in a limited study by McKenniff and her colleagues (McKenniff *et al.*, 1988). They found DME_2 to be a potent contractile agonist which was not antagonized by either the TP-receptor blocking drugs, AH23848 and EP092, or the EP_1-receptor blocking drug, AH6809. These results are consistent with the presence of contractile EP-receptors which are insensitive to AH6809 (i.e. EP_3-receptors, Coleman *et al.*, 1987a). However, we have found that DME_2 is only a very weak contractile agonist on human bronchus. Furthermore, the potent EP_1- and EP_3-receptor selective agonist, sulprostone (Coleman *et al.*, 1987a), is also a very weak contractile agonist, being approximately 1000 times less potent than U-46619. We have therefore been unable to confirm McKenniff's observations with DME_2 and have found no evidence for the presence of either EP_1- or EP_3-receptors mediating the contractile effects of prostanoids in this tissue. Our results with the TP-receptor blocking drug AH23848 are also in disagreement with those obtained by McKenniff. In our experiments, AH23848 is a potent TP-receptor blocking drug, whereas McKenniff found AH23848 to be at least an order of magnitude weaker. A possible explanation for the differences in the results obtained with AH23848 and DME_2 in the two studies may be related to the part of the bronchial tree from which the tissue was taken. In our studies, we have used bronchus of lumen diameter 3–4 mm, whereas McKenniff used bronchus of much larger diameter, between 6–10 mm, presumably from higher up the bronchial tree. Whether receptor differences exist in the prostanoid receptor populations at different levels of the bronchial tree or whether there is some other reason for the differences in the results from the two studies remains to be determined.

In addition to TP-receptors mediating contraction, human bronchial smooth muscle also contains prostanoid receptors mediating relaxation since PGE_2, PGI_2 and DME_2 all caused relaxant responses. Indeed, in the presence of TP-receptor blockade with AH23848, even $PGF_{2\alpha}$ sometimes caused relaxation. An action of PGD_2 and $PGF_{2\alpha}$ at relaxant receptors in human bronchus, coupled with their relatively low potency at TP-receptors may explain the apparent inability of these prostanoids to elicit maximal contractile responses equal to those to U-46619. The identity of such relaxant receptors cannot be determined from the present study. However, other evidence is consistent with these receptors being similar to those mediating relaxation of tracheal smooth muscle from guinea-pig and cat (Gardiner, 1986), which we have previously classified as EP_2-receptors (Coleman *et al.*, 1987b).

In conclusion, the present results suggest that if, as we believe, prostanoids play a role in asthmatic bronchoconstriction, this effect will be mediated by TP-receptors. If so, such bronchoconstriction will be inhibited by TP-receptor blocking drugs like AH23848, irrespective of the particular type of prostanoid concerned. AH23848 and other such drugs should not only serve as tools to determine the extent to which prostanoids contribute to the pathogenesis of bronchial asthma, but may also represent valuable drugs in the treatment of this disease.

References

APPERLEY, E., HUMPHREY, P.P.A. & LEVY, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. *Br. J. Pharmacol.*, **58**, 211–221.

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

BLACK, J.L., ARMOUR, C.L., VINCENC, K.S. & JOHNSON, P.R.A. (1986). A comparison of the contractile activity of PGD_2 and $PGF_{2\alpha}$ on human isolated bronchus. *Prostaglandins*, **32**, 25–31.

BRITTAIN, R.T., BOUTAL, L., CARTER, M.C., COLEMAN, R.A., COLLINGTON, E.W., GEISOW, H.P., HALLETT, P., HORNBY, E.J., HUMPHREY, P.P.A., JACK, D., KENNEDY, I., LUMLEY, P., McCABE, P.J., SKIDMORE, I.F., THOMAS, M. & WALLIS, C.J. (1985). AH23848: a thromboxane receptor-blocking drug that can clarify the pathophysiologic role of thromboxane A_2. *Circulation*, **72**, 1208–1218.

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 prostaglandin H_2 and thromboxane A_2 on some isolated smooth muscle preparations. *Br. J. Pharmacol.*, **73**, 773–778.

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

COLEMAN, R.A., KENNEDY, I. & SHELDRICK, R.L.G. (1987b). New evidence with selective agonists and antagonists for the subclassification of PGE_2-sensitive (EP) receptors. *Adv. Prostaglandin Thromboxane Leukotr. Res.*, **17**, 467–470.

GADDUM, J.H. (1957). Theories of drug antagonism. *Pharmacol. Rev.*, **9**, 211–218.

GARDINER, P.J. (1986). Characterisation of prostanoid relaxant/inhibitory receptors (ψ) using a highly selective agonist, TR4979. *Br. J. Pharmacol.*, **87**, 45–56.

HUMPHREY, P.P.A., LUMLEY, P. & WHITE, B.P. (1986). The agonist action of AH23848 at guinea-pig vascular and airway smooth muscle TP-receptors *in vivo*. *Br. J. Pharmacol.*, **89**, 820P.

KARIM, S.M.M., ADAIKAN, P.G. & KOTTEGODA, S.R. (1980). Prostaglandins and human respiratory tract smooth muscle: structure activity relationship. *Adv. Prostaglandin Thromboxane Res.*, **7**, 969–980.

KENNEDY, I., COLEMAN, R.A., HUMPHREY, P.P.A., LEVY, G.P. & LUMLEY, P. (1982). Studies on the characterisation of prostanoid receptors: a proposed classification. *Prostaglandins*, **24**, 667–689.

MATHÉ, A.A., HEDQVIST, P., HOLMGREN, A. & SVANBORG, N. (1973). Bronchial hyperreactivity to prostaglandin $F_{2\alpha}$ and histamine in patients with asthma. *Br. Med. J.*, **1**, 193–196.

McKENNIFF, M.G., GARDINER, P.J., NORMAN, P. & RODGER, I.W. (1988). Is there a novel contractile prostanoid receptor in human large airways? *Br. J. Pharmacol.*, **93**, 56P.

ROBINSON, C., BEASLEY, C.R.W., VARLEY, J.G. & HOLGATE, S.T. (1987). Effects of inhaled 9α,11β-Prostaglandin F_2 on airway function in man. *Adv. Prostaglandin Thromboxane Leukotr. Res.*, **17**, 1057–1061.

SEIBERT, K., SHELLER, J.R. & ROBERTS, L.J. (1987). (5z,13e)-(15s)-9α,11β,15-Trihydroxyprosta-5,13-dien-1-oic acid (9α,11β-prostaglandin F_2): formation and metabolism by human lung and contractile effects on human bronchial smooth muscle. *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 256–260.

STRANDBERG, K. & HEDQVIST, P. (1977). Bronchial effects of some prostaglandin E and F analogues. *Acta Physiol. Scand.*, **100**, 172–181.

(Received April 4, 1988
Revised August 23, 1988
Accepted October 24, 1988)