

Effect of inhaled prostaglandin E₂ on bronchial reactivity to sodium metabisulphite and methacholine in patients with asthma

I D Pavord, A Wisniewski, R Mathur, I Wahedna, A J Knox, A E Tattersfield

Abstract

Inhaled frusemide protects against the bronchoconstrictor response to a wide range of stimuli that cause bronchoconstriction by indirect mechanisms. One possible explanation for this protection relates to the known ability of frusemide to enhance synthesis of prostaglandin E₂ (PGE₂). Studies in vitro suggest that PGE₂ might protect against indirectly acting bronchoconstrictor challenges rather than those that act directly on airway smooth muscle, though little is known about the effects of PGE₂ in vivo. The effect of inhaled PGE₂ on the bronchoconstrictor response to inhaled sodium metabisulphite (a stimulus with an indirect action) and methacholine (which acts directly on airway smooth muscle) was studied in nine patients with asthma. Subjects were studied on four days, inhaling PGE₂ (100 µg) or placebo in a double blind fashion followed immediately by a cumulative dose challenge with sodium metabisulphite or methacholine. The response to the constrictor stimuli was measured as the provocative dose causing a 20% fall in FEV₁ (PD₂₀). There was no significant change in FEV₁ after inhaled PGE₂ compared with placebo, nor any significant change in the response to methacholine; the geometric mean methacholine PD₂₀ was 0.9 µmol after PGE₂ and 0.56 µmol after placebo (mean difference 0.7 (95% confidence limits -0.1, 1.5) doubling doses). PGE₂, however, protected against sodium metabisulphite, the geometric mean sodium metabisulphite PD₂₀ being 11.8 µmol after PGE₂ and 1.8 µmol after placebo (mean difference 2.5 (95% CL 1.9, 3.1) doubling doses). PGE₂ conferred significantly greater protection against sodium metabisulphite than methacholine (mean difference 1.8 (95% CL 0.8, 2.8) doubling doses). This suggests that PGE₂, like frusemide, has an inhibitory effect on pathways relevant to the bronchoconstriction induced by sodium metabisulphite, with little or no effect on those relevant to methacholine.

The recent finding that inhaled frusemide protects subjects with asthma against the bronchoconstrictor response to stimuli that act indirectly but not directly on airway

smooth muscle has aroused much interest.¹⁻⁵ The effects of frusemide in asthma include protection against stimuli that are thought to cause bronchoconstriction primarily through mast cell mediator release (the early response to allergen,² adenosine,³ and osmolar challenges⁴) and through neural pathways (sodium metabisulphite⁵). In addition, frusemide protects against the late response to allergen,² which is thought to be related to inflammatory events. Any potential explanation for the effects of frusemide must take into account this wide range of action. One possible explanation relates to the known ability of frusemide to stimulate production of prostaglandin E₂ (PGE₂).^{6,7} This hypothesis assumes that PGE₂ is produced in the airway in response to frusemide and that PGE₂ will protect against bronchoconstrictor stimuli that act indirectly but not directly on airway smooth muscle.

There is some circumstantial support for the first assumption. Frusemide has been shown to stimulate release of PGE₂ from renal tubular epithelium⁶ and PGE₂ is a cyclo-oxygenase metabolite of human airway epithelium,⁸ smooth muscle,⁹ alveolar macrophages,¹⁰ and eosinophils.¹¹ Studies in vitro support our second assumption. Although PGE₂ under most circumstances acts as a weak contractile agonist of human airway smooth muscle^{9,12,13} and has no effect on histamine induced contraction,⁹ its effects on other cells are largely inhibitory. These include inhibition of mast cell mediator release,¹⁴ neurally induced airway smooth muscle contraction,¹⁵ and inflammatory cell activation.^{16,17} Thus any protective role PGE₂ may serve in the airway in vivo would be likely to be against indirectly acting bronchoconstrictor challenges rather than directly acting airway smooth muscle spasmogens. Studies in vivo are limited, however. There is indirect evidence that endogenous inhibitory prostaglandin production is responsible for the refractory period commonly observed after exercise¹⁸ and osmolar challenge,¹⁹ and inhaled PGE₂ has been shown to inhibit the bronchoconstrictor response to exercise, allergen, and ultrasonically distilled water in a few subjects with asthma.^{20,21} To test our hypothesis that PGE₂ protects against constrictor stimuli that act indirectly but not those acting directly we have compared the ability of inhaled PGE₂ to protect against methacholine, which acts directly on airway smooth muscle, and sodium

metabisulphite, which is thought to cause bronchoconstriction indirectly via an effect on neural pathways.

Methods

SUBJECTS

We studied nine men, aged 18–52 years, with mild asthma requiring only inhaled drugs. Six were taking regular inhaled corticosteroids (beclomethasone 200–1500 µg daily) and all used an inhaled beta₂ agonist as required (table 1). Eight subjects were atopic and one was a current smoker; all had a forced expiratory volume in one second (FEV₁) above 70% predicted (mean 91%). Bronchodilator medication was withheld for at least six hours before each visit. Subjects gave signed consent to participation in the study, which was approved by the City Hospital ethics committee.

MEASUREMENTS

FEV₁ was measured on a dry bellows spirometer (Vitalograph, Buckingham) and the higher of two successive readings within 100 ml was recorded. Sodium metabisulphite challenge was performed by a method based on that described by Nichol *et al.*²² Serial dilutions, over the range 0.6–160 mg/ml, were made up in normal saline each day. Aerosols were delivered from a nebuliser attached to a breath actuated dosimeter (MEFAR, Brescia, Italy); the nebuliser was set to nebulise for one second with a pause of six seconds at a pressure of 22 lb/in² (152 kPa) and delivered 6.5 µl/puff. Subjects inhaled doubling doses (0.03–64 µmol) of sodium metabisulphite by inspiring rapidly from functional residual capacity to total lung capacity, holding their breath for three seconds and exhaling slowly for three seconds. FEV₁ was measured two minutes after each inhalation. The challenge was discontinued when the FEV₁ had fallen by 20% or more, or when subjects had inhaled the highest cumulative dose of sodium metabisulphite (128 µmol). After completion of the challenge subjects were asked to score the irritancy of the sodium metabisulphite challenge on a nine point scale from 1 (not irritant) to 9 (severely irritant).

Methacholine challenge was performed by a similar method. Serial dilutions of metha-

choline (Sigma, Poole) were made up in normal saline over the range 0.39–25 mg/ml. Doubling doses (0.02–5.12 µmol) were administered via the breath actuated dosimeter every two minutes as in the metabisulphite challenge, except that the output was 10 µl per puff. FEV₁ was measured two minutes after each inhalation. In the main study the starting dose of methacholine and sodium metabisulphite was four doubling doses below the provocative dose causing a 20% fall in FEV₁ (PD₂₀) at an initial assessment visit.

PROTOCOL

Subjects attended on four separate occasions at the same time of day. PGE₂ 100 µg (a dose that causes near maximum bronchodilatation in normal subjects²³) was made up from a concentrated stock solution of Prostin E₂ (UpJohn) diluted to 2 mg/ml in ethanol and further diluted in 4.95 ml normal saline on the day of the challenge. The placebo was 0.05 ml ethanol in 4.95 ml normal saline. Drugs were administered in random order and double blind via a Medix ultrasonic nebuliser (output 1 ml/minute), the subjects inhaling through a face mask at tidal volume until the nebuliser was dry. FEV₁ was measured before and immediately after inhalation. Because cough may occur during inhalation of PGE₂, drugs were administered by a second investigator in a room separate from the challenge laboratory; this investigator also asked the subjects after inhalation about side effects. The sodium metabisulphite or methacholine challenges proceeded immediately after inhalation of PGE₂ or placebo, with the FEV₁ value obtained after PGE₂ or placebo inhalation used as the baseline for the challenge study.

ANALYSIS

FEV₁ before and after inhalation of PGE₂ or placebo and change in FEV₁ after PGE₂ and placebo were compared within subjects by the paired *t* test.

Sodium metabisulphite and methacholine PD₂₀ values were calculated by linear interpolation of the log dose-response curve. When the fall in FEV₁ was less than 20% with the maximum cumulative dose of sodium metabisulphite (128 µmol) this value was assigned as the PD₂₀. The PD₂₀ values were log transformed for analysis and expressed as geometric

Table 1 Details of the subjects

Subject No	Age (y)	FEV ₁ (% pred)	Treatment	Metabisulphite PD ₂₀ (µmol)		Methacholine PD ₂₀ (µmol)	
				PGE ₂	Placebo	PGE ₂	Placebo
1	52	93	S	> 128.00	16.00	1.28	0.76
2	35	90	T, B	46.85	6.96	1.99	1.28
3	37	98	S	5.96	0.75	3.71	1.69
4	18	89	S, B	8.39	1.91	0.91	0.83
5	34	83	S, B	2.32	0.72	0.32	0.96
6	23	110	S, B	4.36	0.97	0.81	0.23
7	50	70	S, B	4.00	1.13	0.32	0.10
8	30	83	S, B	2.95	0.79	0.55	0.22
9	29	105	S	> 128.00	7.76	0.98	0.86
Mean	34	91					
Geometric mean				11.84	1.78	0.90	0.56

S—salbutamol; T—terbutaline; B—beclomethasone.

Table 2 Mean forced expiratory volume in one second (FEV₁) for sodium metabisulphite and methacholine challenges before and after inhalation of prostaglandin E₂ (PGE₂) or placebo: mean (95% confidence limits) within subject differences

Challenge	Inhalation	Mean FEV ₁		Mean difference (l) (95% CL)	Mean difference placebo v PGE ₂ (l) (95% CL)
		Before	After		
Sodium metabisulphite	PGE ₂	3.25	3.34	0.09 (-0.05, 0.24)	0.13 (-0.08, 0.34) p = 0.18
	Placebo	3.21	3.17	-0.04 (-0.11, 0.04)	
Methacholine	PGE ₂	3.14	3.27	0.13 (-0.02, 0.28)	0.14 (-0.01, 0.29) p = 0.06
	Placebo	3.19	3.18	-0.01 (-0.08, 0.06)	

mean values; the differences in PD₂₀ between PGE₂ and placebo for sodium metabisulphite and methacholine were expressed as doubling doses with 95% confidence limits (CL). PD₂₀, difference in PD₂₀, and irritancy scores for the sodium metabisulphite challenge were compared within subjects by the paired *t* test.

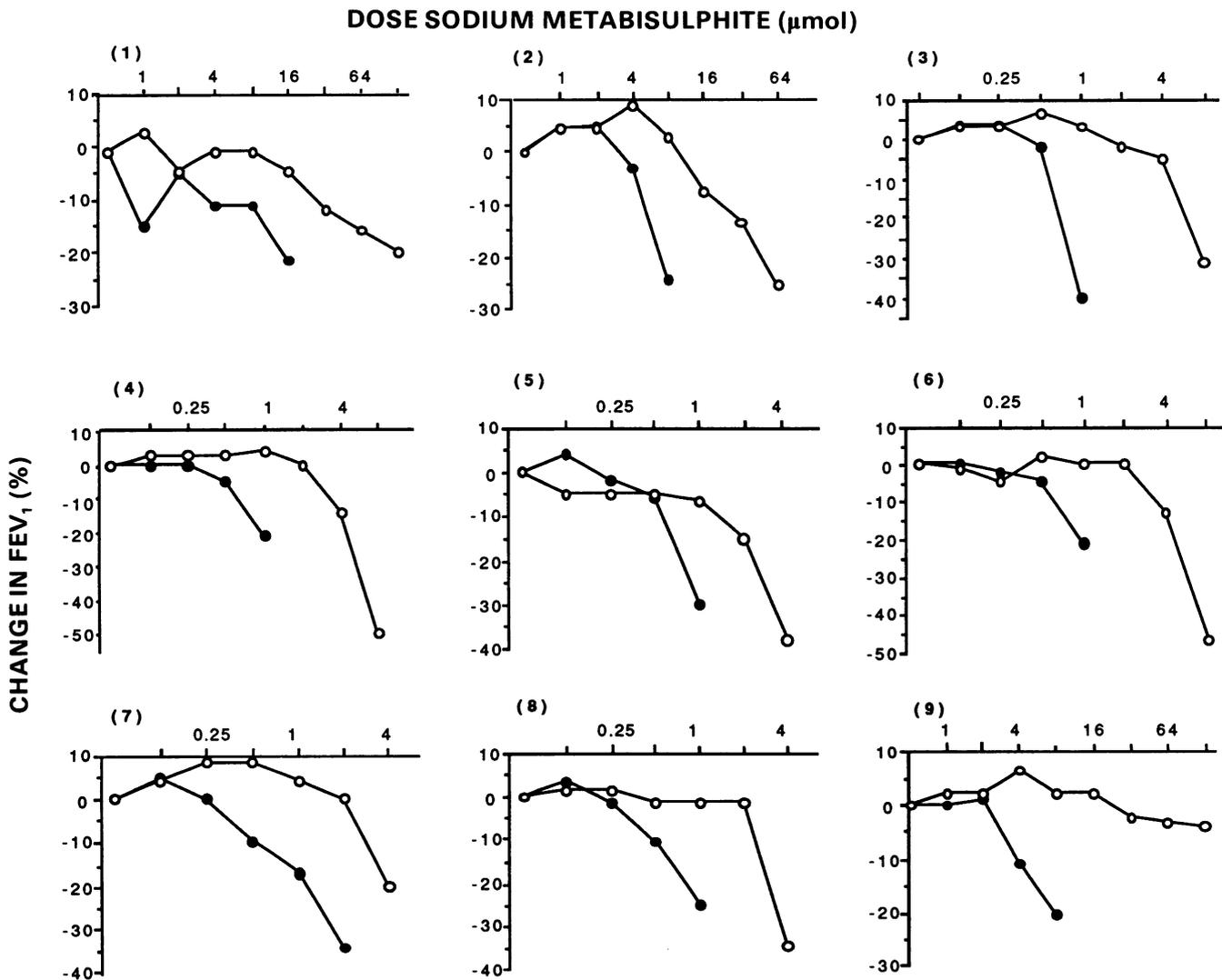
cough and retrosternal soreness in most subjects, though symptoms rapidly subsided as the inhalation proceeded. PGE₂ was otherwise well tolerated.

There was no significant difference in mean FEV₁ before and after inhaled PGE₂ and placebo on either the sodium metabisulphite or methacholine challenge days, nor did the mean change in FEV₁ after inhaled PGE₂ and placebo differ significantly on the two days (table 2).

Results

Inhalation of PGE₂ caused initial transient

The dose-response curve for sodium meta-



Individual cumulative dose-response curves for inhaled sodium metabisulphite after inhalation of placebo (closed circles) and prostaglandin E₂ (PGE₂; open circles).

bisulphite induced bronchoconstriction was displaced to the right in all subjects after inhaled PGE₂ by comparison with placebo. The difference in PD₂₀ ranged from 1.7 to 4 doubling doses (figure, table 1). The geometric mean PD₂₀ sodium metabisulphite was 1.8 μ mol after placebo inhalation and 11.8 μ mol after inhaled PGE₂ (mean difference 2.5 (95% CL 1.9, 3.1) doubling doses; $p < 0.001$). The irritancy score after completion of the metabisulphite challenge was similar after inhaled PGE₂ (mean 4.9) and placebo (mean 4.6).

The dose-response curve for methacholine was displaced to the right in eight of the nine subjects after inhaled PGE₂ (from 0.14 to 1.8 doubling doses); the mean change was not, however, significant. The geometric mean methacholine PD₂₀ was 0.56 μ mol after placebo and 0.9 μ mol after PGE₂, a mean difference of 0.7 (95% CL -0.1, 1.5) doubling doses; $p = 0.08$ —see table 1.

Inhaled PGE₂ conferred significantly greater protection against sodium metabisulphite than against methacholine challenge. The mean difference in PD₂₀ after PGE₂ and placebo was 1.8 (95% CL 0.8, 2.8) doubling doses greater for sodium metabisulphite than for methacholine ($p < 0.005$).

Discussion

PGE₂ provided considerable protection against sodium metabisulphite induced bronchoconstriction in these subjects with mild asthma and this protection was significantly greater than that afforded against methacholine challenge. There was a trend towards bronchodilatation and protection against methacholine after inhalation of PGE₂ in our subjects but neither change was significant. Possibly PGE₂ has a small effect against methacholine that would require more subjects to confirm it, but any effect was very much less than the effect seen against sodium metabisulphite.

PGE₂ is often regarded as a bronchodilator and has caused bronchodilatation consistently when inhaled by normal subjects.²³⁻²⁶ The bronchodilatation may be preceded by transient bronchoconstriction, which has been attributed to a direct contractile effect of PGE₂ on airway smooth muscle,²⁴ as is seen in vitro.^{9,12} The effect of inhaled PGE₂ in asthmatic subjects has been more variable. Smith *et al*²⁵ showed bronchodilatation after 55 μ g inhaled PGE₂ in the four subjects they studied. Mathe and Hedqvist,²⁶ however, showed no change in specific airway conductance over 15 minutes in eight subjects given inhaled PGE₂ in doses (6.25–100 μ g) that caused dose related bronchodilatation in normal subjects. A delayed bronchodilator response to PGE₂ could have occurred in our subjects had they not had a constrictor challenge. Sodium metabisulphite and methacholine were, however, given at the same time after PGE₂ inhalation and both challenges were of similar duration, so any effect of bronchodilatation as such would have affected the two challenges in a similar way.

The protection by PGE₂ against sodium metabisulphite is similar to that seen with

salbutamol 200 μ g²⁷ and considerably more than the protection recorded by others after inhaled sodium cromoglycate,²⁸ frusemide,⁵ or antimuscarinic agents.²² The greater protection against sodium metabisulphite (a stimulus that acts indirectly) than against methacholine (a stimulus that acts directly on airway smooth muscle) resembles the pattern of protection seen with sodium cromoglycate and frusemide. It appears to differ from that seen with beta₂ receptor agonists, where conventional doses have displaced the dose-response curves for methacholine and sodium metabisulphite to a similar degree, albeit in different studies.^{27,29} The difference in response to the two stimuli supports the suggestion that the effects of PGE₂ are indirect and not due to airway smooth muscle relaxation.

An inhibitory effect of PGE₂ on neural pathways is the most likely explanation for our findings. Sodium metabisulphite solutions appear to cause bronchoconstriction through release of sulphur dioxide, because this is released from sodium metabisulphite solutions in a dose dependent manner³⁰ and the response to the two agents is similar in time course and in the way it can be modified by drugs.^{22,30} Bronchoconstriction is thought to be neurally mediated,^{22,30,31} though inhaled antimuscarinic agents have only a weak protective effect, suggesting a role for non-adrenergic, non-cholinergic excitatory nerve pathways in addition to cholinergic pathways.²² The protection afforded by PGE₂ could be due to inhibition of the afferent or efferent limb of these neural pathways.

Irritancy scores after sodium metabisulphite inhalation were similar with inhaled PGE₂ and placebo, despite the larger inhaled dose of sodium metabisulphite on the PGE₂ day. This suggests that PGE₂ may have an inhibitory effect on sensory nerve endings. This is perhaps surprising given that PGE₂ causes cough when inhaled and potentiates cough induced by capsaicin.³² The cough response to inhaled PGE₂ becomes refractory with repeat doses,³² so cross refractoriness might occur between PGE₂ and sodium metabisulphite. An inhibitory effect of PGE₂ on efferent neural activity is suggested by studies showing that low concentrations of PGE₂ inhibit cholinergic contractions of airway smooth muscle stimulated by an electric field in vitro.^{15,33} A similar effect in vivo in man (which might also affect non-adrenergic, non-cholinergic pathways) would provide an attractive explanation for the protection afforded by PGE₂ against sodium metabisulphite induced bronchoconstriction. It would also provide a plausible explanation for the bronchodilatation observed after inhalation of PGE₂ in normal subjects and in some subjects with asthma.²³⁻²⁶ The more variable effects of inhaled PGE₂ on airway tone in subjects with asthma may be due to an exaggerated direct contractile effect of PGE₂ on airway smooth muscle.

PGE₂, like prostacyclin and PGE₁, is a vasodilator. Oral misoprostol (PGE₁)³⁴ and inhaled prostacyclin³⁵ have been shown to provide a small degree of protection (less than one doubling dose change in PD₂₀) against

bronchoconstriction induced by methacholine without altering airway tone. The vasodilatation produced by all three prostaglandins would be expected to increase bronchial blood flow and may increase clearance of inhaled spasmogens.³⁵ This could explain the small effect of the prostaglandins on methacholine induced bronchoconstriction but would not explain the difference in protection against the two stimuli seen in our study.

Thus our finding that inhaled PGE₂ confers considerably greater protection against the bronchoconstrictor response to inhaled sodium metabisulphite than against the airway smooth muscle spasmogen methacholine is consistent with PGE₂ having an indirect effect against neural pathways relevant to sodium metabisulphite induced bronchoconstriction. These data, together with those from earlier studies showing that inhaled PGE₂ protects subjects with asthma against the bronchoconstrictor response to exercise, ultrasonically nebulised distilled water, and allergen,^{20,21} suggest that PGE₂ is capable of modulating asthma induced by a wide range of stimuli that act indirectly. The data also support our hypothesis that the effects of frusemide in asthma are due to stimulation of endogenous production of PGE₂. The role of endogenously produced PGE₂ in modulating the response to indirect challenges and the importance of possible defects in this mechanism in asthma deserve further study.

We thank City Hospital Pharmacy for help with preparing the drugs and Mrs J Williams for help with recruitment. The advice of Dr J Britton is also much appreciated. IDP is supported by the Medical Research Council.

- Bianco S, Vaghi A, Robuschi M, Pasargiklian M. Prevention of exercise-induced bronchoconstriction by inhaled frusemide. *Lancet* 1988;ii:252-5.
- Bianco S, Pieroni MG, Refini RM, Rottoli L, Settini P. Protective effect of inhaled furosemide on allergen-induced early and late asthmatic reactions. *N Engl J Med* 1989;321:1069-70.
- Polosa R, Lau LCK, Holgate ST. Inhibition of adenosine 5'-monophosphate and methacholine-induced bronchoconstriction in asthma by inhaled frusemide. *Eur Respir J* 1990;3:665-72.
- Robuschi M, Gambard G, Spagnotto S, Vaghi A, Bianco S. Inhaled frusemide is highly effective in preventing ultrasonically nebulised water bronchoconstriction. *Pulmon Pharmacol* 1989;1:187-91.
- Nichol GM, Alton EFWF, Nix A, Geddes DM, Chung KF, Barnes PJ. Effect of inhaled furosemide on metabisulfite and methacholine-induced bronchoconstriction and nasal potentials in asthmatic subjects. *Am Rev Respir Dis* 1990;142:576-80.
- Miyanooshita A, Terada M, Endou H. Furosemide directly stimulates prostaglandin E₂ production in the thick ascending limb of Henle's loop. *J Pharmacol Exp Ther* 1989;251:1155-9.
- Johnston GD, Hiatt WR, Nies AS, Payne NA, Murphy RC, Gerber JG. Factors modifying the early non-diuretic vascular effects of frusemide in man. The possible role of renal prostaglandins. *Circ Res* 1983;53:630-5.
- Churchill L, Chilton FH, Resau JH, Bascom R, Hubbard WC, Proud D. Cyclooxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. *Am Rev Respir Dis* 1989;140:449-59.
- Haye-Legrand I, Cerrina J, Raffestin B, Labat C, Boulet C, Bayol A, et al. Histamine contractions of isolated human airway smooth muscle preparations: role of prostaglandins. *J Pharmacol Exp Ther* 1986;239:536-41.
- MacDermot J, Kelsey CR, Waddell KA, Richmond R, Knight RK, Cole PJ, et al. Synthesis of LTB₄ and prostanoids by human alveolar macrophages: analysis by gas chromatography/mass spectrometry. *Prostaglandins* 1984;27:163-79.
- Hubscher T. Role of the eosinophil in the allergic reactions II. Release of prostaglandins from human eosinophilic leukocytes. *J Immunol* 1975;114:1389-93.
- Gardiner PJ. Characterization of prostanoid relaxant/inhibitory receptors using a highly selective agonist TR4947. *Br J Pharmacol* 1986;1:45-56.
- Sweatman WJF, Collier HOJ. Effects of prostaglandins on human bronchial muscle. *Nature* 1968;217:69.
- Peters SP, Schulman ES, MacGlashan DW, Schleimer RP, Newball HH, Lichtenstein LM. Pharmacological and biochemical studies of human lung mast cells. *J Allergy Clin Immunol* 1982;69:150.
- Walters EH, O'Byrne PM, Fabbri LM, Graf PD, Holtzman MJ, Nadel JA. Control of neurotransmission by prostaglandins in canine trachealis smooth muscle. *J Appl Physiol* 1984;57:129-34.
- Giembycz MA, Kroegel C, Barnes PJ. Prostaglandin E₂ inhibits platelet activating factor-induced eosinophil activation [abstract]. *Am Rev Respir Dis* 1990;141(No 4 part 2):A396.
- Christman BW, Christman JW. Prostaglandin E₂ decreases leukotriene B₄ production by A23187-stimulated rat alveolar macrophages. Analysis by gas chromatography/mass spectrometry [abstract]. *Am Rev Respir Dis* 1990;141(No 4 part 2):A393.
- O'Byrne PM, Jones GL. The effect of indomethacin on exercise-induced bronchoconstriction and refractoriness after exercise. *Am Rev Respir Dis* 1986;134:69-72.
- Mattoli S, Foresi A, Corbo GM, Valente S, Ciappi G. The effect of indomethacin on the refractory period occurring after the inhalation of ultrasonically nebulised distilled water. *J Allergy Clin Immunol* 1987;79:678-83.
- Pasargiklian M, Bianco S, Allegra L, Moavero NE, Pettrigni G, Robuschi M, Grugni A. Aspects of bronchial reactivity to prostaglandins and aspirin in asthmatic patients. *Respiration* 1977;34:79-91.
- Pasargiklian M, Bianco S, Allegra L. Clinical, functional and pathogenetic aspects of bronchial reactivity to prostaglandins F_{2,3}, E₁, and E₂. *Adv Prost Thromb Res* 1976;1:461-75.
- Nichol GM, Nix A, Chung KF, Barnes PJ. Characterisation of bronchoconstrictor responses to sodium metabisulphite aerosol in atopic subjects with and without asthma. *Thorax* 1989;44:1009-14.
- Seth RV, Clarke VS, Lewis RA, Tattersfield AE. Effect of propranolol on the airway response to prostaglandin E₂ in normal man. *Br J Clin Pharmacol* 1981;12:731-5.
- Walters EH, Davies BH. Dual effects of prostaglandin E₂ on normal airways smooth muscle in vivo. *Thorax* 1982;37:918-22.
- Smith AP, Cuthbert MF, Dunlop LS. Effects of prostaglandins E₁, E₂, and F_{2,3} on the airway resistance of healthy and asthmatic man. *Clin Sci Mol Med* 1975;48:421-30.
- Mathe AA, Hedqvist P. Effect of prostaglandins F_{2,3} and E₂ on airway conductance in healthy subjects and asthmatic patients. *Am Rev Respir Dis* 1975;111:313-20.
- Dixon CMS, Ind PW. Prevention of metabisulphite induced bronchoconstriction by salbutamol [abstract]. *Thorax* 1989;44:868-9P.
- Dixon CMS, Ind PW. Inhaled sodium metabisulphite induced bronchoconstriction: inhibition by nedocromil sodium and sodium cromoglycate. *Br J Clin Pharmacol* 1990;30:371-6.
- Tattersfield AE. Beta agonists and anticholinergic drugs—effect on bronchial reactivity. *Am Rev Respir Dis* 1987;136:564-8.
- Wright W, Zhang YG, Salome CM, Woolcock AJ. Effect of inhaled preservatives on asthmatic subjects. I. Sodium metabisulfite. *Am Rev Respir Dis* 1990;141:1400-4.
- Nadel JA, Salem H, Tamplin B, Tokiwa Y. Mechanism of bronchoconstriction during inhalation of sulfur dioxide. *J Appl Physiol* 1965;20:164-7.
- Choudry NB, Fuller RW, Pride NB. Sensitivity of the human cough reflex: effect of inflammatory mediators prostaglandin E₂, bradykinin, and histamine. *Am Rev Respir Dis* 1989;140:137-41.
- Ito I, Suzuki H, Aizawa H, Hirose T, Hakoda H. Pre-junctional inhibitory action of prostaglandin E₂ on excitatory neuro-effector transmission in the human bronchus. *Prostaglandins* 1990;39:639-55.
- Manning PJ, Lane CG, O'Byrne PM. The effect of oral prostaglandin E₁ on airway responsiveness in asthmatic subjects. *Pulmon Pharmacol* 1989;2:121-4.
- Hardy CC, Bradding P, Robinson C, Holgate ST. Bronchoconstrictor and anti-bronchoconstrictor properties of inhaled procyclin in asthma. *J Appl Physiol* 1988;64:1567-74.