EFFECT OF PROPRANOLOL ON THE AIRWAY RESPONSE TO PROSTAGLANDIN E₂ IN NORMAL MAN

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- 1 The airway response to inhaled prostaglandin E₂ (PGE₂) and the effect of oral propranolol on this response was studied in eight normal subjects in a double-blind randomised trial. The airway response was measured as specific airway conductance (sGaw).
- 2 Inhalation of PGE₂ caused retrosternal soreness, coughing and an awareness of mucus production. Despite this, PGE₂ caused bronchodilatation and reproducible dose-response curves were obtained, with a maximum increase in sGaw of 53%.
- 3 Inhalation of the diluent of PGE₂, an ethanol/saline mixture, did not cause irritation nor did it alter sGaw.
- 4 Prior administration of propranolol 80 mg did not alter baseline sGaw, nor the response to PGE₂, indicating that the action of PGE₂ *in vivo* is unaffected by bronchial β -adrenoceptor blockade.
- 5 This technique should be of value in studying bronchodilator prostaglandins and their interaction with other drugs.

Introduction

Prostaglandin E₂ (PGE₂), one of several prostaglandins released by the lung, is found predominantly in the bronchi (Karim, Sandlers & Williams, 1967). Like PGE₁ it causes relaxation of isolated animal (Main, 1964) and human (Sweatman & Collier, 1968) airway smooth muscle and bronchodilatation in intact animals (Rosenthale *et al.*, 1970; Wasserman, Griffin & Marsalisi, 1980) and man (Smith, Cuthbert & Dunlop, 1975; Mathé & Hedqvist, 1975). Unfortunately, the irritant effect of both prostaglandins make them unsuitable for use in asthma (Herxheimer & Roetscher, 1971; Smith *et al.*, 1975), though it is not clear whether the irritant effect is related to the prostaglandins or to the ethanol/saline in which they are diluted. Interest in PGE₂ is related to its possible use therapeutically if the irritant effects can be overcome, and to the possible role of prostaglandins in asthma.

Since the action of PGE₁ and PGE₂ on the bronchial smooth muscle is unaffected by vagotomy, α - and β -adrenergic and ganglionic blockade (Rosenthale, Dervinis & Strike, 1976), prostaglandins are thought to act on distinct prostaglandin receptors. Prostaglandin radioligand receptor binding studies (Moore & Wolff, 1973; Rao, 1974) support this view

and suggest that these receptors are coupled to adenylate cyclase. Both PGE₁ and PGE₂ stimulate adenylate cyclase and increase intracellular cyclic-AMP levels in lung tissue (Butcher & Baird, 1968; Weinryb, Michel & Hess, 1973). This receptor is apparently separate from the β -receptor since in most tissues, including lung, neither PGE₁ nor PGE₂ induced smooth muscle relaxation is blocked by β -adrenoceptor blockade (Sheard, 1968; Large, Leswell & Maxwell, 1969; Adolphson & Townley, 1970; Rosenthale *et al.*, 1970). The situation in human airways may be more complicated however since prostaglandins have other actions which could affect airway calibre, in particular their interaction with the autonomic nervous system (Brody & Kadowitz, 1974; Nakanishi, Yoshida & Suzuki, 1976; Hedqvist, 1977). The effect of propranolol on PGE₂ induced bronchodilatation has not been assessed previously in man.

The study was designed to determine whether reproducible cumulative dose-response curves to PGE₂ could be obtained in normal subjects, to investigate the effects of the diluent ethanol/saline and to investigate the effect of propranolol on the airway response to PGE₂. Unlike asthmatic patients,

normal subjects have little if any resting sympathetic tone to their airways so propranolol can be given with safety and without any change in airway resistance (Richardson & Sterling, 1969; Tattersfield, Leaver & Pride, 1973).

Methods

Eight healthy, non-atopic male non-smokers aged 20–25 years took part after giving informed consent. The study was approved by the Southampton Ethical Committee.

Airway resistance and thoracic gas volume were measured in a constant volume body plethysmograph. Results were expressed as specific airway conductance (sGaw), which is airway conductance (Gaw), the reciprocal of airway resistance, divided by the thoracic gas volume at which measurements were made. sGaw should be relatively independent of any change in lung volume during the experiment since airway conductance and thoracic gas volume show a linear relationship in normal subjects. A mean value for sGaw was obtained from ten separate traces. Each set of ten traces was coded and read blind at a later date by the same observer. The subjects were not told which drugs were being administered.

Prostaglandin E_2 solutions were prepared from concentrated PGE_2 (Prostin 10 mg/ml) by double dilution in a normal saline/ethanol mixture, so that all solutions contained the same ratio of ethanol to saline. Ethanol was present in the concentrated stock solution to prevent deterioration. The concentrations ranged from 0.025 to 0.8 mg PGE_2 /ml. Solutions were nebulised in an Inspiron disposable nebuliser and inhaled at 20 breaths/min for 1 min at room temperature. The volume of solution lost from the nebuliser was assessed by weighing the nebuliser before and after inhalation. Solutions containing the same ratio of normal saline to ethanol were prepared for placebo inhalation studies.

Protocol

Each subject attended on six occasions with at least 1 week between visits. On the first visit the time course of response to PGE_2 was assessed by measuring sGaw before and at 5 min intervals after inhalation of 0.3 mg PGE_2 . On the five subsequent visits, cumulative dose-response studies were carried out with either doubling concentrations of PGE_2 or ethanol/saline placebo. Propranolol 80 mg or identical placebo tablets were ingested 90 min before the dose-response study. The effect of PGE_2 after placebo tablet was measured on two occasions to assess reproducibility. Thus, the following five drug combinations were taken in random order:

placebo tablet and PGE_2 inhalation

placebo tablet and PGE_2 inhalation
placebo tablet and ethanol/saline inhalation
propranolol 80 mg and PGE_2 inhalation
propranolol 80 mg and ethanol/saline inhalation.

On each occasion the subjects attended at the same time of day after a light breakfast, having avoided tea or coffee for 8 h. After 5 min rest, baseline sGaw was measured. The subject then took oral propranolol 80 mg or placebo tablet and 90 min later sGaw measurements were repeated. Six solutions of PGE_2 in doubling concentrations or ethanol/saline placebo were then inhaled at 15 min intervals; with sGaw measurements 15 min after each dose. Salbutamol 400 μ g was then administered from a metered aerosol and a final measurement of sGaw made 15 min later.

Dose-response curves were constructed by plotting the mean cumulative weight of nebulised prostaglandin against the mean change in sGaw from baseline (Δ sGaw). Wilcoxon's rank sum test was used to compare baseline sGaw values in each study, to compare baseline sGaw with sGaw after each dose of drug and to compare Δ sGaw at each dose of drug for the five treatment regimes.

Results

Restrosteral soreness, an awareness of mucus production and headache occurred in the majority of subjects following inhalation of PGE_2 . These symptoms were similar after both placebo and propranolol tablets. No side-effects were observed following ethanol/saline inhalation.

Time course (Figure 1)

Following 0.3 mg ($\pm$ 0.04 mg s.e. mean) inhaled PGE_2 , there was a 45% increase in sGaw from a mean baseline of $2.89 \text{ s}^{-1} \text{ kPa}^{-1}$. This peak effect occurred at 15 min and had decreased to 23% at 30 min.

Figure 1 Time course for inhaled PGE_2 . The points represent the mean change in sGaw ($\pm$ s.e. mean) for eight subjects after 0.3 mg PGE_2 .

Figure 2 Cumulative dose-response curves showing mean change in sGaw for eight subjects ($\pm$ s.e. mean) ●—● placebo tablet and PGE₂ inhalation ▲—▲ propranolol tablet and PGE₂ inhalation ○—○ placebo tablet and ethanol/saline inhalation △—△ propranolol tablet and ethanol/saline inhalation.

The response to inhalation of salbutamol 400 μ g at the end of each run is shown (arrowed).

For the ethanol/saline studies the points represent the same volume of ethanol/saline as in the PGE₂ study.

Dose-response studies (Figure 2, Table 2)

There was no significant difference in mean baseline sGaw in the five studies, nor between mean sGaw values before and 90 min after oral propranolol or placebo (Table 1). There was also no significant change in sGaw following ethanol/saline inhalation alone.

Following inhalation of PGE₂ there was a dose-dependent increase in sGaw which was significant for even the lowest concentration ($P < 0.05$). At the highest dose of PGE₂ the mean increase in sGaw was 53% of baseline and the response had reached a plateau. There was no significant difference in the response to each dose of PGE₂ in the two studies after placebo tablet. There was also no significant differ-

ence between these responses and those to PGE₂ following oral propranolol when there was also a 53% increase in sGaw.

Inhalation of 400 μ g salbutamol produced a 35% increase in sGaw ($P < 0.05$) after placebo tablet and ethanol/saline but no significant change after propranolol and ethanol/saline. There was no further change in sGaw when salbutamol was inhaled at the end of the PGE₂ dose-response studies.

Discussion

This study confirms the potency of PGE₂ as a bronchodilator in healthy man, but also confirms the irritant nature of the drug which has precluded its use in clinical practice. It was suggested from laryngeal nerve studies in the cat (Bergren, Vidruk & Sampson, 1979) that the irritant properties of PGE₁ and PGE₂ on the airways may be due to the ethanol solvent. A subsequent study in the dog (Wasserman *et al.*, 1980) showed some airway irritation after ethanol inhalation but also showed a dose-dependent irritant effect after inhalation of a water soluble salt of PGE₂. In our study ethanol/saline alone caused neither irritation nor any change in airway resistance, so we conclude that in normal subjects airway irritation is due to the prostaglandin.

This study was carried out in a double-blind manner though the irritant nature of inhaled PGE₂ meant that most subjects recognised the active drug. Despite some coughing after PGE₂ inhalation, reproducible dose-response curves were obtained. Prostaglandin E₂ was administered at 15 min intervals for the dose-response study on the basis of the results of both our time course study and previous studies (Smith *et al.*, 1975). At the highest dose of PGE₂ a plateau response was seen with no further increase in sGaw after 400 μ g inhaled salbutamol.

Propranolol 80 mg completely blocked the bronchodilator response to the inhaled beta-agonist salbutamol, but had no effect on the airway response to PGE₂. Thus, although both drugs stimulate intracellular adenylate cyclase and increase cyclic-AMP

Table 1 Mean $\pm$ s.e. mean baseline sGaw before and 90 min after placebo or propranolol tablet for each of the five studies. None of the differences in sGaw in the five studies nor the changes after propranolol or placebo were significant.

Tablet	Study Inhalation	Baseline sGaw ($s^{-1}kPa^{-1}$)	
		Before tablet	After tablet
Placebo	PGE ₂	2.64 (0.29)	2.89 (0.38)
Placebo	PGE ₂	2.74 (0.27)	2.86 (0.38)
Placebo	Ethanol/saline	2.51 (0.19)	2.67 (0.28)
Propranolol	PGE ₂	2.61 (0.20)	2.57 (0.17)
Propranolol	Ethanol/saline	2.68 (0.26)	2.59 (0.26)

Table 2 Mean $\pm$ s.e. mean change in sGaw from baseline (Δ sGaw) after each inhalation of PGE₂, ethanol/saline or salbutamol, plus significance values for the differences in Δ sGaw between the five studies.

Study	Tablet	Inhalation	$\Delta sGaw$ ($s^{-1}kPa^{-1}$)						Salbutamol 400 μg
			PGE ₂ or ethanol/saline inhalation number						
			1	2	3	4	5	6	
A	Placebo	PGE ₂	0.83 (0.19)	0.98 (0.17)	1.17 (0.15)	1.41 (0.19)	1.41 (0.22)	1.33 (0.18)	1.26 (0.34)
B	Placebo	PGE ₂	0.56 (0.17)	0.66 (0.19)	0.85 (0.21)	1.24 (0.19)	1.54 (0.33)	1.51 (0.29)	1.12 (0.22)
C	Placebo	Ethanol/saline	-0.03 (0.06)	0.05 (0.10)	0.09 (0.10)	0.14 (0.11)	0.05 (0.11)	0.10 (0.11)	1.06 (0.22)
D	Propranolol	PGE ₂	0.64 (0.13)	1.02 (0.20)	1.13 (0.24)	1.25 (0.17)	1.29 (0.23)	1.14 (0.27)	1.19 (0.23)
E	Propranolol	Ethanol/saline	0.10 (0.20)	0.07 (0.11)	0.20 (0.15)	0.06 (0.12)	0.22 (0.16)	0.26 (0.15)	0.36 (0.21)
Significance of difference (Wilcoxon's paired rank sum test)									
Comparison of studies									
A	v	B	NS	NS	NS	NS	NS	NS	NS
A	v	D	NS	NS	NS	NS	NS	NS	NS
B	v	D	NS	NS	NS	NS	NS	NS	NS
C	v	E	NS	NS	NS	NS	NS	NS	NS
A	v	C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
B	v	C	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	NS
D	v	E	<0.05	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

NS = not significant at 5% level. <0.01 and <0.05 = significant at the 1% and 5% level respectively.

levels (Butcher & Baird, 1968; Burges & Blackburn, 1972; Weinryb *et al.*, 1973) they appear to be doing so via separate receptors. Our results are similar to several *in vitro* studies where the effect of PGE₁ and PGE₂ on smooth muscle was unaltered by propranolol administration (Sheard, 1968; Large *et al.*, 1969; Adolphson & Townley, 1970; Rosenthale *et al.*, 1970).

Our findings are consistent with a direct action of PGE₂ on a prostaglandin receptor on bronchial smooth muscle, although other possibilities such as inhibition of cholinergic neurotransmission (Nakanishi *et al.*, 1976) are not excluded. Several studies in animals suggest that prostaglandins interact with the autonomic nervous system, prostaglandins of the E series usually inhibiting the release of noradrenaline or depressing the response to noradrenaline (Brody & Kadowitz, 1974; Hedqvist, 1977). We would not expect to detect any interaction with β -adrenoceptor agonist activity in this study, since normal subjects have little resting sympathetic stimulation to airways as judged by the lack of any significant change in sGaw after propranolol. In patients with asthma, where resting sympathetic activity to the airways may be important, interaction between PGE₂ and noradrenaline may be more pertinent. This would be difficult to confirm *in vivo*, since propranolol itself will alter baseline sGaw in asthmatic patients.

This study has shown that a cumulative technique can be used to provide airway dose-response curves for PGE₂ in normal subjects and that reproducible dose-response curves can be obtained. This technique in normal subjects has obvious potential for the study of other bronchodilator prostaglandins.

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