The action of prazosin and propylene glycol on methoxamine-induced bronchoconstriction in asthmatic subjects

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1   The effect of 1 mg inhaled prazosin on bronchoconstriction induced by methoxamine was investigated in seven asthmatic subjects.

2   Prazosin caused significant inhibition of the methoxamine-induced bronchoconstriction in six of the seven patients.

3   These findings suggest that methoxamine produces bronchoconstriction in asthmatic subjects via stimulation of α-adrenoceptors.

4   In previous studies propylene glycol has been used as a vehicle for delivery of prazosin. This substance was found to cause significant inhibition of methoxamine effects and to shift the dose response curve to histamine to the right in four of seven patients.

Keywords α-adrenoceptors   asthma   prazosin   methoxamine   propylene glycol

Introduction

The existence of α-adrenoceptors in human airways and their contribution to respiratory disease such as asthma remains controversial. We have previously reported (Black *et al.*, 1982) that methoxamine, an α-adrenoceptor agonist, induced bronchoconstriction in 10 asthmatic subjects. No such reaction occurred, however, in 10 subjects with no clinical history of asthma, although four of these exhibited bronchoconstriction in response to inhaled histamine.

α-adrenoceptor antagonists have been used in studies in asthmatics to determine whether a particular response, e.g. that induced by exercise, has an α-adrenoceptor-mediated component. The results of those studies have been conflicting (Sly *et al.*, 1967; Barnes *et al.*, 1981b) and this may reflect the nature of the α-adrenoceptor antagonists used. Phentolamine, an α-adrenoceptor antagonist which blocks both α_1- and α_2-adrenoceptors, is known to possess histamine H_1-receptor antagonist properties, releases catecholamines from the adrenal medulla and has been reported to cause relaxation of bronchial and vascular smooth muscle (Kerr *et al.*, 1970; Lish *et al.*, 1968;

Taylor *et al.*, 1965). Thus, on the basis of studies using antagonists such as phentolamine, it has been difficult to identify responses as being mediated by α-adrenoceptors.

Prazosin is a specific α_1-adrenoceptor antagonist which is remarkably free from activity other than at α-adrenoceptor sites (Cambridge *et al.*, 1977; Graham & Pettinger, 1979). This study reports the use of inhaled prazosin to antagonize bronchoconstrictor effects of methoxamine. Prazosin is not sufficiently soluble in water or saline and therefore we intended to use propylene glycol, a vehicle used by others for administration of prazosin (Barnes *et al.*, 1981b). During the course of the study it became apparent that propylene glycol was unsuitable for this purpose. The actions of propylene glycol on methoxamine and histamine-induced bronchoconstriction are therefore also reported.

Methods

We studied nine patients, seven female and two

male aged 24–72 years (mean ± s.d., 42 ± 14.9 years). The patients all had clinically recognized asthma and were taking aerosol β-adrenoceptor agonists regularly for control of their symptoms. None required oral steroid therapy. The protocol was approved by the Ethics Review Committee of Royal Prince Alfred Hospital and informed consent was obtained. The patients agreed to withhold all medications for 4–6 h prior to the challenge procedures. Details of the patients are given in Table 1.

Measurements of forced expiratory volume in 1 s (FEV_1) were made by means of a Vitalograph dry spirometer. Each measurement was repeated until values were reproducible within 200 ml and then the highest reproducible value was recorded.

Methoxamine challenge

This was carried out as previously described (Black *et al.*, 1982). Methoxamine hydrochloride was weighed each challenge day and dissolved in 0.9% w/v saline to give solutions of 10 mg/ml and 1 mg/ml. Two to three ml of these solutions, at room temperature, were placed in a de Vilbiss no. 646 nebulizer, connected via a nebulization dosimeter (Rosenthal-French, USA) to compressed air at 20 p.s.i. (138 kPa). The dosimeter was set to produce a 3-second delivery. Patients inhaled slowly from functional residual capacity to total lung capacity. One, three or five inhalations of the 1 mg/ml and then 10 mg/ml solutions were successively administered to the patient and the FEV_1 was measured 60 s later. The challenge was stopped when the FEV_1 had fallen by 20% from the prechallenge value, or the maximum cumulative dose of 32 μmol had been delivered. Any bronchoconstriction induced by inhalational challenge was reversed by means of two inhalations of salbutamol aerosol.

Antagonism of methoxamine responses

(i) *Propylene glycol studies* In order to investigate the effect of prazosin on methoxamine-induced bronchoconstriction, prazosin was dissolved in 20% aqueous propylene glycol as previously described (Barnes *et al.*, 1981b). In our initial control experiments, 2 ml of aqueous propylene glycol alone was administered via a Hudson nebulizer and face mask using tidal breathing. Spirometry was repeated 2–3 min later and then the methoxamine challenge carried out.

(ii) *Administration of prazosin via spinhaler* Prazosin (1 mg) was weighed and combined in a spin cap with 20 mg of lactose powder. The capsule was then inserted into a spinhaler and the contents administered to the patient over the course of 2–3 short inhalations. After 15 min (Anderson *et al.*, 1983), the FEV_1 was measured and the methoxamine challenge commenced. Blood pressure and pulse were measured immediately prior to and 15 min following prazosin administration.

Histamine inhalation challenge

Histamine acid phosphate was weighed and dissolved in 0.9% w/v saline to produce solutions of 0.625, 2.5 and 5.0% w/v. Histamine inhalation tests were carried out as described by Yan *et al.* (1983). Discrete doses of histamine solutions of increasing concentrations were administered via a hand-held de Vilbiss no. 40 nebulizer and the FEV_1 measured 60 s after each dose. Maximum cumulative dose administered was 3.9 μmol.

Analysis of results

The dose of methoxamine or histamine required to induce a 20% reduction in FEV_1 (PD_{20}) was determined by interpolation from a curve relating change in FEV_1 to the cumulative dose of agonist inhaled. Mean values for PD_{20} and 95% confidence limits were determined from log values. Paired *t*-tests performed on log-transformed data, were used to determine the significance of differences and these were considered significant when $P < 0.05$.

Table 1 Details of patients studied

Patient	Sex	Age (years)	Treatment
1	F	55	S, D
2	M	42	S
3	F	33	S, B
4	F	70	S, B, D
5	M	52	S, B, D
6	F	27	S, B
7	F	32	S, T, B
8	F	43	S
9	F	24	S, B, D

S Salbutamol
D Disodium cromoglycate
B Beclomethasone dipropionate
T Theophylline

Results

The nine patients visited the laboratory on two to five occasions. All nine patients responded with bronchoconstriction to methoxamine. The mean PD_{20} with 95% confidence limits (CL) for the group was 2.4 (CL 0.7–8.0) μmol. One patient had been challenged with methoxamine on six occasions over 12 months and the mean PD_{20} was 0.6 (CL 0.4–1.0) μmol.

When the 20% aqueous propylene glycol solution was administered to the first patient, prior to prazosin testing, it was found that a much larger concentration of methoxamine was necessary before bronchoconstriction occurred. A subsequent methoxamine test with no propylene glycol pretreatment indicated that propylene glycol had caused significant inhibition of the methoxamine-induced bronchoconstriction. Methoxamine inhalation tests were then repeated in a further six patients. In each case the PD_{20} for methoxamine after propylene glycol was greater than that in its absence. The mean PD_{20} for methoxamine following propylene glycol was 9.2 (CL 2.3–37) μmol and this was significantly different from that obtained without propylene glycol pretreatment—1.8 (CL 0.4–7.4) μmol. These values are shown in Table 2. To determine if this effect of propylene glycol was specific for methoxamine-induced bronchoconstriction, a histamine inhalation test with and without prior administration of propylene glycol was carried out in the same seven patients. In four of the seven patients the PD_{20} for histamine after propylene glycol was greater than that before, but the differences in PD_{20} for the group were not significant. These values are shown in Table 2. Prazosin 1 mg, combined with 20 mg of lactose powder was administered via a spinahaler to seven patients. There was no significant difference in the FEV_1 expressed as a percentage of predicted values prior to, or 15 min following prazosin. In six of seven patients the PD_{20} for methoxamine was greater after prazosin than in its absence, increasing from 2.9 (CL 1.1–7.7) to 10.1 (CL 3.3–31.2) μmol. This represents a significant difference for the group ($P = 0.008$), and these data are shown in Table 3. Prazosin did not produce any change in blood pressure measured 15 min after administration.

Discussion

There are two important observations from this study. Firstly, that response to methoxamine may be markedly attenuated when propylene glycol is used as a vehicle for prazosin. Secondly,

Table 2 Effect of propylene glycol (PG) on the dose of methoxamine and histamine (PD_{20}) producing a 20% fall in forced expiratory volume in 1 s (FEV_1) and the FEV_1 expressed as a percentage of predicted values (FEV_1 R%P)

Patient	Methoxamine		Methoxamine after PG		Histamine		Histamine after PG	
	FEV_1 R%P	PD_{20} (μmol)	FEV_1 R%P	PD_{20} (μmol)	FEV_1 R%P	PD_{20} (μmol)	FEV_1 R%P	PD_{20} (μmol)
1	67	0.37	73	18	69	0.03	67	0.14
2	76	1.1	74	4.6	58	0.08	63	0.15
3	64	3.3	76	30	76	0.27	71	0.27
4	93	5.6	76	>32	79	0.30	79	0.39
5	50	3.6	60	5.6	42	0.24	45	0.26
6	100	15	97	21	102	0.185	102	0.215
7	49	0.15	48	0.62	48	≪0.06	46	0.018
Mean	73.3†	1.79*	72†	9.2*	67.7‡	0.09§	67.6‡	0.16§
s.e. mean	7.5		5.7		7.7		7.4	

†, §, ‡ denote pairs of values which are not significantly different.
* These values are significantly different, $P = 0.01$, (paired *t*-tests performed after logarithmic transformation).

Table 3 Effect of prazosin on the dose of methoxamine (PD_{20}) producing a 20% fall in forced expiratory volume in 1 second (FEV_1) and the FEV_1 expressed as a percentage of predicted values (FEV_1 R%P)

| Patient | Methoxamine | | Methoxamine after prazosin | |
	FEV_1 R%P	PD_{20} (μmol)	FEV_1 R%P	PD_{20} (μmol)
1	81	0.72	93	3.3
2	76	1.1	70	1.1
3	64	3.3	64	9.5
4	93	5.6	82	30
5	50	3.6	40	24
7	105	15	112	19
9	88	2.4	97	23
Mean	79.6†	2.94*	79.7†	10.1*
s.e. mean	7.0		9.0	

† denotes values which are not significantly different.
* These values are significantly different, $P = 0.008$ (paired t-tests performed after logarithmic transformation).

when prazosin is administered by a spin cap directly into the airways, there is a significant inhibition of methoxamine-induced broncho-constriction. This provides direct evidence that α-adrenoceptors are present in the airways of our asthmatic patients. It is not clear why methoxamine-induced bronchoconstriction was not inhibited by prazosin in one of the subjects. The most likely explanation is that, although the patient received the contents of the spin cap, a larger than usual percentage of the powder passed into the gastrointestinal rather than respiratory tract and insufficient drug reached the active site.

It is unlikely that the effect of prazosin was due to direct bronchodilatation. Although Maril et al. (1982) found that 1 mg prazosin induced bronchodilatation in patients with asthma, prazosin was administered orally in their study, and was associated with hypotension. It is therefore likely that the small bronchodilator effect observed in their patients resulted from baroreflex sympathetic stimulation leading to bronchodilatation. In two studies in which prazosin was administered by inhalation (Barnes et al., 1981a, b) there was no evidence of a direct bronchodilator effect. The results of the present study in which there was no change in resting FEV_1 after inhaled prazosin would seem to support this finding. The use of the FEV_1 may not always be adequate for the detection of small direct effects on airway smooth muscle, however, the effect of prazosin in the study by Marlin et al. (1982) was detected by the FEV_1.

It is unlikely that the action of prazosin in inhibiting methoxamine-induced bronchocon-striction results from an effect other than that at α1-adrenoceptors. Prazosin is free of the pharmacological activity characteristic of other α-adrenoceptor antagonists such as histamine H1-receptor antagonism and inhibition of α2-adrenoceptors. In the present study we did not determine the effect of prazosin on histamine-induced bronchoconstriction, but others have investigated this using a higher dose of prazosin (Barnes et al., 1981b) and found no histamine H1-receptor antagonism.

On finding that propylene glycol was unsatisfactory as a vehicle for prazosin in our study we attempted to use an alternative diluent. Prazosin was dissolved in lactic acid 0.04 mol/l. However, when 2 ml of this solution were administered via a Hudson nebulizer and face mask to one of our patients, a 27% fall in FEV_1 from resting values was recorded 2–3 min later. It is likely that this effect was due to the low pH (approximately 4) of the lactic acid solution and this too was abandoned as a vehicle.

In the present investigation we did not use a placebo in determining the effect of prazosin on methoxamine-induced bronchoconstriction. Shifts in bronchoconstrictor dose response curves may result from suggestion (Luparello et al., 1968) although, in a study in which we suggested to our patients that inhibition of bronchoconstriction would occur, we failed to demonstrate this (unpublished). It is unlikely that the shift to the right in the methoxamine response curve resulted from the lactose present with prazosin in the spin cap. Indeed, lactose inhalation has been previously associated with bronchoconstriction (Tattersall, personal communication) on one occasion and

thus is unlikely to have caused bronchodilatation.

The results of our investigations with propylene are interesting. This allegedly inert vehicle caused shifts to the right of the dose response curves to both histamine and methoxamine. Barnes *et al.* (1981b) used propylene glycol as the diluent for prazosin to investigate the role of α-adrenoceptors in exercise-induced bronchospasm. In determining whether prazosin affected histamine-induced bronchoconstriction, they reported no significant differences between propylene glycol and prazosin dissolved in propylene glycol. However, a comparison between histamine provocation tests performed in the absence and presence of propylene glycol cannot be made in their study. In addition there is no information provided concerning the severity of the bronchoconstrictor response to exercise in the absence of propylene glycol.

The mechanism of action of propylene glycol is unclear. It is a surface wetting agent and may have coated the airway mucosa—thus preventing access of bronchoactive agents to bronchial smooth muscle, and altering airway mucosal permeability. We have found (unpublished observations) that propylene glycol 1% v/v produced, in isolated human bronchial smooth muscle strips, 15-25% decreases in resting tone which were sustained for up to 1 h. Propylene glycol however did not affect the subsequent histamine dose response curve *in vitro*. Although this relaxant action *in vitro* would indicate a direct inhibitory action on airway tone *in vivo* this was not reflected in any change in FEV_1 measured after propylene glycol administration. It may be, however, as previously mentioned, that the FEV_1 is not sufficiently sensitive to detect these changes.

The present study provides evidence that methoxamine-induced bronchoconstriction is mediated via α-adrenoceptors. In does not, however, indicate the site of these receptors which could be on mast cells, on nerve endings or on bronchial smooth muscle.

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