accessibility to the circulation is greater than elsewhere in the bronchial tree. The plateau phase following this suggests a subsequent slower absorption, possibly from the bronchial mucosa of larger airways. It would seem unlikely that there is significant absorption from the gut and oropharynx as less than 2% of nebulised solution enters this region, whilst 11% or greater enters the airways (Lewis, personal communication; Lewis *et al.*, 1981).

We would recommend that at least 500 mg can be nebulised with safety and that it is probably safe to give 1000 mg particularly if one were to use a conventional nebuliser. At both doses it is doubtful if significant blood levels would be achieved.

These studies show that much larger doses than have been previously used can be successfully inhaled without inducing significant systemic or local side-effects in normal subjects. In particular no bronchoconstriction was seen. This study supports the possibility of achieving substantial local levels of drug with concomitantly low levels in the blood. Therefore we are now undertaking further studies to assess the feasibility of using this method of medication in treating asthmatic subjects. The major limitations for this may well be the taste and the potential hazard of sensitization to the ethylenediamine component of aminophylline.

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COMPARISON BETWEEN AIRWAYS RESPONSE TO AN α-ADRENOCEPTOR AGONIST AND HISTAMINE IN ASTHMATIC AND NON-ASTHMATIC SUBJECTS

There is now *in vitro* evidence for the existence of a population of α-adrenoceptors in human lung tissue, both central (Kneussl & Richardson, 1971) and peripheral (Black *et al.*, 1981). There is, in addition, evidence for a change in α-receptor number in pulmonary disease states on the basis of animal studies (Barnes *et al.*, 1980). The results of *in vivo* studies however, have been conflicting. There are reports of the existence of α-adrenoceptors in the airways of asthmatic subjects (Snashall *et al.*, 1978), non-asthmatics (Anthracite *et al.*, 1971) and neither (Stone *et al.*, 1973).

Bronchoconstriction in response to inhalation challenge with histamine has become a diagnostic feature of asthma (Salome *et al.*, 1980). However, there is a certain population of non-asthmatic subjects who bronchoconstrict in response to histamine challenge. A positive response to histamine challenge thus does not always serve to distinguish asthmatic from non-asthmatic subjects.

This study examines the airways response to inhalation of methoxamine in both asthmatics and non-asthmatic subjects and compares it to the responses to histamine provocation.

The subjects were 10 known asthmatics, 4 females and 6 males aged 21 to 54 years, and 10 non-asthmatics, 5 females and 5 males, aged 24 to 56 years. All the asthmatic subjects were taking regular bronchodilator aerosol therapy, but none was steroid dependent. Nine of the asthmatic subjects and 5 of the non-asthmatics were atopic (that is, they reacted with a positive skin prick test to one or more 8 standard allergens). Informed consent was obtained from all subjects and the protocol was authorised by the

Ethics Review Committee of Sydney University. All asthmatic subjects agreed to withhold all medications for 6 h prior to the challenge.

Challenges with histamine diphosphate, dissolved in 0.9% w/v NaCl solution and at room temperature, were carried out using the method of Chai *et al.* (1975). Briefly, histamine in doses of 0.006–4.0 μmol was administered with a De Vilbiss No. 646 nebuliser attached to medical air at 20 p.s.i. The length of each nebulization was controlled by a Nebulization-Dosimeter (Rosenthal-French, U.S.A.) at a setting of 0.6 s per inhalation. Each dose of histamine was administered over five inhalations.

Challenges with methoxamine hydrochloride were conducted on a separate day. Methoxamine 0.02–20 μmol was administered as above except that the dosimeter was set at 3 s. This method of delivery has been shown to ensure peripheral deposition of the agonist (Yan *et al.*, 1981) and was chosen since our *in vitro* work had been carried out in peripheral lung (Black *et al.*, 1981).

Initial measurements for forced expiratory volume in one second (FEV_1) were made and then repeated 90 s after each dose of agonist. The challenge was stopped when a 20% or greater fall in FEV_1 had occurred or when the maximum dose of agonist had been administered. For each patient results were expressed graphically as percentage fall in FEV_1 versus log dose of agonist in μmol. The PD_{20} FEV_1 i.e. the dose required to produce a 20% fall in FEV_1 from initial value was determined from the dose-response curve.

All asthmatics responded to histamine challenge with a 20% or greater fall in FEV_1-mean PD_{20} FEV_1 was 0.08 μmol (range 0.01–0.56). In addition, four non-asthmatics exhibited a dose-dependent fall in FEV_1-mean PD_{20} FEV_1 1.2 μmol (range 0.52–1.5). Three of these subjects were atopic.

All the asthmatic subjects but none of the non-asthmatics experienced a 20% fall in FEV_1 in response to methoxamine—mean PD_{20} FEV_1 0.83 μmol (range 0.03–20.0). The four non-asthmatics who reacted to histamine did not respond to methoxamine. The three different types of responses to histamine and methoxamine are seen in Figure 1.

The investigation has demonstrated that in the subjects selected for study, asthmatics but not non-asthmatics, responded with dose dependent falls in FEV_1 to inhalation challenge with methoxamine, an α-adrenoceptor agonist. These findings are in agreement with those of Snashall *et al.* (1978) but differ from those of Anthracite *et al.* (1971) who studied only non-asthmatics and found bronchoconstriction in response to methoxamine. The differences between these findings may be explained on the basis of the use of different parameters to measure change in lung function (*viz* airway conductance and FEV_1).

Although methoxamine has no β-adrenoceptor

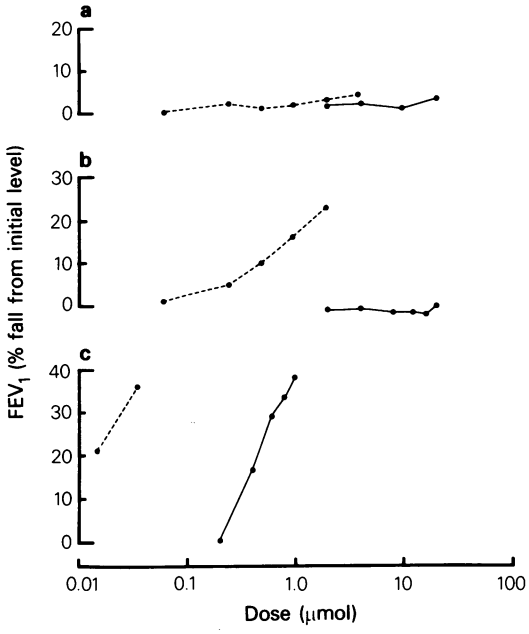

Figure 1 The effect on forced expiratory volume in one second (FEV_1) of inhalation challenge with increasing doses of histamine (----) and methoxamine (——) in three subjects, (a) one normal, (b) one with asymptomatic bronchial hyperreactivity and (c) one with clinical asthma.

agonist activity, it has been reported to be a weak β-adrenoceptor antagonist (Karim, 1965). It is unlikely that the effects observed in the present study were the result of β-adrenoceptor blockade as the dose of methoxamine was similar to that used by Snashall *et al.* (1978) who found no evidence of β-adrenoceptor blockade.

It is interesting that although four of the non-asthmatic subjects were hyperreactive to histamine, methoxamine did not cause bronchoconstriction. Thus it appears that response to α-receptor stimulation may distinguish asthmatics from those with asymptomatic bronchial hyperreactivity.

It has been suggested that the response in asthmatics to α-adrenoceptor antagonists, and presumably agonists is heterogeneous (Black *et al.*, 1978; Spector, 1979). The present study identifies a group of subjects in whom it may be possible to assess the therapeutic benefit of α-adrenoceptor antagonists.

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THE EFFECTS OF FENFLURAMINE ON *IN VITRO* PLATELET AGGREGATION

Fatal cardiac dysrrhythmias are known to occur following overdoses of fenfluramine and possibly during anaesthesia in patients who had been taking this drug (Bennett & Eltringham, 1977). Since fenfluramine is structurally similar to adrenaline (Bennett & Eltringham, 1977) it may be expected that the toxic effects result from adrenergic properties or from adrenoceptor blockade. Since platelets have adrenergic receptors, and adrenaline is a potent platelet aggregator (Grant & Scrutton, 1979) we undertook an investigation to determine whether fenfluramine affected adrenaline-induced platelet aggregation.

Such an experiment would allow us to comment on the action of fenfluramine in a system other than the heart. It would also enable us to assess whether platelets are a practical biological model for adrenoceptor studies in humans.

Platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared from eight healthy volunteers (four male, four female) as previously described (Mikhailidis *et al.*, 1982). None of these subjects had ingested any drugs for a period of 2 weeks prior to sampling.

Various amounts of fenfluramine were dissolved in phosphate buffer (pH 7.4, 0.1 mmol/l) such that when 5 μl of these solutions were added to 450 μl of PRP, final concentrations of 20, 2 and 0.2 μg/ml were achieved. Following the addition of fenfluramine, PRP was incubated at 37°C for 10 min.

Platelet aggregation, carried out in a chronolog aggregometer, was initiated by the addition of 50 μl of adenosine diphosphate (ADP) or adrenaline (final concentrations are shown in the table). We selected appropriate concentrations of these agonists in order to achieve 'low' and 'high' levels of aggregation. 'Low' levels would be more appropriate to demonstrate enhancement effects, while 'high' levels would be needed to demonstrate inhibition. The 'low' levels of ADP and adrenaline were selected so as to give near maximum first phase aggregation. Any further enhancement by fenfluramine would then be likely to induce the secondary phase of aggregation. Aggregation was recorded as the percentage fall in optical density 3 or 6 min after initiating aggregation (Table 1). Buffer (5 μl) was added in place of the drug to provide control values. Values obtained from samples incubated with buffer (controls) and from samples incubated with fenfluramine were compared using a paired Wilcoxon rank sum test. *P* values are shown in Table 1.

The presence or absence of spontaneous aggregation was assessed during incubation by monitoring the baseline tracing on the aggregometer.

Adrenaline induced platelet aggregation at 3 min was significantly inhibited at the two higher concentrations of fenfluramine (Table 1). However, when aggregation was recorded at 6 min, fenfluramine induced inhibition was significant only at the highest concentration of 20 μg/ml (Table 1). When the areas under the aggregation curves were analysed, the statistical significance of our results was