The effect of inhaled 5-hydroxytryptamine (5-HT, serotonin) on airway calibre in man

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Since platelet activation has been incriminated in the pathogenesis of asthma, we have investigated the effect of one of its major granule-derived mediators, 5-hydroxytryptamine (5-HT, serotonin) on airway calibre in normal (12), atopic non-asthmatic (12) and atopic asthmatic subjects (16). On separate days subjects inhaled increasing concentrations of 5-HT and methacholine, and airway response was measured as FEV₁, Vmax₃₀ and sGaw. All subjects bronchoconstricted with methacholine, geometric mean provocation concentrations causing a 20% fall in FEV₁ (PC₂₀) for the normal, atopic non-asthmatic and atopic asthmatic subjects being 28.6, 18.3 and 0.71 mM respectively. In contrast, 5-HT up to a maximum concentration of 77 mM had no consistent effect on FEV₁, Vmax₃₀ or sGaw in any of the subject groups. Thus, in contrast to a variety of animals, 5-HT is unlikely to serve as a significant bronchoconstrictor mediator in man.

Keywords 5-hydroxytryptamine reactivity asthma

Introduction

Since its discovery in the late 1940s 5-hydroxytryptamine (5-HT, serotonin) has been proposed as a bronchoconstrictor mediator of asthma. A possible relationship between 5-HT and airway obstruction was suggested by the association of wheezing with the carcinoid syndrome, although it is now appreciated that other mediators such as histamine and bradykinin are also released in this situation. In man 5-HT is principally localised to nerve terminals, the argentaffin cells of the gastrointestinal and respiratory tract and the dense secretory granules of platelets (Sirek & Sirek, 1970). Recently the role of platelets in the pathogenesis of allergic reactions, in particular asthma, has been reappraised with the identification of a platelet activating factor, PAF-acether (acetyl-glycerol-ether-phosphorylcholine, AGEPC), generated by mast cells, basophils and alveolar macrophages upon IgE-dependent activation (Demopoulos *et al.*, 1979; Page *et al.*, 1984). Morley has proposed that platelet activation mediated by PAF-acether may contribute

to bronchoconstriction through the release of platelet derived mediators such as 5-HT, histamine and thromboxane (Morley *et al.*, 1984).

The effect of 5-HT on airway calibre has been extensively studied in animals *in vitro* and *in vivo*. In a number of species including rat, dog and monkey 5-HT causes contraction of tracheal and bronchial smooth muscle (Brocklehurst, 1963) and *in vivo* it produces bronchoconstriction in the guinea-pig, cat and dog (Simonsson & Svedmyr, 1981). The situation is less clear in man. *In vitro* 5-HT has been reported to produce both contraction (Brocklehurst, 1963) and relaxation (Mathe *et al.*, 1971) of respiratory smooth muscle. Several studies in the 1960s also suggested that 5-HT was a bronchoconstrictor in man, although the number of subjects who achieved significant airflow limitation with inhaled 5-HT (i.e. 20% fall of FEV₁) varied from 16% (Panzani, 1962) to 65% (Girard & Moret, 1962) of those challenged. In view of the potentially important role of platelets in asthma and

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the confusion over the airway effects of 5-HT in man, the present study was undertaken to assess the effects of 5-HT on the airways of normal and asthmatic subjects in a controlled study in which non-specific airways reactivity was measured with methacholine.

Methods

Three groups of subjects were studied—12 normal non-atopic, 12 atopic non-asthmatic, and 16 atopic subjects with mild asthma (Table 1). None of the subjects were taking regular medication. Two asthmatic subjects were on intermittent inhaled β_2 -adrenoceptor agonists and these were discontinued 12 h prior to study. Informed consent was obtained and the study approved by the Southampton University and Hospitals Ethics Sub-committee.

Subjects attended on two occasions to inhale, in random order, doubling concentrations of 5-HT creatinine sulphate, 1.21–77.0 mM, or methacholine, 0.08–409.0 mM (Sigma, Poole, Dorset). All solutions were prepared in 0.9% sodium chloride. 5-HT was poorly soluble at room temperature and was therefore warmed to 25° C to achieve a maximum concentration of 77 mM. Analysis of a 5-HT solution treated in this manner using reverse phase high pressure liquid chromatography (h.p.l.c.) showed a single peak of UV absorbance at 229 nm that had an identical retention time to a 5-HT solution that had not been heated. This confirmed that heating of 5-HT to 25° C did not result in chemical degradation of the amine. In addition, bioassay using rat isolated ileum showed similar contractile effects for the heated and unheated 5-HT solutions. Inhalations of aerosols were for 1 min from disposable Mini-neb nebulisers (Inspiron,

C. R. Bard Ltd, Sunderland) driven by compressed air at 8 l min⁻¹. Each series of inhalations was preceded by a single inhalation of 0.9% sodium chloride and subjects were to be excluded from further study if this produced a greater than 10% fall of the forced expiratory volume in one second (FEV₁). The airways response to provocation was assessed as FEV₁ and maximum expiratory flow at 30% of vital capacity (Vmax₃₀) using a rolling seal spirometer on line to a microprocessor (No. 050184, Collingwood Measurement, Leicester), and as specific airways conductance (sGaw) using a pressure compensated whole body plethysmograph (Fenyes & Gut, Basle, Switzerland). Results for methacholine were expressed as the provocation concentrations (PC) producing a 20% fall in FEV₁ (PC₂₀), 40% fall in Vmax₃₀ (PC_{v40}) and 35% fall in sGaw (PC_{s35}) from the mean post-saline value. For 5-HT, results were expressed as the maximum bronchoconstriction achieved with the highest concentration of 5-HT administered.

Differences between baseline observations and between the maximum bronchoconstriction for 5-HT for the normal, atopic and asthmatic subjects were analysed using Student's paired and unpaired *t*-tests for FEV₁ and Wilcoxon's signed rank test and rank sum test for sGaw and Vmax₃₀. PC values for methacholine were compared using Student's unpaired *t*-test and the association between airways reactivity to methacholine and response to inhaled 5-HT was determined using least mean squares regression analysis.

Results

There was no significant difference between baseline values of FEV₁, Vmax₃₀ or sGaw within

Table 1 Subject characteristics

	Sex M,F	Age (years)	FEV ₁ (l)	% predicted FEV ₁	sGaw (s ⁻¹ kPa ⁻¹)	Vmax ₃₀ (l min ⁻¹)
Normal	5,7	24.0 (21–33)	3.57 (0.19)	97.8 (2.9)	1.82 (0.14)	154.4 (11.9)
*Atopic non-asthma	9,3	23.2 (21–30)	3.85 (0.19)	95.8 (4.1)	1.84 (0.19)	133.1 (12.4)
Atopic asthma	8,8	21.5 (17–30)	3.35 (0.15)	89.5 (3.1)	1.53 (0.18)	109.4** (11.5)

All values are given as mean (s.e. mean) with the exception of age which is shown as mean and range.

*Atopic = positive skin test reaction to two or more of the following allergens; house dust, *Dermatophagoides pteronyssinus*, cat fur, dog hair, grass pollens, tree and shrub pollens, mixed feathers, *Aspergillus fumigatus*.

**Significantly ($P < 0.05$, Wilcoxon's signed rank test) lower than the normal and atopic non-asthmatic subjects.

each group of subjects on the two study days, nor was there any significant difference between pre- and post-saline values. Mean baseline values of FEV₁ and sGaw were not significantly different between the three groups of subjects but the mean baseline Vmax₃₀ was significantly lower in the asthmatic subjects (109.4 l min⁻¹) compared to the normal (154.4 l min⁻¹) and atopic non-asthmatic subjects (133.1 l min⁻¹) ($P < 0.05$). No subject had a greater than 10% fall of FEV₁ following inhalation of saline. Inhalation of methacholine produced concentration-dependent bronchoconstriction in all subjects though one normal non-atopic subject only achieved a 15% fall of FEV₁. The respective geometric mean (range) PC₂₀, PC₃₅ and PC₄₀ values for methacholine (mm) were 28.6 (6.2–250.0), 17.1 (5.6–79.2), 10.4 (4.2–29.6) for the normal subjects, 18.3 (4.5–63.9), 10.9 (2.0–42.9), 7.0 (1.8–15.0) for the atopic non-asthmatic subjects and 0.71 (0.1–2.97), 0.52 (0.13–2.30), 0.35 (0.06–1.25) for the asthmatic subjects. The asthmatic subjects were significantly more reactive to methacholine than the atopic non-asthmatic ($P < 0.01$) or normal subjects ($P < 0.01$) irrespective of the method used to measure airway calibre. There was no significant difference between the geometric mean PC values for the atopic non-asthmatic and the normal subjects.

In contrast to the airways response to methacholine, there was no consistent pattern of re-

sponse to inhaled 5-HT in any of the three groups of subjects studied (Figure 1). Even with the highest concentration of 5-HT inhaled (77.0 mm) none of the subjects achieved the specified fall in all three measures of airway calibre. Vmax₃₀ fell by > 40% in three asthmatic subjects, and one of these also demonstrated > 35% fall in sGaw. No relationship was established between reactivity to methacholine and the airway response to the highest concentration of 5-HT in any group of subjects irrespective of the method used to measure airflow resistance.

Discussion

Inhalation of 5-HT up to a concentration of 77 mm had no significant effect on airway calibre of normal, atopic non-asthmatic or atopic asthmatic subjects. This is in agreement with a recent study by Tonnesen (1985) showing that 5-HT in concentrations up to 20 mg ml⁻¹ (52 mm) had no effect on FEV₁ or FVC in 20 stable asthmatic subjects. It is not apparent why our results differ from previously reported studies showing a minimum of 16% of asthmatic subjects responding to 5-HT with bronchoconstriction (Panzani, 1962). The concentration of 5-HT used in the present study was slightly higher than previously used but the major difference is more likely to lie in the type of asthmatic patients studied. Our

Figure 1 The airway response to inhalation of 5-HT in normal (N, $\blacktriangle$), atopic non-asthmatic (ANA, ∇) and asthmatic subjects (A, $\bullet$). Results expressed as percentage change from baseline for FEV₁ (vertical axis left) and Vmax₃₀ and sGaw (vertical axis right).

subjects all had mild asthma (FEV₁ greater than 70% predicted) although they had a wide range of non-specific airways reactivity reflected by methacholine PC values of 0.10–2.97 mM (0.02–0.58 mg ml⁻¹). Asthmatic subjects with a similar range of methacholine responsiveness have previously been shown to respond to other forms of bronchial provocation, such as histamine, adenosine and exercise (Holgate *et al.*, 1984). Previous studies have been less clear in defining

the severity of asthma in their subjects, but when recorded, they had a wide spectrum of disease severity with FEV₁ being as low as 17% predicted (Booij-Noord *et al.*, 1969). We conclude from our study that in normal and in asthmatic subjects with a wide range of non-specific bronchial reactivity, 5-HT has little effect on airway calibre. Thus, if this mediator is released from activated platelets in asthma it is unlikely to contribute significantly to bronchoconstriction.

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