

# Bronchial Challenge with Serotonin in Asthmatics

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Bronchial challenge with serotonin creatinine in phosphate buffer was performed in 20 stable asthmatics. In contrast to some earlier studies we observed no changes in FEV<sub>1</sub> or FVC 0 and 2 min after provocation with increasing concentrations of serotonin (maximal concentration 20 mg/ml). In the first eight patients the PC<sub>20</sub> histamine was 0.125–0.65 mg/ml, performed on a separate day. In three other asthmatics serotonin chloride 20 mg/ml did not affect lung function or produce any subjective symptoms. Serotonin can not be used to evaluate non-specific bronchial hyperreactivity in asthmatics.

*Key words:* asthma; bronchial challenge; lung function; serotonin.

*Accepted for publication 15 September 1984*

The most characteristic abnormal finding in bronchial asthma is bronchial non-specific hyperreactivity, which can be demonstrated by bronchial challenge with histamine and methacholine (3, 6, 15).

Some earlier investigators have shown that serotonin inhalations induced an acute fall in lung function (> 20% fall in FEV<sub>1</sub>) in 10–65% of asthmatics (1, 5, 7), while others have found no response (8, 9).

If serotonin can induce bronchoconstriction, it could be used to measure bronchial hyperreactivity, and its role in the pathophysiology of asthma would be a hypothetical possibility. To examine this closer we performed bronchial challenge with serotonin creatinine in 20 stable asthmatics, but we observed no significant fall in lung function. In three other asthmatics serotonin chloride neither influenced lung function, nor provoked any subjective symptoms.

## MATERIAL AND METHODS

### *Patients*

The study comprised 23 stable asthmatics (10 women and 13 men; age range 16–60 years,

mean 55 years) were recruited consecutively from our allergy clinic. Twenty had known external inhalant allergies and three intrinsic asthma. All had at least 20% increase in FEV<sub>1</sub> after inhalation of a beta<sub>2</sub>-agonist spray. Basal PEF and FEV<sub>1</sub> were > 65% of predicted normal values. Patients with chronic bronchitis, emphysema or abnormal chest-X-ray were excluded, as were patients with > 10% variability in FEV<sub>1</sub> and/or FVC in three subsequent trials. Patients receiving oral steroids and patients with acute exacerbation in their asthma within the last 2 months were not included.

### *Medication*

The use of antihistamines was stopped 3 days before the trial, inhalant steroid, sodium chromoglycate and theophyllines were withheld for 24 h. After 8 p.m., the day before challenge, beta<sub>2</sub>-agonists were stopped, as well as other anti-asthmatic drugs, and all medications were withheld on the day of testing

### *Bronchoprovocation*

The routine method of the clinic was used, modified according to Cockcroft et al. (3). In the

sitting position the patient placed the face mask over mouth and nose and breathed quietly at tidal volume through the mouth. Aerosols were generated with a nebulizer operated with an airflow from an electric pump (Pariinhalerboy 036). 1 ml of the drug was placed in the nebulizer chamber, which was only activated during inspiration. During the 2-min inhalation the nebulizer delivered an output of 0.15 ml/min, with a particle diameter of 0.5–5.5  $\mu\text{m}$ . The first eight patients started with a control inhalation (isotonic saline), and then received either histamine or serotonin creatinine inhalations in doubling concentrations at 5-min intervals on 2 separate days in random order with at least a 24 h interval. The inhalation was stopped when a 20% fall in post saline  $\text{FEV}_1$  was observed or the highest concentration was reached. The next 12 patients received inhalations with only serotonin creatinine at maximal concentration (20 mg/ml).

The last three patients with extrinsic asthma

received serotonin chloride (20 mg/ml) inhalations. The lung function was measured with a Vitalograph® by separately recording three forced expirograms, i.e. before challenge, just after, and 2 min after challenge. The maximal values of  $\text{FEV}_1$  and FVC were recorded.

### Reagents

Histamine chloride in phosphate buffer: 0.03, 0.06, 0.12, 0.25, 0.5, 1.0, 2.0, 4.0 mg/ml (pH 6.10–7.40). (Osmolarity calculated 255–333 mOsm). Serotonin creatinine sulphate (Sigma, USA) in phosphate buffer: 1.25, 2.5, 5.0, 10.0, 20.0 mg/ml (pH 6.62–7.28). (Osmolarity calculated: 240 mOsm). Both preparations were freshly prepared each week and stored at 5°C. The preparations were used at room temperature. No preservatives were added, but the solutions were filtered through 0.22  $\mu$  Millipore®. Isotonic saline was used as control solu-

Table 1  
Patients' clinical data ( $n = 23$ )

| Age<br>$\bar{x}$ (range) | Rhinitis | IgE<br>$\bar{x}$ (range) | Eos.<br>Mia/l<br>$\bar{x}$ (range) | External<br>allergy | FVC (% of<br>predicted)<br>$\bar{x}$ (range) | $\text{FEV}_1$ (% of<br>predicted)<br>$\bar{x}$ (range) | Daily<br>medicine |
|--------------------------|----------|--------------------------|------------------------------------|---------------------|----------------------------------------------|---------------------------------------------------------|-------------------|
| 55 years<br>(16–60)      | 15       | 324<br>(29–1508)         | 0.52<br>(0.02–1.55)                | 20                  | 100.2<br>(81–117)                            | 96.7<br>(66–127)                                        | 10                |

Table 2  
Lung function values after serotonin bronchial challenge in 23 asthmatics

|                   | Post saline |      | 0 min after serotonin creatinine |      | 2 min after serotonin creatinine |      |
|-------------------|-------------|------|----------------------------------|------|----------------------------------|------|
|                   | $\bar{x}$   | SD   | $\bar{x}$                        | SD   | $\bar{x}$                        | SD   |
| ( $n = 20$ )      |             |      |                                  |      |                                  |      |
| $\text{FEV}_1$    | 3.50        | 1.12 | 3.55                             | 1.16 | 3.53                             | 1.17 |
| FVC               | 4.42        | 1.24 | 4.38                             | 1.25 | 4.39                             | 1.25 |
| ( $n = 3$ )       |             |      |                                  |      |                                  |      |
| $\text{FEV}_{1*}$ | 4.62        |      | 4.63                             |      | 4.65                             |      |
| FVC*              | 5.25        |      | 5.22                             |      | 5.17                             |      |

\* = After serotonin chloride.

† values:  $-0.647$ – $-0.922$ , all  $P$  values  $> 0.3$ .

tion. Serotonin chloride 20.0 mg/ml was used in phosphate buffer (pH 6.5). (Osmolarity calculated: 496 mOsm).

## RESULTS

Table 1 shows the patients' clinical data. No significant changes were observed in FEV<sub>1</sub> or FVC (Student's t-test for paired data,  $P > 0.3$ ) after challenge with serotonin creatinine (Table 2). The lower confidence limit of the difference is a fall in FVC/FEV<sub>1</sub> of up to 4% ( $P = 5\%$ ). The first eight patients were given increasing doses of serotonin, and in two of the patients a minor fall in FEV<sub>1</sub> (17%, 5%) and FVC (7%, 9%) was observed, without any dose-dependent decrease in lung function. None of the eight patients had a subjective feeling of dyspnoea or other respiratory complaints. The histamine concentration producing a 20% FEV<sub>1</sub> fall (PC<sub>20</sub>) was calculated from the dose-response curves with histamine in the above eight patients. The PC<sub>20</sub> histamine was 0.125–0.65 mg/ml.

In the following 12 patients 20 mg/ml serotonin neither influenced lung function nor produced any subjective respiratory symptoms from the lower airways, except in one person who had a 10% fall in FEV<sub>1</sub> and 19% fall in FVC.

In two of the patients we performed partial flow-volume curves after serotonin provocation, but did not observe any change in forced expiratory flow at 20% of FVC (before 0.54 and 0.81, after 2 ml serotonin 20 mg/ml 0.63 and 0.81 l/s).

In three other asthmatics serotonin chloride bronchial challenge did not influence lung function nor produce any subjective respiratory symptoms.

Nine patients, who suffered from both asthma and rhinitis, reacted with rhinitis symptoms after serotonin inhalation, i.e. sneezing, tickling and hypersecretion. In another patient, who suffered only from asthma, the lowest serotonin concentration produced very severe sneezing, and it was impossible to continue with the serotonin challenge.

Table 3

Serotonin bronchial challenge reports in asthmatics. Positive challenge:  $> 20\%$  fall in FEV<sub>1</sub>

| No. | Positive BPT serotonin | Positive BPT histamine | Serotonin creatinine solution | Buffered | Type of asthma                                            | References                 |
|-----|------------------------|------------------------|-------------------------------|----------|-----------------------------------------------------------|----------------------------|
| 6   | 5 (83%)                | —                      | 0.67%                         | no       | ?                                                         | Herxheimer (7) 1953        |
| 3   | 0 (0%)                 | —                      | 0.67%                         | yes      | ?                                                         | Herxheimer (8) 1955        |
| 20  | many                   | —                      | 1% (serotonin chloride)       | no       | ?                                                         | Michelson (10) 1956        |
| 43  | 8 (19%)                | —                      | 1%                            | no       | Positive patients had permanent dyspnoea                  | Panzani & Pascal (12) 1961 |
| 246 | 146 (59%)              | 126 (51%)              | 2%                            | yes      | ?                                                         | Hajos (5) 1962             |
| 60  | 20 (33%)               | —                      | 1%                            | no       | Many chronic asthmatics                                   | Panzani (11) 1962          |
| 40  | 26 (65%)               | —                      | 1%                            | no       |                                                           | Girard (4) 1963            |
| 11  | 2 (18%)                | —                      | 2%                            | no       | ?                                                         | Marcelle (9) 1968          |
| 23  | 14 (61%)               | 14 (61%)               | 0.125–2.0%                    | yes      | Some patients severe reduced FEV <sub>1</sub>             | Booij-Nord (1) 1968        |
| 23  | 0 (0%)                 | 8/8 (100%)             | 0.125–2.0%                    | yes      | Post saline FEV <sub>1</sub> $> 65\%$ of predicted values | Tønnesen (14) 1984         |

## DISCUSSION

Serotonin bronchial challenge did not produce a significant or clinically relevant fall in lung function in 23 asthmatics. As shown in Table 3 several investigators have reported a fall in  $FEV_1$  after serotonin inhalations in asthmatics (1, 5, 10, 12). In some of these studies the serotonin solutions were not buffered ( $pH < 6.5$ ) and it is known that acid solutions can induce bronchospasm. This is usually only a mild reaction (2), and can only partly explain the relatively large fall after serotonin described.

Some authors used buffered serotonin solutions with  $pH$  7.6–8.4, but Booij Noord et al. (1) did not find any difference between buffered ( $pH$  8.4) and un-buffered serotonin creatinine inhalations. Other factors might account for the difference in response. In some of the studies the asthmatics had severe chronic asthma with permanently decreased lung function, but Girard & Moret (4) did not find any relation between predicted basal  $FEV_1$  and the reaction after serotonin. In some of the earlier studies lung function values are not described in detail, and it is not possible to separate the patients' individual responses.

We have, of course, speculated about any methodological failure done by us. The inhalation technique was correct because our patients responded to the histamine inhalations. To rule out inactivation of serotonin we measured the serotonin content in the solutions used by us, which showed no loss of activity during 1 week. It has not been possible to dissolve more than 20 mg/ml of serotonin in water, but even this concentration is highly supraphysiological.

We have used serotonin in the same concentrations and doses as others, but could not reproduce the earlier findings. In contrast to many older studies our patients are well characterized asthma patients in stable conditions, and we have started every patient with a control inhalation to exclude patients who respond to saline.

We have not examined whether serotonin and histamine could have a potentiating effect on bronchoconstriction, but as serotonin did not potentiate the wheal and flare reaction of

histamine by skin prick testing, this seems unlikely (13).

From our study we can conclude that serotonin bronchial inhalation did not alter lung function in 23 stable asthmatics, and that serotonin therefore can not be used to evaluate bronchial hyperreactivity. We are now doing serotonin challenges on a greater number of asthmatics to evaluate this further.

As an original finding we observed that some patients got rhinitis symptoms after serotonin, and this finding has been confirmed in normal persons by nasal challenge with serotonin. Reflex-mediated symptoms, i.e. sneezing, tickling and hypersecretion were induced after serotonin in the same degree as after histamine (14). Because of nasal hyperreactivity, rhinitis patients might be more sensitive to serotonin than normal individuals, but it has not been possible to elucidate this problem in the present study.

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