The effects of 5-HT on cholinergic contraction in human airways in vitro

L.J. Dupont*, J.L. Pype*, M.G. Demedts*, P. De Leyn**, G. Deneffe**, G.M. Verleden*


ABSTRACT: Inhaled 5-hydroxytryptamine (5-HT) causes bronchoconstriction in asthmatics, and 5-HT plasma levels are elevated in asthma. Electrical field stimulation (EFS) of human airways, in vitro, evokes cholinergic contraction mediated by the release of acetylcholine (Ach) from postganglionic cholinergic nerves. The present study investigates whether selective 5-HT agonists and antagonists can modulate EFS-induced cholinergic contraction in human airways in vitro.

Human airways, obtained from resections for bronchial carcinoma or organ transplant donors, were suspended under 2-g tension, between two platinum wire electrodes, in carbogenated Krebs solution at 37 °C and EFS was applied (1–32 Hz, 50 V, 0.5 ms, 15 s every 4 min) to elicit cholinergic contractions. 5-HT (10 μM–0.3 mM) produced frequency- and concentration-dependent facilitation of cholinergic contraction, but did not displace the concentration/response curve to Ach. Tropisetron (1 μM), a 5-HT3 and 5-HT4 antagonist, completely blocked the facilitatory effect of 5-HT (100 μM), whereas both ondansetron (1 μM) and GR 125478D (1 μM), a selective 5-HT1 and 5-HT3 antagonist, respectively, also attenuated the 5-HT-enhanced induction of cholinergic contraction. This facilitatory effect of 5-HT was partially mimicked by both selective 5-HT3 (2-methyl-5-HT) and 5-HT4 (RS 125478D) agonists. Fluoxetine (10 μM), a 5-HT uptake inhibitor, had no effect on the 5-HT (10–100 μM) induced potentiation of cholinergic contraction.

These findings suggest that 5-HT facilitates cholinergic contraction in human airways in vitro through stimulation of both prejunctional 5-HT1 and 5-HT4 receptors. This may implicate a role of 5-HT in asthma.


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Following the isolation and synthesis of 5-hydroxytryptamine (5-HT) in the early 1950s, there has been increasing interest and research into the physiological functions of this biogenic amine. 5-HT is found in large amounts in enterochromaffin cells throughout the gastrointestinal tract, in blood platelets and in specific regions of the central nervous system [1]. The presence of 5-HT has also been demonstrated in the lung, although its exact role in the respiratory system remains unclear [2].

5-HT exhibits a broad diversity of effects on airway smooth muscle contraction, which seems to implicate the presence of a wide variety of 5-HT receptor subtypes in both airway smooth muscle and effluent nerves and which also appears to be species-dependent. In several animal airways, 5-HT acts directly on airway smooth muscle, causing contraction at low doses and relaxation at high doses. Both contraction and relaxation are mediated by stimulation of the 5-HT2A receptor on airway smooth muscle [3]. The effects of 5-HT on airway smooth muscle contraction may also be attributed, in part, to the ability of 5-HT to modulate the contractile and relaxing response to other neurotransmitters. 5-HT has been shown to modulate nonadrenergic noncholinergic (NANC) contraction in guinea-pig airways in vitro. This contraction, mediated by the release of neuropeptides from sensory nerve endings [4], was shown to be significantly inhibited by stimulation of a prejunctional 5-HT receptor, which was originally described as a 5-HT1-like subtype [5] but should probably be considered as a 5-HT2 receptor on the basis of its pharmacological profile [6]. 5-HT has also been shown to modulate cholinergic neural responses in a number of species by interacting with presynaptic neuronal 5-HT receptors. The 5-HT receptor subtype involved appears to differ according to the nature of the species. In mouse isolated tracheal segments, 5-HT potentiates cholinergic contraction by activating presynaptic 5-HT1-like receptors [7]. In rat bronchi 5-HT enhances cholinergic contraction by stimulation of 5-HT3 receptors [8]. Conversely, in guinea-pig airways, several authors have demonstrated facilitatory effects of 5-HT on postganglionic cholinergic neurotransmission by stimulation of 5-HT3 receptors [9, 10].

Although the effects of 5-HT on airway calibre have been extensively studied in several animal species, both in vivo and in vitro, the situation is less well established in humans. A possible relationship between 5-HT and airway obstruction has been suggested on the basis of the association of wheezing with carcinoid syndrome, although it...
is now obvious that other mediators such as histamine, bradykinin and tachykinins are also released in this pathology [11]. Inhaled 5-HT does not produce bronchoconstriction in normal subjects [12]. It has been demonstrated in some studies, however, that inhalation of 5-HT causes bronchoconstriction in 10–65% of asthmatic patients, whereas other investigators have found no response [12]. An elevated plasma level of 5-HT has been documented in symptomatic asthmatic patients when compared to non-asthmatics. In the former group, the 5-HT level significantly correlated with clinical severity rating and forced expiratory volume in one second (FEV1) [13]. Despite these findings, 5-HT antagonists are not routinely used in asthma therapy, although beneficial effects of ketanserin, a 5-HT2 antagonist, have been noted on FEV1 in patients with chronic airflow limitation [14]. In excised human airways in vitro facilitatory effects of relatively high concentrations of 5-HT were observed, which were, in accordance with the receptor subtype found in guinea-pig airways, attributed to an effect on 5-HT3 receptors [10]. However, in this study, only a limited range of 5-HT agonists and antagonists was used and it remains to be established whether the 5-HT3 receptor subtype is exclusively responsible for the observed effects of 5-HT on cholinergic contraction in human airways.

The aim of the present study was to investigate the effects of 5-HT on cholinergic contraction elicited by electrical field stimulation (EFS) at different frequencies of stimulation in human airways in vitro and to characterize the receptor subtype involved by using selective 5-HT agonists and antagonists, listed in table 1. The effects of pretreatment with fluoxetine, a selective 5-HT reuptake inhibitor, on the 5-HT-induced effect on cholinergic contraction were also of interest, as it has previously been demonstrated that fluoxetine enhances 5-HT inhibition of NANC contraction in guinea-pig airways in vitro [17].

### Methods

#### Preparation of tissue

Macroscopically normal bronchial tissue was obtained from thoracotomy specimens from patients (three females, 17 males; mean±SEM age 65±8 yrs, 14 smokers 45±6 (pack-yrs)) undergoing surgery for resection of bronchial carcinoma. Main bronchi were also obtained from three patients (one female, two males) who were used as transplant donors. None of the patients showed characteristics of asthma. Immediately after surgical resection, a macroscopically normal part of the lung tissue was immersed in cooled (4°C) and carbogenated (5% CO2, 95% O2) modified Krebs-Henseleit buffer solution of the following composition (mM): NaCl 118, KCl 5.9, MgSO4 1.2, CaCl2 2.5, NaH2PO4 1.2, NaHCO3 25.5, and dextrose 5.5 (pH 7.4). The tissue remained in fresh carbogenated buffer throughout the dissection procedure and during the experiments and was used 1–18 h after resection. Just prior to the experiments, the airways were carefully stripped from surrounding lung tissue and cut into strips (main bronchi) or ring segments (segmental and subsegmental bronchi). The bronchial preparations were mounted between two platinum wire electrodes (separated by 1.0 cm) in 10-mL organ baths containing modified Krebs-Henseleit solution, which was maintained at 37°C and continuously bubbled with 5% CO2 in O2. Thin silk threads were tied to both ends of the strips and passed through and tied to the bronchial rings. One thread was connected to a steel hook at the bottom of the organ bath and the other was connected a Grass FT 03 force-displacement transducer (Stag Instruments, Chalgrove, UK). The preparations contracted against a load of 2 g, which has previously been shown to produce optimal repeatable responses in similar preparations [18]. While being washed with fresh buffer solution every 20 min, tissues were allowed to equilibrate under tension for ≥60 min before the experimental protocols were started, during which time a stable baseline tension was achieved.

#### Experimental protocol

The experimental protocol was identical for bronchial strip and bronchial ring preparations. Isometric contractile responses, induced by either EFS or adding acetylcholine (Ach), were measured using a force-displacement transducer. The traces were visualized on a computer screen after digitalization of the signal (Codas; Dalaq Instrument, Inc., Akron, OH, USA) and recorded on a personal computer.

**Electrical field stimulation.** EFS was produced using a Harvard student stimulator (Harvard Apparatus, Edenbridge, UK). Biphasic square-wave pulses at a supramaximal voltage of 50 V at source and a pulse duration of 0.5 ms were delivered for 15 s every 4 min at frequencies ranging 1–32 Hz.

Eight tissues were simultaneously tested with at least one time control tissue per experiment. These time control tissues were treated with EFS and served to demonstrate the stability of the response. After the equilibration period, a frequency/response curve (1–32 Hz) was constructed and discarded. After washing the tissues, a control frequency/response curve was constructed. In a first set of experiments, 5-HT (3–300 μM) was added to the organ baths, using one concentration of drug with each tissue preparation. After an incubation period of 15 min, a third frequency/response curve was obtained. Preliminary experiments involving the time course of the stimulatory effects of

### Table 1. Selective agonists and antagonists and their potency at human 5-hydroxytryptamine (5-HT)3 and 5-HT4 receptors.

<table>
<thead>
<tr>
<th></th>
<th>5-HT3</th>
<th>5-HT4</th>
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<tbody>
<tr>
<td><strong>Agonists</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT</td>
<td>NA</td>
<td>8.2</td>
</tr>
<tr>
<td>2-Methyl-5-HT</td>
<td>7.7</td>
<td>&lt;4</td>
</tr>
<tr>
<td>5-Methoxytryptamine</td>
<td>Inactive</td>
<td>7</td>
</tr>
<tr>
<td>RS67333</td>
<td>NA</td>
<td>8.7</td>
</tr>
<tr>
<td>RU24969</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>Sumatriptan</td>
<td>Inactive</td>
<td>Inactive</td>
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<tr>
<td><strong>Antagonists</strong></td>
<td></td>
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</tr>
<tr>
<td>Tropisetron</td>
<td>10.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Ondansetron</td>
<td>8.6</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Methysergide</td>
<td>Inactive</td>
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</table>

*: pEC50, the negative logarithm of the molar concentration of an agonist that produces 50% of the maximal effect; #: pA2, the negative logarithm of the molar concentration of an antagonist that produces a two-fold shift to the right in the agonist dose-response curve; NA: not available. (Adapted from [15, 16]).

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</tr>
</tbody>
</table>

*: pEC50, the negative logarithm of the molar concentration of an agonist that produces 50% of the maximal effect; #: pA2, the negative logarithm of the molar concentration of an antagonist that produces a two-fold shift to the right in the agonist dose-response curve; NA: not available. (Adapted from [15, 16]).
5-HT demonstrated no further facilitation of the cholinergic contraction with longer incubation time.

In a second set of experiments, the tissues were pretreated with the specific 5-HT antagonists methysergide (1 μM, 5-HT1A/1D selective), tropisetron (1 μM, 5-HT2, 5-HT4 selective), ondansetron (1 μM, 5-HT3 selective) and GR 125487D (1 μM, 5-HT4 selective), each for 15 min, before 5-HT was added. The same protocol was used as above. Control tissues were treated with antagonists alone.

In a third set of experiments, the effect of the selective 5-HT agonists 2-methyl-5-HT (1–100 μM, 5-HT1 selective), 5-methoxytryptamine (1–100 μM, 5-HT2, 5-HT4 selective), RS 67333 (1–100 μM, 5-HT3 selective), sumatriptan (1–100 μM, 5-HT1/1D selective) and RU-24969 (1–100 μM, 5-HT3A/3B selective), was studied on the cholinergic contraction elicited by EFS.

A fourth set of experiments investigated the effect of pretreatment with fluoxetine (10 μM, a selective 5-HT uptake inhibitor) on the 5-HT-induced effects on cholinergic contraction in the same manner as above.

Cumulative concentration/response curve to acetylcholine. To determine whether the effects of 5-HT on cholinergic contraction were due to activation of pre- or postjunctional receptors, the effect of a 15-min incubation period with 5-HT (300 μM) was studied on the cumulative concentration/contraction relationship to exogenously applied Ach and was compared to contractile responses to Ach in untreated tissues from the same specimen. A cumulative concentration/efficacy curve to Ach was obtained by adding incremental concentrations, spaced at half log10 intervals (30 nM–30 mM), to the organ bath. Increasing concentrations of Ach were added until a plateau contraction was reached. The results were expressed as a percentage of the maximum contraction response to Ach (10 μM), which was determined at the beginning of the experiment.

Drugs

Drugs used in these experiments were obtained from the following sources: 5-HT, Ach (Sigma Chemical Co., Eupen, Belgium); hexamethonium, tetrodotoxin ((Biomol), Sanver Tech, Boechout, Belgium); methysergide maleate, 2-methyl-5-HT, 5-methoxytryptamine ((Research Biochemicals International), Sanver Tech, Boechout, Belgium); RS67333 (Tocris Cookson, Langford, UK); tropisetron (ICS205-930) (a kind gift from Sandoz-Novartis, Basle, Switzerland); fluoxetine (LY110140) (a kind gift from Lilly Research Laboratories, Indianapolis, IN, USA); and ondansetron (GR 38032F), GR125478D, sumatriptan (GR 43175C) (a kind gift from GlaxoWellcome, Stevenage, UK). Compound RU-24969 was provided by Research Biochemicals International as part of the Chemical Synthesis Program of the National Institute of Mental Health, contract N01MH30003. Tropisetron was dissolved in dimethyl sulfoxide. All other drugs were dissolved in distilled water. Fresh drug solutions were made up daily. Drug additions did not exceed 1% of the organ bath volume. All concentrations refer to the final organ bath concentration.

Analysis of results

Results are expressed as mean±SEM. All contractile responses were measured as the difference between the peak tension and the resting tension that developed. The effects of single concentrations of 5-HT or 5-HT agonists with or without antagonist or fluoxetine were expressed as percentage facilitations, comparing the contractile responses at each stimulation frequency after pretreatment with the contraction at the same frequency in the control. Since each tissue acted as its own control, analysis of data was possible using a Student’s t-test for paired data. The significance of difference between tissues treated with 5-HT with or without antagonists or fluoxetine was assessed using a Student’s t-test for unpaired data. The same test was used to assess the effect of 5-HT versus control on the cumulative dose/response curve to exogenous Ach. A p-value of <0.05 was considered significant.

Results

In isolated human airways, EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) caused a rapid contraction that was abolished by pretreatment of the tissues for 10 min with atropine (1 μM), confirming that the contractile response was caused by the release of Ach. Incubation with hexamethonium (10 μM), a ganglion blocker, for 10 min had no effect on cholinergic contraction elicited by EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min), confirming that the contractile responses were mediated by the release of Ach from postganglionic cholinergic nerves. The responses to EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) were also completely blocked after 10 min incubation with tetrodotoxin (1 μM), confirming their neuronal origin.

Effect of 5-hydroxytryptamine on cholinergic contraction in human airways in vitro

EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) of human airways resulted in rapid cholinergic contraction,

Fig. 1. – Trace showing the effect of 5-hydroxytryptamine (5-HT; 100 μM) on cholinergic contraction elicited by electrical field stimulation (EFS) (50 V at source, 0.5 ms, 1–32 Hz for 15 s every 4 min) in human airways in vitro. a) 5-HT produced marked facilitation of cholinergic contraction. b) The response to EFS in control tissues remained stable.
Table 2. – The effect of 5-hydroxytryptamine (5-HT) on electrical field stimulation (EFS)-induced contractile responses at different frequencies of stimulation in human airways in vitro

<table>
<thead>
<tr>
<th>Contractile response* %</th>
<th>1 Hz</th>
<th>2 Hz</th>
<th>4 Hz</th>
<th>8 Hz</th>
<th>16 Hz</th>
<th>32 Hz</th>
<th>5-HT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>5-HT</td>
<td>300 μM</td>
<td>8.5±3.8</td>
<td>104±6.6</td>
<td>19.4±8.4</td>
<td>104±15.9</td>
<td>35.6±11.2</td>
<td>104±15.9</td>
</tr>
<tr>
<td>5-HT</td>
<td>100 μM</td>
<td>4.7±1.0</td>
<td>104±6.3</td>
<td>12.1±2.9</td>
<td>104±4.0</td>
<td>23.4±3.9</td>
<td>104±2.9</td>
</tr>
<tr>
<td>5-HT</td>
<td>30 μM</td>
<td>12.2±2.7</td>
<td>104±6.8</td>
<td>18.8±3.9</td>
<td>104±5.7</td>
<td>28.4±5.7</td>
<td>104±5.7</td>
</tr>
<tr>
<td>5-HT</td>
<td>10 μM</td>
<td>13.2±2.5</td>
<td>104±6.5</td>
<td>15.4±2.4</td>
<td>104±5.8</td>
<td>23.6±3.9</td>
<td>104±5.5</td>
</tr>
</tbody>
</table>

Time control | 6.7±1.7 | 104±7.6 | 7.0±3.1 | 104±6.8 | 15.9±2.7 | 104±6.7 | 17.4±4.2 | 104±6.6 | 34.6±2.2 | 104±6.5 | 36.7±3.7 | 104±6.7 |

Data are presented as mean±SEM. *: % of maximal cholinergic contraction caused by EFS at 32 Hz; all stimulation at 50 V at source, 0.5 ms, 0.5±32 Hz for 15 s every 4 min. Pre: baseline response; Post: response after incubation with 5-HT. *: p<0.05; **: p<0.01; ***: p<0.001 versus control.

which increased in amplitude with increasing frequencies of stimulation. A typical trace is shown in figure 1, which also demonstrates the effect of 5-HT (100 μM) on the baseline tension as well as on cholinergic response. 5-HT produced no statistically significant effect on baseline tension in human airways, although there was a trend towards a small relaxatory effect at high concentrations (>100 μM). 5-HT (100 μM) facilitated cholinergic contraction at all frequencies of stimulation. When results were expressed as % facilitation, there was a more pronounced effect at lower frequencies of stimulation. However, when expressing the results as a percentage of the maximal contraction at 32 Hz, the increase was more pronounced at higher frequencies (table 2).

5-HT (10–300 μM, n=5) produced a concentration-dependent increase in cholinergic contraction induced by EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in human airways in vitro, with a maximum facilitatory effect of 104±13% at 1 Hz at a concentration of 300 μM (fig. 2).

The responses to EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in vehicle-treated tissues (n=8) remained stable throughout the period of the experiment. The different airway preparations behaved similarly in relation to the degree of facilitation of cholinergic contraction induced by 5-HT, as was evident from our data: 5-HT 100 μM at 16 Hz in airway strips versus bronchial ring segments: 31±9 (n=3) versus 27±5% facilitation (n=5, NS).

Effect of 5-hydroxytryptamine antagonists on 5-hydroxytryptamine-facilitation of cholinergic contraction in human airways in vitro

Addition of methysergide (1 μM, n=5), a 5-HT1,5-HT3, 5-HT7 antagonist, alone had no significant effect on either

Fig. 2. – Effect of 5-hydroxytryptamine (5-HT; 10–300 μM) on cholinergic contraction elicited by electrical field stimulation (EFS) (50 V at source, 0.5 ms, 0.5–32 Hz for 15 s every 4 min) in human airways in vitro. 5-HT produced a concentration-dependent facilitation of cholinergic contraction. Data are presented as mean±SEM. ●: 300 μM (n=5); ■: 100 μM (n=8); ▲: 30 μM (n=6); ▼: 10 μM (n=6); ◆: control tissues (n=10).

Fig. 3. – Facilitatory effect of 5-hydroxytryptamine (5-HT) 100 μM (■, n=8) on cholinergic contraction elicited by electrical field stimulation (EFS) (50 V at source, 0.5 ms, 0.5–32 Hz for 15 s every 4 min) in human airways in vitro and effect of pretreatment with selective 5-HT antagonists. Tropisetron 1 μM (○, n=5) inhibited the 5-HT (100 μM)-induced effect. Methysergide 1 μM (△, n=5) had no effect on facilitation of cholinergic contraction by 5-HT (100 μM). Pretreatment with either ondansetron 1 μM (▲, n=6) or GR 125478D 1 μM (▼, n=6) also had a significant inhibitory effect on facilitation of cholinergic contraction by 5-HT. The responses in control tissues (○, n=8) remained stable. Data are presented as mean±SEM. *: p<0.05; **: p<0.01; ***: p<0.001 versus 5-HT.
basal tone or cholinergic contraction elicited by EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in human airways in vitro. Methysergide also failed to prevent the 5-HT-induced increase in cholinergic contraction (fig. 3).

Tropisetron (1 μM, n=5), a 5-HT₃ antagonist, alone had no significant effect on either basal tone or cholinergic contraction induced by EFS in human airways in vitro (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) but completely blocked the facilitatory effect of 5-HT (100 μM, n=5) on cholinergic contraction (fig. 3).

Ondansetron (1 μM, n=6), a 5-HT₃ antagonist, and GR 125478D (1 μM, n=6), a 5-HT₄ antagonist, alone did not significantly affect basal tone or cholinergic contraction produced by EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in human airways in vitro. However, both antagonists attenuated the facilitation of cholinergic contraction by 5-HT (100 μM, n=6) (fig. 3).

The responses to EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in vehicle-treated tissues (n=6) remained stable throughout the period of the experiment.

**Effect of 5-hydroxytryptamine agonists on cholinergic contraction in human airways in vitro**

The effects of selective 5-HT agonists are shown in figure 4, which depicts the % facilitation of cholinergic contraction elicited by EFS at 2 Hz (50 V, 0.5 ms, 15 s) in human airways in vitro. 5-Methoxytryptamine (1–100 μM, n=6) and RS67333 (1–100 μM, n=6), both agonists at the 5-HT₄ receptor, and also 2-methyl-5-HT (100 μM, n=6), a 5-HT₃ agonist, produced concentration-dependent facilitation of cholinergic contraction similar to the effects of 5-HT (fig. 4). Sumatriptan (100 μM, n=5), a 5-HT₁₈/₁₉D agonist, and RU-24969 (100 μM), a 5-HT₁₅/₁₁B agonist, had no effect on cholinergic contraction (data not shown).

The responses to EFS (50 V, 0.5 ms, 2 Hz for 15 s) in vehicle-treated tissues (n=6) remained stable throughout the period of the experiment.

**Effect of fluoxetine and 5-hydroxytryptamine on cholinergic contraction in human airways in vitro**

Fluoxetine (10 μM, n=6) alone had no effect on either basal tone or on cholinergic contraction elicited by EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in human airways in vitro. Fluoxetine (10 μM, n=5) also failed to modulate 5-HT (10–100 μM)-induced facilitation of cholinergic contraction in human airways in vitro (fig. 5). The responses to EFS (50 V, 0.5 ms, 1–32 Hz for 15 s every 4 min) in vehicle-treated tissues (n=6) remained stable throughout the period of the experiment.

**Effect of 5-hydroxytryptamine on the concentration/response curve to acetylcholine in human airways in vitro**

Ach (30 nM–30 mM) produced a concentration-dependent contraction of human airways with a maximum contraction tension of 1.9±0.2 g. Pretreatment with 5-HT (300 μM) had no significant effect on contractile responses to incremental concentrations of Ach (30 nM–30 mM) in human airways in vitro (n=5, NS) compared to contraction to Ach in control tissues (fig. 6).

**Discussion**

Activation of parasympathetic nerves results in the release of acetylcholine, which induces airway smooth muscle contraction through stimulation of muscarinic M₃ receptors [19]. Release of Ach upon nerve activation has been shown to be modulated by several endogenous substances including certain peptides and autacoids, such
as 5-HT [19]. Numerous reports have demonstrated that 5-HT is able to potentiate parasympathetic effects on airway smooth muscle via the efferent vagal pathway. 5-HT induced bronchoconstriction in dogs in vivo, which was inhibited by cooling of the vagus nerve [20] or by atropine pretreatment [21]. It was also shown that 5-HT enhanced bronchoconstriction induced by vagal stimulation in dogs in vivo, whereas the bronchoconstrictor response to exogenous Ach remained unchanged [22]. These data suggested an interaction of 5-HT with the parasympathetic nerves in human airways, confirmed by the lack of effect of methysergide on 5-HT-induced facilitation of the cholinergic contraction by both selective 5-HT3 and 5-HT4 antagonists. 5-HT1-selective agonists mimicked the effect of 5-HT on cholinergic contraction in human airways, but this effect was only evident at relatively high concentrations. Although 5-HT4 agonists (5-methoxytryptamine, RS67333) were somewhat more potent than the 5-HT3 agonist (2-methyl-5-HT), the pharmacological profile of the receptor involved in the present study does not agree exactly with the profile of a 5-HT4 receptor subtype, as a facilitatory effect by selective 5-HT3 agonists was also noted. This is in agreement with the significant inhibition of 5-HT-induced facilitation of the cholinergic contraction by both selective 5-HT3 and 5-HT4 antagonists. 5-HT1-selective agonists did not affect cholinergic contraction in human airways, confirmed by the lack of effect of methysergide on 5-HT-induced facilitation. Contrary to the assumption of Takahashi et al. [10], these data, therefore, suggest involvement of both prejunctional 5-HT3 and 5-HT4 receptors located on the postganglionic cholinergic nerves in the potentiation of cholinergic contraction.

Excitatory 5-HT3 and 5-HT4 receptors on postganglionic parasympathetic nerves have also been demonstrated in isolated guinea-pig colon [28], ileal circular muscle [29] and stomach preparations [30]. Similarly, facilitation of cholinergic neurotransmission in strips of isolated human detrusor muscle [31] as well as in rat stomach fundus [32] is mediated through stimulation of 5-HT4 receptors.

In the present study, 5-HT had no significant effect on baseline tension nor on Ach-induced contractions. These findings are in agreement with previous data from Lulich and Paterson [33] who also failed to demonstrate a significant response to 5-HT (1–100 µM) in bronchial smooth muscle preparations. Raffestin et al. [34], on the other
hand, observed that 5-HT (100 nM–10 μM) caused relaxation of bronchial preparations precontracted with Ach (50 μM). In this study, however, large bronchial preparations were treated under an initial load of 5 g, in contrast to 2 g in the present study. As demonstrated previously, relaxant responses in human airways in vitro increase in magnitude with increasing resting tension [35], which could account for this discrepancy from the present findings.

Significant neuronal uptake of 5-HT into sympathetic nerve terminals as well as extraneuronal uptake of 5-HT has been shown in various preparations [36]. In mouse trachea, pretreatment with the 5-HT uptake inhibitor fluvoxamine tended to increase the potentiation of cholinergic contraction by 5-HT, although this effect did not reach significance [7]. In guinea-pig airways in vitro fluoxetine, a selective 5-HT uptake inhibitor, significantly facilitated 5-HT-induced inhibition of NANC contraction [17]. In the present study, however, fluoxetine, at a concentration which was much higher than the concentration required to inhibit 3H-5-HT uptake in rat brain synaptosomal preparations [37], failed to modulate 5-HT-induced facilitation of cholinergic contraction in human airways in vitro. These data argue against the presence of an important 5-HT uptake mechanism in human airways. In conclusion, evidence has been found for the existence of both prejunctional 5-HT₃ and 5-HT₄ receptors on postganglionic cholinergic nerves, which enhance the EFS-induced cholinergic contraction in human airways in vitro.

Although the significance of 5-hydroxytryptamine in the pathophysiology of obstructive airway diseases remains to be elucidated, the elevated plasma level in asthmatic patients [13] and the release of 5-hydroxytryptamine from platelets during allergic reactions [38] may have a role in facilitating bronchoconstriction in these conditions.

References

28. Brieger MR, Schuurkes JA. 5-HT₃ and 5-HT₄ receptors and cholinergic and tachykinergic neurotransmission in


