

Modifications of plasma 5-HT concentrations during the allergic bronchoconstriction in guinea pigs

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ABSTRACT

Several contractile mediators involved in the antigen-induced airway obstruction have been identified, but the role of 5-HT (5-hydroxytryptamine or serotonin) has been scantily investigated. In this work, the potential role of 5-HT in the allergic bronchoconstriction was evaluated through a pharmacological approach and plasma 5-HT measurement in blood samples from the right and left ventricles of anesthetized guinea-pigs. Intravenous 5-HT caused a dose-dependent increase of the lung resistance in anesthetized, nonsensitized guinea pigs. Likewise, in sensitized animals the antigenic challenge with ovalbumin also caused a transient bronchoconstriction ($356 \pm 60\%$ the basal value), which was largely inhibited by the blockade of serotonergic receptors with methiothepin plus tropisetron ($134 \pm 10\%$, $P = .007$). Sensitized animals tended to have plasma 5-HT concentrations higher than nonsensitized controls, and shortly after the peak of the allergic bronchoconstriction the 5-HT levels in the left ventricle (blood flowing out from lungs) tended to be higher than in the right ventricle (blood entering the lungs), although data dispersion precluded the obtaining of statistical significance. Interestingly, the degree of bronchoconstriction highly correlated with the concentrations of 5-HT found in the left ventricle and measured either in platelet-rich plasma ($r = 0.97$, $P = .007$) or platelet-poor plasma ($r = 0.97$, $P = .006$). After the obstructive response subsided these correlations were lost, but now the degree of bronchoconstriction turned to be correlated with 5-HT concentration in platelet concentrate ($r = 0.76$, $P = .03$). In conclusion, our results suggested that 5-HT is actively released from lungs during the antigenic challenge and that this autacoid is involved in the generation of the airway obstruction.

KEYWORDS 5-hydroxytryptamine, asthma, bronchoconstriction, bronchospasm, serotonin

INTRODUCTION

Asthma is a highly prevalent disease in many regions of the world, being estimated that ~235 million people suffer this disease worldwide [1]. In roughly, half of them an underlying mechanism of allergy is involved not only in the persistence of airway inflammation but also in the triggering of acute airway obstruction after the exposure to the offending

allergen [2]. In experimental conditions asthmatic patients submitted to an antigenic bronchial challenge usually develop a bronchoconstriction which progressively subside in the next few hours [3]. Animal models of allergic bronchoconstriction closely resemble this phenomenon [4].

A number of mediators potentially involved in the development of this acute obstructive response have been identified, including histamine, acetylcholine, prostaglandin D₂ and cysteinyl leukotrienes, but the role of 5-HT (5-hydroxytryptamine or serotonin) has been scantily investigated. In in vitro conditions 5-HT causes airway smooth muscle contraction in humans and several animal species through direct stimulation of 5-HT_{2A} receptors in myocytes [5, 6],

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but also by indirect means such as facilitation of acetylcholine release through stimulation of prejunctional 5-HT₄ and 5-HT₇ receptors [7, 8]. Although inhaled 5-HT does not cause bronchoconstriction in healthy, atopic, or asthmatic subjects [9], there are some reports suggesting that this autacoid participates in asthmatic responses. Thus, Lechin *et al.* [10] found that plasma concentrations of free 5-HT notably increased during an asthmatic exacerbation, and this increment was related to the severity of bronchoconstriction. This same research group also found that tianeptine, a drug that lowers plasma 5-HT, improves pulmonary function in asthmatic children [11]. Additionally, in a recent work, Dürk *et al.* reported increased levels of 5-HT in the bronchoalveolar lavage of sensitized mice or allergic asthma patients 24 hours after being submitted to an antigenic challenge [12]. Finally, in a previous study, we found that 5-HT participates in the generation of airway hyperresponsiveness developed after an antigenic challenge in sensitized guinea pigs [8].

Therefore, the aim of the present work was to evaluate the potential role of 5-HT during the allergic bronchoconstriction in guinea pigs by using a pharmacological approach and by measuring 5-HT concentrations in blood samples from the right and left ventricles (*i.e.*, blood entering and leaving the lungs, respectively).

MATERIALS AND METHODS

Study Design

In all experiments, bronchoconstrictor responses were evaluated by continuously measuring lung resistance (RL) in a plethysmograph for anesthetized animals, as described below.

In a first set of experiments, a group of non-sensitized guinea pigs received noncumulative doses of 5-HT (0.18–5.6 µg/kg) in order to evaluate the bronchoconstrictor effect of this autacoid. The dose-response curve was finished when RL was threefold the basal value. In the remaining experiments, guinea pigs sensitized to ovalbumin (OA) were submitted to an antigenic challenge with 400 µg/kg OA *i.v.*, a dose that in this animal model usually produces an acute maximal bronchoconstriction in 2–6 min, with a gradual return to basal values in ~15–30 min. To determine the functional role of 5-HT in the allergic bronchoconstriction, sensitized guinea pigs received a combination of 300 µg/kg methiothepin (antagonist of 5-HT₁, 5-HT₂, 5-HT₅, 5-HT₆, and 5-HT₇ receptors) [13, 14] plus 3 mg/kg tropisetron (antagonist of 5-HT₃ and 5-HT₄ receptors) [15–17], both admin-

istered by the *i.v.* route approximately 10 min before the antigenic challenge. Finally, to quantify possible changes in 5-HT concentrations blood samples were obtained from sensitized guinea pigs before the antigenic challenge (*prechallenge group*), 10 min after the antigenic challenge (*challenge group*), or 1 hour after the RL returned to basal values (*postchallenge group*). Additionally, a *control group* of nonsensitized guinea pigs (with similar weight as the other groups) were also studied.

Animals

Male Hartley guinea pigs (320–400 g body weight) bred in our laboratory animal facilities (conditioned filtered air, 21°C ± 1°C, 50%–70% humidity, sterilized bed, 12/12 hours light/dark cycles) and fed *ad libitum* were used. The protocol was approved by the Scientific and Ethics Committee (approval B32–10) of the Instituto Nacional de Enfermedades Respiratorias.

Sensitization Procedure

On day 0, animals received 60 µg OA plus 1 mg aluminum hydroxide as adjuvant, both dissolved in saline solution and equally applied by the intraperitoneal and subcutaneous routes. On days 7 and 15, booster inhalations of OA in saline were administered (3 mg/mL during 15 min and 1 µg/mL OA during 60 s, respectively). All sensitized animals were studied at day 21.

Measurement of Lung Resistance

Guinea pigs were anesthetized with 35 mg/kg sodium pentobarbital intraperitoneally, and once a jugular vein was cannulated the deep of the anesthesia was maintained through additional hourly doses (~9 mg/kg, *i.v.*). Trachea was cannulated and connected to a mechanical ventilator (model 50–1700, Harvard Apparatus, Bournemouth, England) to receive a tidal volume of 10 mL/kg at 48 breaths/min. Pancuronium bromide (0.06 mg/kg *i.v.*) was administered to each guinea pig to suppress voluntary movements. Esophagus was also cannulated to obtain a surrogate of intrapleural pressure, and pressure signals from the trachea and esophagus were related through a differential pressure transducer (SX01DN SenSym, CA, USA) to obtain transpulmonary pressure. The guinea pig was then introduced into a plethysmograph chamber for anesthetized animals (Buxco, Wilmington, NC, USA). A second differential pressure transducer (SCXL004DN SenSym, CA, USA) was connected to the chamber to measure

flow, and finally both differential pressure signals were related through a software (Buxco Biosystem XA v1, Wilmington, NC, USA) to obtain RL.

Blood Sampling

Blood samples from the right and left ventricles were obtained, allowing us to evaluate 5-HT concentrations in blood before and after passing through the pulmonary vascular bed, respectively. To this end, after medial sternotomy and heart exposure, a direct cardiac puncture to each ventricle was performed. Because 5-HT is a photosensitive drug, samples were immediately put in 1.5 mL amber microtubes (model MCT-150-X, Axygen, Union City, CA, USA) to protect them from light. Each tube had ACD anticoagulant (citric acid, sodium citrate and dextrose) and pargyline (inhibitor of the monoamine oxidase B, final concentration 19 $\mu\text{g/mL}$). Samples were deproteinized by adding a mixture of 0.1 M perchloric acid and 4 nM sodium metabisulphite (1:1 ratio). Afterward, the tubes were centrifuged at 2500 rpm during 3 min to obtain the platelet-rich plasma (PRP). The platelet-poor plasma (PPP) was obtained by further centrifugation of an aliquot of the PRP at 6000 rpm during 15 min. The pellet obtained after this last procedure represents a platelet concentrate (PC), and was resuspended in 500 μL of perchloric acid and submitted to sonication (model 100, Fisher Scientific, Hanover Park, IL, USA). Finally, all samples were filtered through 45 μm hydrophilic membranes (Millipore Corporation, Billerica, MA, USA) and stored at -80°C until their study.

Measurement of 5-HT Concentration

The 5-HT concentrations were determined by fluorometric detection according to the method described by Peat and Gibb [18]. An HPLC (model 510, Waters, Milford, MA, USA) with an injection valve (Valco Instrument Co., Houston, TX, USA) and a fluorometer (model 474, Waters) was used with an 18 $\mu\text{L/min}$ flow. All experiments were carried out with excitation/emission wavelengths of 290/330 nm. Detection signal was registered at 10 mV input. A reverse phase, 150 mm $\times$ 3.9 mm, 5 μm particle size, Ultraesfera ODS column was used (model Symmetry C18, WAT046980, Waters). No precolumn was used. The mobile phase was separated with 0.02 M potassium phosphate monobasic, 1 g/L heptasulphonic acid, adjusting pH to 3.3 using phosphoric acid, in addition to a dilution of methanol in distilled water (3:2), the latter representing 34% of the phase. A standard curve to 5-HT diluted in 0.1 M perchloric acid and 4 mM metabisulphite sodium was per-

formed with 5-HT concentrations of 0.4, 0.2 and 0.002 ng/ μL .

Drugs

Ovalbumin (grade II), 5-HT (serotonin hydrochloride), methiothepin mesilate, and tropisetron chlorhydrate were acquired from Sigma-Aldrich Co. (St. Louis, MO, USA).

Data Analysis

After normal distribution of interval variables was corroborated, a parametric approach for data analysis was used. Thus, data was expressed as mean $\pm$ standard error of the mean, and statistical differences were estimated through paired Student's *t*-test and analysis of variance followed by Tukey test. Associations were evaluated through Pearson correlation coefficient. Statistical significance was set at $P < .05$ bimarginally.

RESULTS

In nonsensitized guinea pigs, i.v. administration of increasing doses of 5-HT produced a dose-dependent bronchoconstriction, with a maximum RL of 2.23 ± 0.42 cm $\text{H}_2\text{O/mL/second}$ (mean $\pm$ standard error, $n = 4$), which was statistically higher than the basal RL value (0.54 ± 0.11 cm $\text{H}_2\text{O/mL/second}$, $P = .01$, Figure 1). The degree of this bronchoconstriction corresponded to ~ 4 fold the basal RL value (i.e., 416%).

Concerning the allergic bronchoconstriction, i.v. administration of the specific allergen (OA, 400 $\mu\text{g/kg}$) to sensitized animals caused a sudden RL increment of $356 \pm 60\%$ the basal value ($n = 5$) in the next 2–6 mins. administration of 300 $\mu\text{g/kg}$ methiothepin plus 3 mg/kg tropisetron before the antigenic challenge importantly diminished the bronchoconstrictor response ($134 \pm 10\%$, $n = 5$, $P = .007$, not illustrated), pointing out that 5-HT played a central role in this response.

Plasma concentration of 5-HT showed a relatively high dispersion of values in PRP, PPP, and PC; which precluded the obtaining of statistical significance. Nevertheless, there were some tendencies worthy to be mentioned. Once the sensitization procedure was concluded (day 21) the 5-HT concentration in the prechallenge group tended to be higher than the levels found in nonsensitized control animals, especially in samples of PRP and PPP (Figure 2A and 2B). Likewise, during the antigenic challenge 5-HT concentrations in blood samples obtained

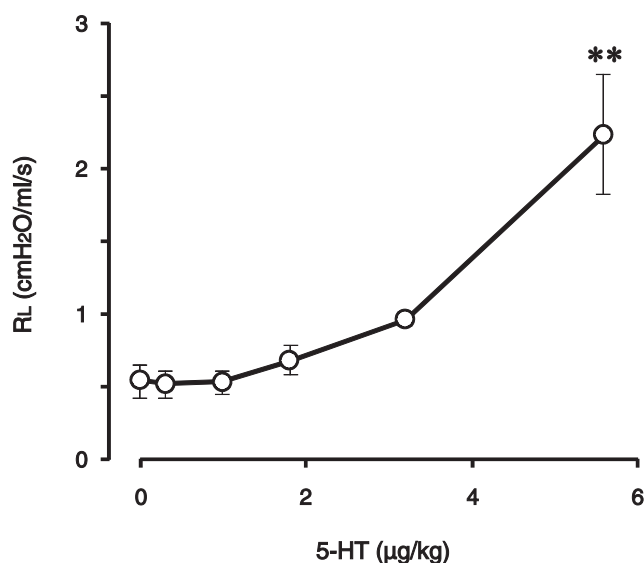


FIGURE 1. Bronchoconstrictor effect of 5-HT in guinea pigs. Administration of non-cumulative i.v. doses of 5-HT caused a progressive increment of lung resistance (RL). Symbols correspond to mean, and vertical lines to standard error of 4 animals. ** $P = .01$ (ANOVA followed by Tukey test).

from the left ventricle (blood flowing out from lungs) tended to be higher than those from the right ventricle (blood entering the lungs), which was more noticeable in the PC compartment (Figure 2C).

The 5-HT concentrations measured in samples of blood coming from lungs had a high correlation with the maximum bronchoconstriction observed during antigenic challenge (challenge group), either in PRP ($r = 0.97$, $P = .007$, $n = 5$, Figure 3A) or PPP ($r = 0.97$, $P = .006$, $n = 5$, Figure 3C), suggesting that total or free 5-HT played a role in the generation of allergic bronchoconstriction. After the allergic bronchoconstriction subsided (postchallenge group) these relationships were lost, but the 5-HT concentration in PC now became positively correlated with the degree of bronchoconstriction ($r = 0.76$, $P = .03$, Figure 3F).

DISCUSSION

In the present work, we found data suggesting that 5-HT participates in the allergic bronchoconstriction. We showed that 5-HT is capable to produce an acute airway obstruction in guinea pigs, and that blockade of serotonergic receptors almost fully abolished the bronchoconstrictor response to the antigenic challenge. On the other hand, we found that plasma concentrations of 5-HT, measured shortly after the peak of an allergic bronchoconstriction, had a

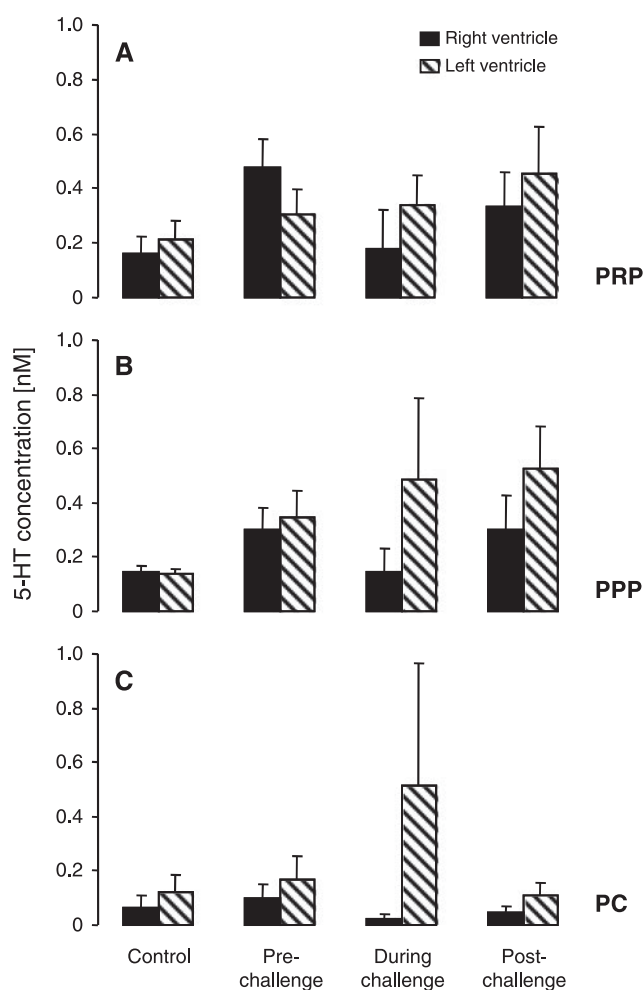


FIGURE 2. Effect of antigenic challenge on the plasma 5-HT concentrations. After i.v. administration of 400 µg/kg OA to nonsensitized (control group) or sensitized (remaining groups) guinea pigs, blood samples were obtained from right and left ventricles and 5-HT measured in platelet-rich plasma (PRP, panel A), platelet-poor plasma (PPP, panel B), and platelet concentrate (PC, panel C). Bars correspond to mean, and vertical lines to standard error of 5–9 animals. There were no differences among groups (ANOVA and Tukey tests).

very high correlation with the maximum increase of RL. Interestingly, this association was only observed with 5-HT levels quantified in the blood coming from lungs but not in the blood collected before entering the lungs, which suggests that 5-HT was actively released by lung tissue. This speculation is in agreement with a previous study showing that sensitization per se caused a ninefold increase in the 5-HT concentration present in the lung parenchyma, with a sharp drop after the antigenic challenge [8]. In such experiments, intravascular blood was completely removed from the lungs, and thus the 5-HT source was the pulmonary tissue. The specific cell responsible of this

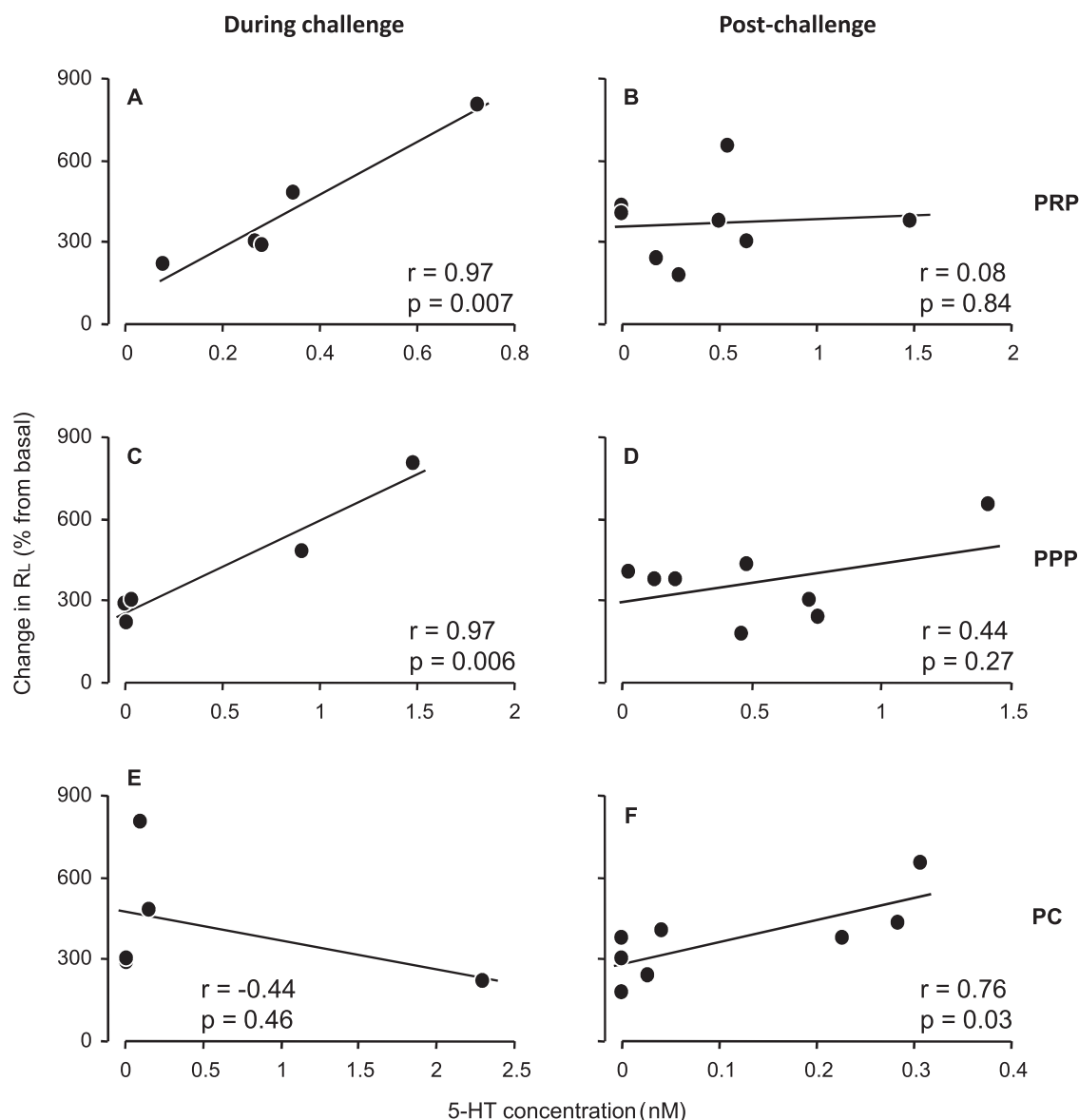


FIGURE 3. Relationship between plasma 5-HT concentrations and maximum allergic bronchoconstriction. All blood samples were obtained from the left ventricle shortly after the maximum increase of lung resistance (RL) was reached (A, C, E), or 1 hour after the allergic bronchoconstriction was subsided (B, D, F). The 5-HT concentrations were measured in platelet-rich plasma (PRP, panels A and B), platelet-poor plasma (PPP, panels C and D), and platelet concentrate (PC, panels E and F). The Pearson correlation coefficient (r) and its statistical significance are showed in each panel.

5-HT release is unknown at present. Among potential candidates, neuroendocrine cells or neuroepithelial bodies have a well-known capacity to produce 5-HT [19], and thus might play a major role in 5-HT release. In the other hand, although in some animal species pulmonary mast cell are capable to produce and release 5-HT [20], such production has not been convincingly described in guinea pigs, and thus in our study a possible role of mast cell as source of 5-HT remains speculative.

Recently, Dürk et al. elegantly demonstrated a pivotal role of 5-HT released by platelets in the development of allergic inflammation and airway hyper-responsiveness in sensitized mice. In this same work, authors also studied patients with allergic asthma and found that a local antigenic challenge was accompanied by increased 5-HT levels detected in the bronchoalveolar lavage. Interestingly, they found a simultaneous decrease in serum 5-HT levels, suggesting that 5-HT was translocated from platelet stores into

the lung [9]. Thus, in our study platelets might have been an alternative source of 5-HT. This possibility would be in line with a study by Yoshida *et al.* [21] who found that in sensitized mice platelets almost immediately accumulate in the lungs after an antigenic challenge, with subsequent release of 5-HT.

An additional finding from our study was that the correlation of 5-HT levels and the degree of bronchoconstriction was only observed in plasma samples (PRP and PPP), but not in platelets (PC). However, 1 hour after the obstruction was finished the maximal bronchoconstriction was related only with 5-HT levels contained in platelets, but not in plasma. It is well known that platelets are the main elements responsible of the reuptake of intravascular 5-HT [22], so these results may imply that 5-HT released during the antigenic challenge was actively reuptaked by platelets.

In our study, the antigenic challenge was accomplished through the i.v. route, which of course could release 5-HT from wider sources than if it were by inhalation. Thus, our conclusions may differ from those obtained with an antigenic challenge delivered by the inhalatory route, and further studies are required to elucidate this.

In conclusion, our results suggested that 5-HT is actively released from lungs during the antigenic challenge and that this autacoid is involved in the generation of the airway obstruction.

Declaration of interest: Authors have no conflict of interests of any kind to declare in relation to this manuscript. The authors alone are responsible for the content and writing of the paper.

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