

Allergic sensitization modifies the pulmonary expression of 5-hydroxytryptamine receptors in guinea pigs

Guadalupe Córdoba-Rodríguez^a, Mario H. Vargas^a, Víctor Ruiz^b, Verónica Carbajal^a, Patricia Campos-Bedolla^c, Paulina Mercadillo-Herrera^d, José Luis Arreola-Ramírez^a, Patricia Segura-Medina^{a,*}

^a Departamento de Investigación en Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias, México DF, Mexico

^b Laboratorio de Biología Molecular, Departamento de Investigación en Fibrosis Pulmonar, Instituto Nacional de Enfermedades Respiratorias, México DF, Mexico

^c Unidad de Investigación Médica en Enfermedades Neurológicas, Hospital de Especialidades, Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México DF, Mexico

^d Instituto Tecnológico y de Estudios Superiores de Monterrey, Campus Ciudad de México, México DF, Mexico

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ABSTRACT

There is mounting evidence that 5-hydroxytryptamine (5-HT) plays a role in asthma. However, scarce information exists about the pulmonary expression of 5-HT receptors and its modification after allergic sensitization. In the present work, we explored the expression of 5-HT_{1A}, 5-HT_{2A}, 5-HT₃, 5-HT₄, 5-HT_{5A}, 5-HT₆, and 5-HT₇ receptors in lungs from control and sensitized guinea pigs through qPCR and Western blot. In control animals, mRNA from all receptors was detectable in lung homogenates, especially from 5-HT_{2A} and 5-HT₄ receptors. Sensitized animals had decreased mRNA expression of 5-HT_{2A} and 5-HT₄ receptors and increased that of 5-HT₇ receptor. In contrast, they had increased protein expression of 5-HT_{2A} receptor in bronchial epithelium and of 5-HT₄ receptor in lung parenchyma. The degree of airway response to the allergic challenge was inversely correlated with mRNA expression of the 5-HT_{1A} receptor. In summary, our results showed that major 5-HT receptor subtypes are constitutively expressed in the guinea pig lung, and that allergic sensitization modifies the expression of 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors.

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1. Introduction

5-Hydroxytryptamine (5-HT, serotonin) is a neurotransmitter synthesized in the central nervous system, which is involved in numerous physiological responses and behavioral disorders (Filip and Bader, 2009). In non-neural tissues, 5-HT is synthesized, stored, and released mainly by enterochromaffin cells (Gershon and Tack, 2007). The majority of 5-HT released into the blood is captured by and stored in platelets, from which it can be released in any organ (Duerschmied et al., 2013). 5-HT plays a fundamental role in the functioning of the gastrointestinal tract and vascular system, and in the lungs it appears to be involved in chronic inflammatory diseases (Nichols and Nichols, 2008). Nearly all of the 5-HT found in the lung

is released by intravascular platelets, but an additional source of 5-HT can be the neuroendocrine cells/neuroepithelial bodies located in airway epithelia (Cazzola and Matera, 2000; Livermore et al., 2015).

There are seven families of 5-HT receptors (5-HT_{1–7}), comprising 15 receptor subtypes, through which 5-HT exerts its physiological or pathological effects (IUPHAR/BPS, 2015). All 5-HT receptors are metabotropic receptors, i.e., associated with G proteins, except for 5-HT₃, which is a ligand-gated ionotropic receptor. The type of G protein coupled to the 5-HT receptor determines its mode of action. 5-HT₁ and 5-HT₅ receptors are coupled to G_{i/o}, thus inducing diminution of cAMP. 5-HT₂ receptor is coupled to G_{q/11}, thus leading to Ca²⁺ mobilization. Finally, 5-HT₄, 5-HT₆, and 5-HT₇ receptors are coupled to G_s, hence favoring cAMP production.

From the pathophysiological point of view, asthma is characterized by two phenomena, namely increased airway responsiveness and intermittent periods of acute or subacute airway obstruction. Some human and animal studies suggest that 5-HT might play a role in both phenomena. With respect to airway responsiveness, Lechin et al. (1998) found that tianeptine, a drug that lowers plasma 5-

* Corresponding author at: Departamento de Investigación en Hiperreactividad Bronquial, Instituto Nacional de Enfermedades Respiratorias, Calzada de Tlalpan 4502, CP 14080, México DF, Mexico.

E-mail addresses: psegura@unam.mx, psegura@iner.gob.mx (P. Segura-Medina).

HT levels, improved pulmonary function in children with asthma. In guinea pigs sensitized to ovalbumin (OA), we found that 5-HT levels in lung homogenates increased 9-fold as compared with non-sensitized animals (Segura et al., 2010). In this same study, we found that 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors were involved in the development of non-specific, allergen-induced airway hyper-responsiveness.

Regarding the potential involvement of 5-HT in acute or sub-acute airway obstruction, it has been found that during an asthma exacerbation, plasma concentrations of free 5-HT notably increase and correlate with the severity of airway narrowing (Lechin et al., 1996). In another study, a local antigenic challenge in patients with allergic asthma was associated with an increment of 5-HT levels in the bronchoalveolar lavage 24 h later (Durk et al., 2013). Finally, we found that 5-HT was actively released by guinea pig lung during an antigenic challenge, and that this release was related with the degree of the bronchoconstriction (Arreola-Ramírez et al., 2013). Although these last studies suggest that 5-HT may be involved in the development of airway obstruction, there is scant published information about the pulmonary expression of 5-HT receptors, either under healthy conditions or in the presence of allergic sensitization, and on how this expression is related with the degree of allergic airway obstruction. Therefore, in the present study, we used a guinea pig model of allergic asthma to explore the mRNA and protein expression of major 5-HT receptors, and to evaluate to what extent mRNA expression correlates with the degree of acute allergic bronchoconstriction. Taking into account our previous studies, we hypothesized that the same 5-HT receptors found to be involved in airway hyperresponsiveness, i.e., 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors (Segura et al., 2010), may be also involved in the acute airway obstruction.

2. Materials and methods

Male Hartley guinea pigs bred at our laboratory animal facilities under conventional conditions (conditioned filtered air, $21 \pm 1^\circ\text{C}$, 50–70% humidity, sterilized bed, and 12/12 h light/dark cycles) were utilized. The experimental group comprised guinea pigs (350–400 g body weight) submitted to the sensitization procedure described in the following section, while the non-sensitized control group comprised healthy guinea pigs exclusively used for the purpose of the present study and weighing 500–650 g, a body weight similar to that of experimental animals at the end of the sensitization procedure. For the analysis of mRNA expression, lung homogenates from 12 control and 12 sensitized guinea pigs were used (each individual lung homogenate served for the analysis of several 5-HT receptors). Regarding protein determination, tissues from 15 control and 12 sensitized animals were employed, taking into account that each Western blot determination required the pooled tissues from three guinea pigs. While both control and sensitized animals provided tissue for qPCR and Western blot analyses, only the latter group was submitted to barometric plethysmography to evaluate the airway response to an antigenic challenge. The protocol was approved by the institutional review boards of the Instituto Nacional de Enfermedades Respiratorias (approval B17-12) and the Instituto Mexicano del Seguro Social (approval 2010-785-033). The study was conducted following the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health, revision 2011.

2.1. Sensitization procedure and barometric plethysmography

At commencement of the sensitization process (day 0), animals received 60 µg OA (cat. A-5253; Sigma-Aldrich Co., St. Louis, MO, USA) plus 1 mg aluminum hydroxide as adjuvant, both

dissolved in 1 ml saline and administered in equal proportions by intraperitoneal and subcutaneous routes. On days 7 and 15, booster inhalations of aerosolized OA in saline were administered (3 mg/ml during 5 min, and 1 mg/ml during 60 s, respectively, both delivered at a flow rate of 2 ml/min). Aerosols were generated with an ultrasonic nebulizer (model WH-2000, Guangdong Yuehua Medical Instrument Factory Co., Ltd., Guangdong, China, particle diameter 1–5 µm). These booster stimuli were conducted with the animal placed inside a whole-body, single-chamber, barometric plethysmograph for freely moving animals (Buxco Electronics Inc., Troy, NY, USA). The basis of barometric plethysmography has been comprehensively described elsewhere (Drorbaugh and Fenn, 1955; Hamelmann et al., 1997). In this technique, the equipment records several respiratory parameters in a non-invasive fashion, and from these it calculates a unitless index known as enhanced pause (Penh), which has been shown to be an acceptable surrogate of airway resistance (Vargas et al., 2010). Two 5-min periods of Penh measurements were recorded under baseline conditions and shortly (1 min) after OA inhalation, respectively. In our experience, at day 15 the OA challenge usually causes a 2- to 6-fold increment of the Penh with respect to their baseline value (which typically ranges from 0.22 to 0.32), and this increment is associated with some degree of respiratory distress (thoraco-abdominal dissociation). This airway response (ΔPenh , i.e., post-challenge Penh minus baseline Penh) was considered an indicator of the degree of allergic sensitization. Because the equipment calculates the Penh for each breath, adjustments were made to the software in order to average Penh values from all breaths occurring during 15 s, and then to average these values during the 5-min period. All animals were studied for expression of 5-HT receptors on days 21–24 (at this time period, animals are considered to be fully sensitized, and any potential change caused by antigen exposure at day 15 has subsided). All solutions were prepared on the same day of their use.

2.2. Tissue procurement

On the study day, the guinea pig was deeply anesthetized with intraperitoneal injection of pentobarbital sodium (35 mg/kg), the chest was opened, and the pulmonary artery was cannulated through the right ventricle. After the left atrium was cut, a low-pressure constant flow of ~18 ml/min saline was delivered to the lungs until intravascular blood was completely drawn. Thereafter, the lungs were obtained and the right cranial lobe was excised, immediately placed into an RNAlater® solution (cat. AM7021; Ambion, Austin, Texas, USA) and in less than an hour it was stored at -70°C until its analysis by quantitative real-time polymerase chain reaction (qPCR).

On the other hand, for protein content determination by Western blot, the tracheal epithelium, the tracheal smooth muscle, and the bronchial epithelium were obtained as follow. The whole trachea was excised, opened by a longitudinal cut through the cartilage rings, and fixed with pins in a Petri dish with agar containing Krebs solution (composition [mM]: NaCl 120, NaHCO₃ 25, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, CaCl₂ 2.5, and glucose 11) aerated with oxygen. Under these conditions, the epithelium was obtained by lifting the most superficial membrane with surgical forceps and gently pulling it apart from the trachea. From the remaining tracheal tissue, the smooth muscle was dissected after removing the excessive connective tissue. The bronchial tree was dissected up to the third or fourth bronchi generation by eliminating the surrounding lung parenchyma, and it was subsequently processed as described for the trachea until the epithelium was obtained. Finally, a fragment of lung parenchyma was obtained from the left diaphragmatic lobe. Because the available amount of tracheal and bronchial tissue from each animal was so scant, each Western blot determination was performed in a pool from tissues obtained from three guinea pigs.

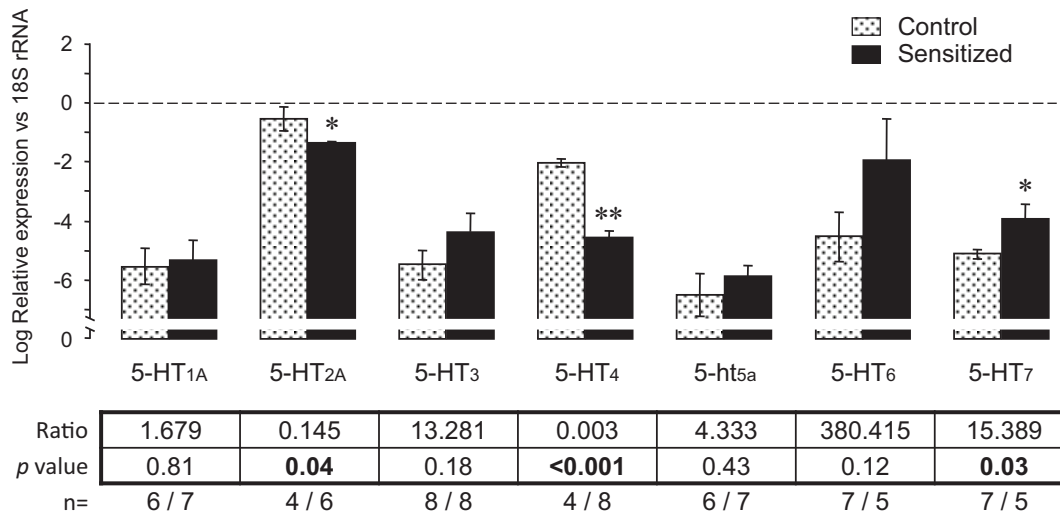


Fig. 1. Relative expression of 5-HT receptors mRNA in lung homogenates from guinea pigs with or without sensitization to ovalbumin. Each bar corresponds to the expression of a 5-HT receptor mRNA in either non-sensitized control (grey bars) or sensitized (closed bars) animals, normalized by the constitutive gene (ribosomal 18S rRNA) and calculated with the $2^{-\Delta CT}$ method (geometric mean $\pm$ standard error). The inset table shows the corresponding normalized expression of 5-HT receptor mRNA (sensitized/control ratio) calculated with the $2^{-\Delta\Delta CT}$ method. The number of control and sensitized animals for each group is showed at the bottom of the table (n). The horizontal broken line indicates the baseline level of expression of the constitutive gene (18S rRNA).

In some animals, a sample of brain tissue was obtained to serve as a positive control for Western blotting of 5-HT₇. All of these tissues were placed separately into cryogenic tubes, immediately frozen in liquid nitrogen, and then stored at -70°C until their analysis by Western blot.

2.3. Quantitative PCR

Total RNA was extracted from lung tissue using TRIzol reagent (cat. 10296028; Life Technologies, Grand Island, NY, USA). RNA quality was assessed by resolution on denatured 1% agarose gels and measurement of absorbance ratios at 260/280 nm. Complementary DNA (cDNA) was synthesized using 1 μg of total RNA, 1 μg of DNase, and 1 μl Buffer DNase (cat. EN0523; Fermentas, Waltham, MA, USA). cDNA was reverse transcribed utilizing Moloney murine leukemia virus reverse transcriptase according to the manufacturer's protocol (High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, cat. 4374966; Applied Biosystems, Carlsbad, CA, USA). qPCR amplification was performed in an i-Cycler iQ Detection System (Bio-Rad, Hercules, CA, USA) utilizing cDNA working mixture in a 15- μl reaction volume containing 4 μl cDNA, 2 mM dNTP (cat. 10297-018; Invitrogen, Carlsbad, CA, USA), 10 μM of specific 5' and 3' primers, 50 mM MgCl₂, 10 $\times$ PCR Buffer, 1.5 U Taq DNA polymerase (cat. 10966-018; Invitrogen), and 500 nM fluorescein (cat. 11636154910; Roche, Indianapolis, IN, USA) and dye SYBR Green I Dye 1 $\times$ (cat. 4707516001, Roche). Pairs of primers for 5-HT receptors were designed in Profinder (<http://qpcr.profinder.com/organism.jsp>) (Table 1). Amplification of 18S rRNA was used as internal control gene (cat.4352930E; Applied Biosystems). All qPCR were performed in triplicate. Results were normalized by log-

transforming individual values, and relative expression of the 5-HT receptor, as compared with the expression of the internal control gene (18S rRNA) in the same tissue, was calculated by the $2^{-\Delta CT}$ formula. In addition, the relative expressions of 5-HT receptors in tissues from sensitized guinea pigs were compared with those observed in non-sensitized animals by using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.4. Western blot

Tissues from three guinea pigs were pooled to obtain tracheal smooth muscle ($\sim 12\text{ mg}$), tracheal epithelium ($\sim 6\text{ mg}$), bronchial epithelium ($\sim 18\text{ mg}$), and lung parenchyma ($\sim 30\text{ mg}$), which were separately submerged into 500 μl of lysis solution containing 50 mM Tris-HCl, 0.1 mM EDTA, 0.1 mM EGTA, 120 mM NaCl, 0.5% Igepal[®], 1 mM Na₂VO₄, 5 mM NaF, 0.5% sodium deoxycholate, 10 mg phenylmethylsulfonyl fluoride (PMSF) and a protease inhibitor cocktail (Set III, cat. 539134; Calbiochem, La Jolla, CA, USA), 1:200 v/v pH 7.4. Tissues were homogenized (Polytron PT 1200 E, Kinematica, Luzern, Switzerland) and then centrifuged at 4°C and 3000 rpm during 5 min. Total protein content was determined in the supernatant using a commercial kit (DC Protein Assay, cat. 500-0116; Bio-Rad). Forty micrograms of this supernatant were loaded into different lanes of a 10% SDS-polyacrylamide gel and subjected to electrophoresis under reducing conditions. Afterward, proteins were transferred onto a nitrocellulose membrane and blocked with 5% nonfat dry milk in TBS-Tween (Tween 20, 0.1%) during 1.5 h at room temperature. Membranes were incubated overnight at 4°C with rabbit polyclonal antibody raised against 5-HT_{2A} (cat. ab16028; Abcam, Cambridge, MA, USA) 1:200 v/v, 5-HT₄ (cat. ab87337; Abcam) 2 $\mu\text{g}/\text{ml}$, or 5-HT₇ (cat. ab13898; Abcam) 1:200 v/v. Finally, membranes were incubated with a horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (cat. 12-348; Millipore, CA, USA) 1:1000 v/v during 1 h, at 37°C . Immunoblots were developed employing an enhanced chemiluminescent reagent (Super Signal West Femto Maximum Sensitivity Substrate, cat. 34096; Thermo Scientific, Rockford, IL, USA). 5-HT receptors immunoblots were analyzed by densitometry (Molecular Imaging ChemiDoc XRS; Bio-Rad). α -Actin was blotted as a control for protein load by using a mouse monoclonal antibody

Table 1
Primers sequences for 5-HT receptors used for the qPCR.

Receptor	Primer sense	Primer antisense
5-HT _{1A}	5'CACTCTGTTGGGTGCCATAA/3	5'TGACGGGGTTTAGCAGAGAG/3
5-HT _{2A}	5'CTGCAGGATGATCCAAGGT/3	5'AAATGCCACGAAAGAACCAA/3
5-HT ₃	5'CTACGTGGTCATCCGCCGGC/3	5'CCGGGGCAGGTAGAAGCCC/3
5-HT ₄	5'AATAAGATGACCCCTCTGCGTA/3	5'CATGGGGATAACCCAGCA/3
5-HT _{5a}	5'CGCATCAATGTATATGATCG/3	5'GGGGCCAGAGAGATGACAG/3
5-HT ₆	5'CTGCCCTTCGTCTGTG/3	5'TGCAGTAAGTGAAGCAGATGC/3
5-HT ₇	5'CACAGCGGTGGCATTITAC/3	5'GCAGCCTTGTAATCCGGTA/3

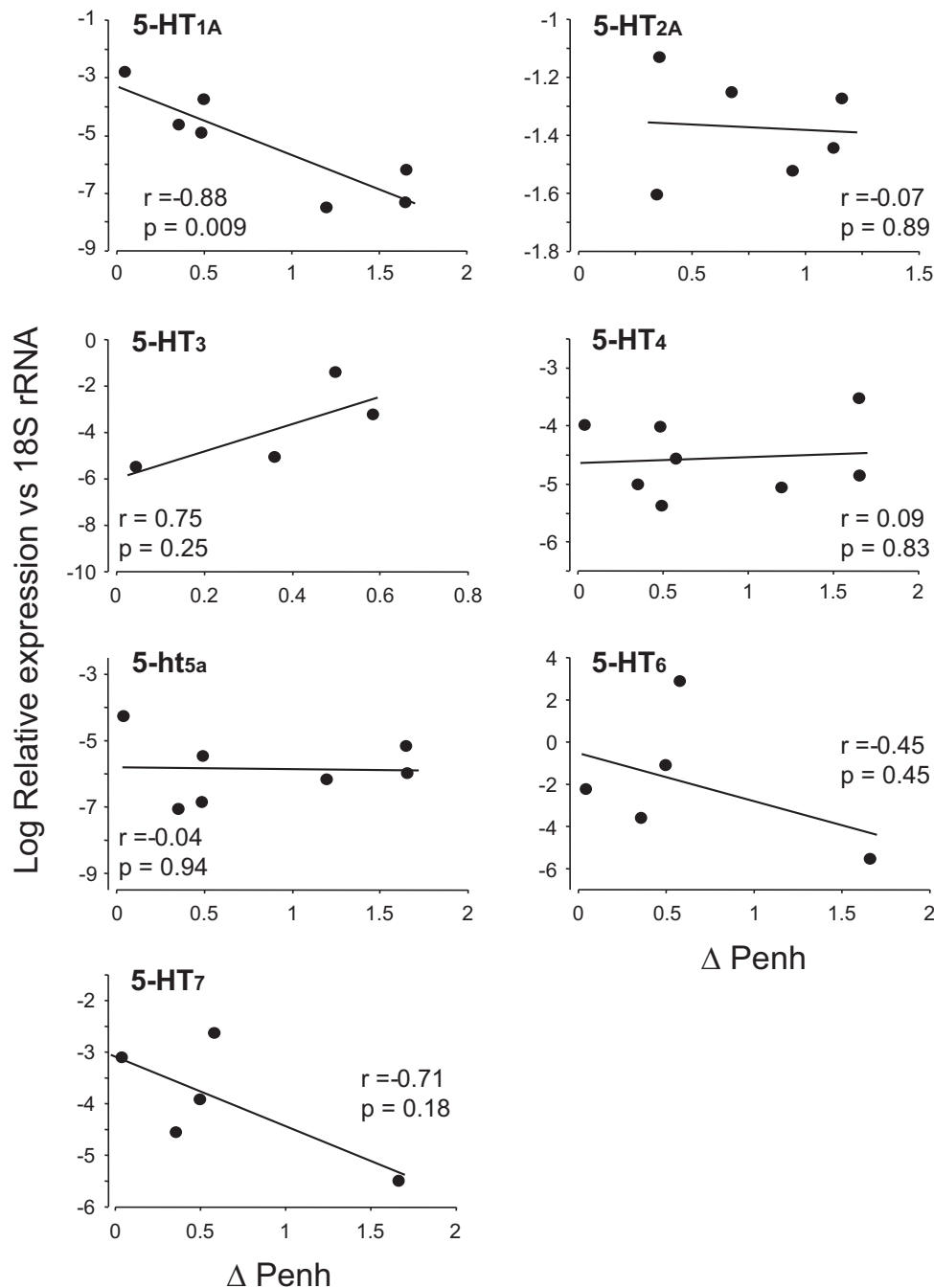


Fig. 2. Relationship between intensity of response to the antigenic challenge at day 15 of sensitization, and level of lung parenchyma expression of 5-HT receptors mRNA at day 21–24 of sensitization. The airway response developed after the inhaled antigen challenge was evaluated by the increase of the enhanced pause index (Penh) through barometric plethysmography.

(cat. A5228; Sigma–Aldrich) 1:1000 v/v. These latter membranes were incubated with a horseradish peroxidase-conjugated sheep anti-mouse IgG secondary antibody (cat. NA9310 V; Amersham, Buckinghamshire, UK) 1:2000 v/v. To determine immunolabeling specificity, negative controls were performed by utilizing the blocking peptides for 5-HT_{2A} (cat. ab161959; Abcam) and 5-HT₄ (cat. ab102731; Abcam) to saturate the primary antibody (2 μ g peptide/1 μ g antibody). Because blocking peptide for 5-HT₇ was not available, we used guinea pig brain as positive control.

2.5. Statistical analysis

Differences between sensitized and non-sensitized animals regarding mRNA and protein expression levels were evaluated using the unpaired Student's *t*-test. Data in the text and illustrations correspond to mean $\pm$ standard error. Statistical significance was set at two-tailed $p < 0.05$.

3. Results

We found that in the baseline state, lung parenchyma from control guinea pigs constitutively expressed mRNA for the majority of

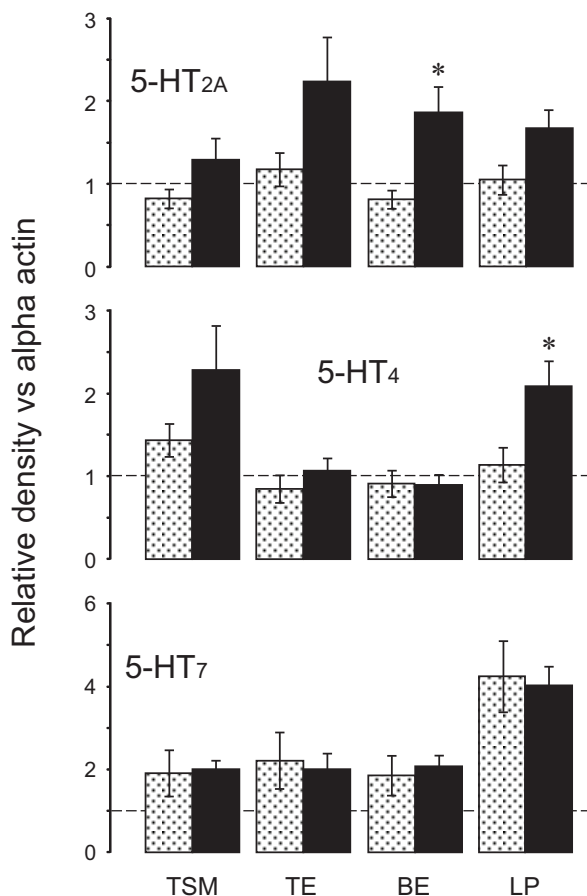


Fig. 3. Protein expression of 5-HT_{2A}, 5-HT₄ and 5-HT₇ receptors, as analyzed by Western blot in lung tissues from guinea pigs with or without sensitization to ovalbumin. All data are expressed as the relative density (5-HT receptor/ α actin densities ratio) in tracheal smooth muscle (TSM), tracheal epithelium (TE), bronchial epithelium (BE) and lung parenchyma (LP) from non-sensitized control (grey bars) or sensitized guinea pigs (closed bars). Bars represent the mean $\pm$ standard error of $n = 4$ –5 Western blot determinations (each determination was performed in tissues pooled from three animals). Horizontal broken lines indicate the baseline level of expression of the constitutive protein (actin). * $p < 0.05$ vs the non-sensitized group.

5-HT receptors, especially for 5-HT_{2A} and 5-HT₄ receptors (Fig. 1). The full order of relative mRNA expression in healthy lungs was 5-HT_{2A} > 5-HT₄ > 5-HT₆ $\geq$ 5-HT₇ > 5-HT_{1A} = 5-HT₃ > 5-HT_{5A}. The only 5-HT receptor for which relative mRNA expression was greater in sensitized guinea pigs was 5-HT₇, with an approximately 15-fold higher expression than in non-sensitized animals ($p = 0.03$). In contrast, 5-HT_{2A} and 5-HT₄ receptors exhibited lower mRNA expression in sensitized guinea pigs (~14.5% and ~0.3% of the respective mRNA expression in non-sensitized animals; $p = 0.04$ and $p < 0.001$, respectively). Finally, 5-HT_{1A}, 5-HT₃, 5-HT_{5A}, and 5-HT₆ mRNA levels in sensitized animals were not statistically different from mRNA levels in control animals.

When the degree of airway response (i.e., Δ Penh) developed after OA inhalation at day 15 of sensitization was plotted against the level of 5-HT receptors mRNA expressed at day 21–24 of sensitization, a statistically significant inverse correlation was observed for the 5-HT_{1A} receptor ($r = -0.88$, $p = 0.009$), i.e., guinea pigs with the greatest response to OA had the lowest mRNA expression of 5-HT_{1A} receptor (Fig. 2).

For those 5-HT receptors that reached statistically significant changes in their lung mRNA levels after sensitization, protein expression was explored by Western blot in different locations, namely tracheal smooth muscle, tracheal and bronchial epithelia, and lung parenchyma homogenate (Fig. 3). We found that pro-

teins of 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors were constitutively expressed in all of the lung tissues studied, especially 5-HT₇ in lung parenchyma. In general terms, the expression of 5-HT_{2A} receptor protein tended to be greater in sensitized guinea pigs, as compared with non-sensitized animals, although a statistical significant difference was only evident in the bronchial epithelium ($p = 0.02$). Expression of 5-HT₄ receptor was higher in tracheal smooth muscle and lung parenchyma homogenates from sensitized guinea pigs, but a statistical significance was only observed in the latter tissue ($p = 0.048$). Finally, expression of the 5-HT₇ receptor protein did not change with sensitization in any tissue.

4. Discussion

Asthma remains a rather complex disease in which a large number of mediators appear to be involved in its pathophysiology (Holgate, 2011). Although earlier studies exploring a possible role of 5-HT in asthma were disappointing (So et al., 1985; Tonnesen, 1985), there is now mounting evidence from clinical studies and animal models that this mediator may play a role in some aspects of the disease (Arreola-Ramírez et al., 2013; Durk et al., 2013; Lechin et al., 2004, 1998, 1996; Segura et al., 2010).

In the present study, we found evidence that all major subtypes of 5-HT receptors are expressed in lungs from healthy guinea pigs. To our knowledge, detection of 5-HT receptors in pulmonary tissue from animal species has been poorly described, and this is especially true for the guinea pig. Among these studies, Baez et al. (1994) described that guinea pig and rat tracheas expressed 5-HT_{2A} receptor mRNA, and these authors demonstrated that receptor density coincided with increased contractile responses to 5-HT. Ullmer et al. (1995) explored the expression of 5-HT receptors in several types of blood vessels; with respect to the lung, these authors detected 5-HT_{2A} > 5-HT_{2B} = 5-HT₇ > 5-HT₄ = 5-HT_{1B/D β} mRNA in pig pulmonary artery, 5-HT_{2B} = 5-HT_{1B/D β} > 5-HT₄ mRNA in endothelial cells from human pulmonary artery, and 5-HT_{2A} > 5-HT_{1B/D β} = 5-HT₇ > 5-HT_{2B} mRNA in smooth muscle from human pulmonary artery. Wang et al. (1999) described the expression of the 5-HT_{2C} receptor in type II pneumocytes from rats. More recently, Hofmann et al. (2014) found that both mRNA and protein expression of 5-HT_{2A} receptor increased in lung tissue from a rat model of congenital diaphragmatic hernia, with pulmonary arteries the main site of expression.

In addition to the localization of pulmonary 5-HT receptors, some authors have also analyzed their modification after exposure to a noxious stimulus. In this respect, after describing the baseline expression of 5-HT_{2A} receptor in rat bronchial segments, Cao et al. (2012) found that mRNA and protein expression of this receptor significantly increased after exposure of animals to secondhand cigarette smoke. Likewise, Dupont et al. (2014) described the presence of 5-HT₄ receptor mRNA in mouse lung, and these authors found that such expression notably increased after exposure to tobacco smoke. Murphy et al. (2013) found that mRNA and protein expression of 5-HT_{2A} and 5-HT₄ receptors in the airway epithelium of central and peripheral airways in Rhesus monkeys tended to increase after acute or intermittent exposure to ozone. Finally, Cadirci et al. (2013) detected by immunohistochemistry a low level of expression of 5-HT₇ receptor in the lung interstitium of rats, but such expression was more widely distributed and at a greater level in a rat model of septic shock.

Human studies have found that, compared with healthy control subjects, patients with asthma had increased mRNA expression of 5-HT_{2A} receptor (Ahangari et al., 2015; Sheikhha et al., 2014), as well as of 5-HT_{3A} receptor (Ahangari et al., 2013; Mohammadi Amirabad et al., 2014) in their peripheral blood mononuclear cells.

In agreement with these studies, in the present work we found that the development of pulmonary allergic inflammation was accompanied by changes in relative mRNA expression of pulmonary 5-HT receptors. Thus, the sensitization process significantly decreased 5-HT_{2A} and 5-HT₄ receptors mRNA, while 5-HT₇ receptor mRNA notably increased. In order to better localize the specific tissue where possible changes occurred, we also analyzed the protein expression of these latter receptors by Western blot in airway epithelium, airway smooth muscle and lung parenchyma. To our surprise, although we detected that sensitization modified the protein expression of these receptors, these changes were not fully concordant with changes observed in mRNA expression. Thus, while mRNA levels of 5-HT_{2A} and 5-HT₄ receptors decreased with sensitization, protein expression tended to increase in the majority of lung tissues studied. On the other hand, increased expression of the 5-HT₇ receptor mRNA was not accompanied by detectable changes in protein expression. Although at first glance it may be expected that the level of mRNA expression must correlate with the level of protein synthesis, this appears not to be the rule and studies exploring this issue have found only a minimal or modest correlation (Anderson and Seilhamer, 1997; Greenbaum et al., 2003; Ideker et al., 2001; Nie et al., 2006). Potential explanations for the disagreement between mRNA and protein expression include the existence of complex transcriptional regulatory mechanisms, differences in protein half-lives, and the degree of mRNA stability (Esquerré et al., 2015; Greenbaum et al., 2003; Nie et al., 2006). For example, transcriptional efficiency is enhanced by the action of YTHDF1 in the presence of N⁶-methyladenosine (Wang et al., 2015), and by tRNA regulation of ribosome flux to the mRNA (Gorgoni et al., 2014). Moreover, Esquerré et al. (2015) reviewed some mRNA half-lives datasets for *Escherichia coli*, and they found that the major factor determining mRNA half-life (which ranged from 1 to 53 min) was its own mRNA concentration; hence, the lower the mRNA concentration, the longer the mRNA half-life. In this regard, in our study the higher protein expression of 5-HT_{2A} and 5-HT₄ receptors despite a lower mRNA expression could be due, at least hypothetically, to a longer mRNA half-life and a better chance of being translated into protein. In fact, the occurrence of these types of mechanisms in 5-HT receptors has been demonstrated in some studies, where increased stability and half-life of the 5-HT_{2A} mRNA occurring after a brief stimulation with 5-HT was found in vitro (Ferry et al., 1994; Wohlpert and Molinoff, 1998).

In a previous study, we found that administration of selective antagonists of 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors to sensitized guinea pigs fully abolished the hyperresponsiveness to acetylcholine usually observed after an antigenic challenge (Segura et al., 2010). This finding implied that these 5-HT receptors were involved in the development of the antigen-induced unspecific airway hyperresponsiveness. In this context, the greater protein expression of 5-HT_{2A} and 5-HT₄ receptors found in the present study is in full agreement with the preeminent role of these receptors in the guinea pig asthma model.

We do not have a clear explanation for the inverse correlation between the degree of sensitization (airway response to the antigen at day 15) and the expression of 5-HT_{1A} receptor mRNA. If this association is indeed a cause-effect relationship, then it may simply reflect that the sensitization process induced downregulation of the mRNA. The alternative possibility, that the 5-HT_{1A} receptor mRNA level determined the degree of sensitization, appears to be less probable. Besides its important role in the central nervous system, 5-HT_{1A} receptor is widely distributed in inflammatory cells such as monocytes/macrophages, mast cells, eosinophils, dendritic cells, and lymphocytes but, in general terms, its activation leads to proinflammatory effects (Arreola et al., 2015). Thus, it would be expected that an enhanced expression of 5-HT_{1A} receptor mRNA, if translated into a greater amount of protein, would be associ-

ated with greater sensitization, which was not the case. A potential limitation of this part of the study was the temporal dissociation between physiological response to the antigen (Δ Penh measured at day 15) and pulmonary expression of 5-HT receptors (measured at days 21–24). Because our aim was to evaluate the expression of 5-HT receptors in a stable state, the lag time with the allergen challenge was necessary to avoid any potential residual effect of this acute allergen exposure on the expression of 5-HT receptors.

Two statistical issues must be acknowledged in our study. First, it has been claimed that any study must perform *p*-value adjustments (such as the Bonferroni correction) according to the number of outcome variables tested (Bland and Altman, 1995). Although this approach is still debated (Perneger, 1998; Rothman, 2014), if it were applied to our mRNA comparisons, the only 5-HT receptor remaining statistically different between sensitized and control animals would be 5-HT₄ receptor, while 5-HT_{2A} and 5-HT₇ would be devoid of statistical significance. Second, we found that mRNA levels of 5-HT_{1A}, 5-HT₃, 5-HT_{5A}, and 5-HT₆ receptors were not different between sensitized and control animals, but the power analysis revealed that statistical tests had relatively low power values (9%, 41%, 21%, and 52%, respectively). Thus, it could not be discarded that the lack of difference for some of these receptors could be explained by a small number of experiments.

In summary, our results showed that major 5-HT receptor subtypes are constitutively expressed in the guinea pig lung, and that allergic sensitization modifies the expression of 5-HT_{2A}, 5-HT₄, and 5-HT₇ receptors.

Conflicts of interests

The authors report no conflicts of interests.

Acknowledgments

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