SHORT COMMUNICATION

Role of 5-HT$_2$ Receptors Family in the Allergy-Induced Increased Aorta Contractile Responses to 5-HT

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Summary

Asthma poses an increased risk for cardiovascular disorders, suggesting that allergy, which is an underlying process in asthma, causes atypical functioning of organs other than lungs. In a previous study in a guinea pig asthma model, we concluded that allergic sensitization increased aorta contractile responses to 5-HT. To further characterize these responses, here we explored the role of the 5-HT$_2$ receptors family. We found that TCB-2 (5-HT$_{2A}$ agonist) and WAY161503 (5-HT$_{2C}$ agonist) induced aorta contractions resembling those elicited by 5-HT but less intense (~43 % and ~25 %, respectively). In these experiments, aortas from sensitized guinea pigs showed increased contractions to TCB-2, but not to WAY161503. In turn, MDL 100907 (5-HT$_{2A}$ antagonist) and RS-102221 (5-HT$_{2C}$ antagonist) caused a notably and a mild reduction of the 5-HT-induced contractions, respectively, with no differences seen between sensitized and non-sensitized tissues. BW723C86 (5-HT$_{2B}$ agonist) did not induce contractile responses and RS-127445 (5-HT$_{2B}$ antagonist) did not modify the contractile responses to 5-HT. In non-sensitized aortas, the pattern of protein expression of receptors was 5HT$_{2B}$>5-HT$_{2A}$=5-HT$_{2C}$, which did not change in sensitized animals. In conclusion, we found that allergic sensitization increased the aorta contractile responses to 5-HT, partly mediated by enhanced responses of 5-HT$_{2A}$ receptors, which was unrelated to changes in the expression of these receptors.

Key words

5-HT$_2$ receptors • Asthma model • Thoracic aorta • Serotonin • Vascular smooth muscle

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Allergic sensitization is a major pathophysiological mechanism present in both the atopic and non-atopic asthma phenotypes [1]. It has been demonstrated that patients with asthma have increased cardiovascular risk [2,3], which suggests that allergy causes abnormal functioning of organs other than lungs. The origin of this increased risk remains mostly unexplored. 5-Hydroxytryptamine (5-HT, serotonin) is involved in many physiological processes, and it has also been implicated in asthma and cardiovascular diseases

[4,5]. Thus, abnormal responsiveness to 5-HT induced by allergy may be the link between asthma and cardiovascular disorders. In a previous study, we found that aorta rings from guinea pigs sensitized to ovalbumin (OVA) had increased contractile responses to 5-HT compared with aortas from non-sensitized animals [6]. In order to better understand the causes of this allergy-induced increased vascular responses to 5-HT, in the present study, we explored the role of the 5-HT_2 receptors family.

The protocol was approved by the scientific and bioethics committees of the Instituto Mexicano del Seguro Social (approval No. 2017-785-003) and the Instituto Nacional de Enfermedades Respiratorias (approval No. B19-16). Experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals [7].

Male Hartley guinea pigs bred under conventional conditions and weighing 400-550 g were used in accordance with international guidelines. The allergic sensitization was carried out with 60 mg OVA and 1 mg $Al(OH)_3$ equally administered through the intraperitoneal and subcutaneous routes (day 0), followed by two booster nebulizations with 3 $mg \cdot ml^{-1}$ OVA for 5 min (day 8), and 0.5 $mg \cdot ml^{-1}$ OVA for 1 min (day 15). Guinea pigs were studied on days 21-25. Guinea pigs not submitted to this sensitization protocol and having the same weight as experimental animals were used as controls.

On the day of the study, animals were deeply anesthetized with pentobarbital sodium and exsanguinated, and four rings were obtained from the thoracic aorta. For the *in vitro* experiments, each tissue was hung in an organ bath containing Krebs solution at physiological conditions and attached to an isometric transducer (model TSD125B, Biopac Systems Inc., Santa Barbara, CA, USA) connected to a digitizer (model MP150, Biopac). Signals were monitored through the Acknowledge 3.9.1 software (Biopac). We evaluated contractile responses of aorta rings to equimolar concentrations (100 µM) of each one of the following agonists: 5-HT, and 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors agonists (TCB2, BW723C86, and WAY161503, respectively). In separate aorta rings, potent and selective antagonists of these receptors (MDL 100907, RS-127445, and RS-102221, respectively) were preincubated (10 nM) 20 min before 5-HT. Each study group comprised n=8 experiments (all tissues within each group were obtained from different animals). Because 5-HT_{2B} receptor agonists produce endothelium-dependent relaxation [8], in a separate set of experiments we corroborated the functionality of the endothelial layer by inducing muscarinic (acetylcholine or carbachol) relaxation in guinea pig aorta rings pre-contracted by K^+, phenylephrine, or 5-HT (Fig. S1).

Western blotting for detecting 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors was performed in non-sensitized (n=5) and sensitized (n=5) aortas, obtained as described above. Each tissue was lysed, homogenized, and centrifuged, and 30 µg of total protein were separated by SDS-PAGE using a 13 % acrylamide gel and electroblotted onto PVDF membranes (Merck, Darmstadt, Germany) blocked in 5 % (w/v) milk/TBS-Tween. Primary antibodies were: mouse anti-SR-2A (1:500, sc-166775, Santa Cruz Biotechnology Inc., Dallas, TX, USA), mouse anti-SR-2B (1:500, sc-376878), mouse anti-SR-2C (1:750, sc-17797), and mouse anti-GAPDH (1:3000, MAB374 Millipore Corporation, Billerica, MA, USA). Species-specific HRP-conjugated secondary antibodies (Santa Cruz Biotechnology Inc.) were applied at 1:5000 dilutions during 1 h. Peroxidase activity was visualized using Immobilon Western Chemiluminescent HRP Substrate (Merck). Each 5-HT_2 receptor and its load control protein (GAPDH) were run in the same membrane, so the analysis and normalization of each receptor was done independently from each other. Densitometric analyses were performed using ImageJ software.

Our results showed that administration of 100 µM 5-HT produced a sustained contraction in aortas from non-sensitized guinea pigs that reached a plateau after ~6 min (Fig. 1A). Sensitization increased the 5-HT-induced contraction, mainly in the second half of the response.

Activation of 5-HT_{2A} receptors by TCB-2 mostly reproduced the pattern of responses to 5-HT, although with less than half potency, i.e. in non-sensitized aortas, TCB-2 produced sustained contractile responses that were heightened in sensitized tissues (Fig. 1B). The 5-HT_{2B} agonist BW723C86 did not cause any contraction response, neither in sensitized nor in non-sensitized aortas (Fig. 1C). In non-sensitized tissues, activation of the 5-HT_{2C} receptors by WAY161503 produced aorta contractions but the response was not increased in sensitized aortas (Fig. 1D).

Antagonism of 5-HT_{2A} receptors by MDL 100907 notably reduced the aorta contraction in both sensitized and non-sensitized tissues, but the response was no longer increased by sensitization (Fig. 1E). The antagonism of the 5-HT_{2B} receptors by RS-127445 did not modify contractile responses to 5-HT in sensitized and

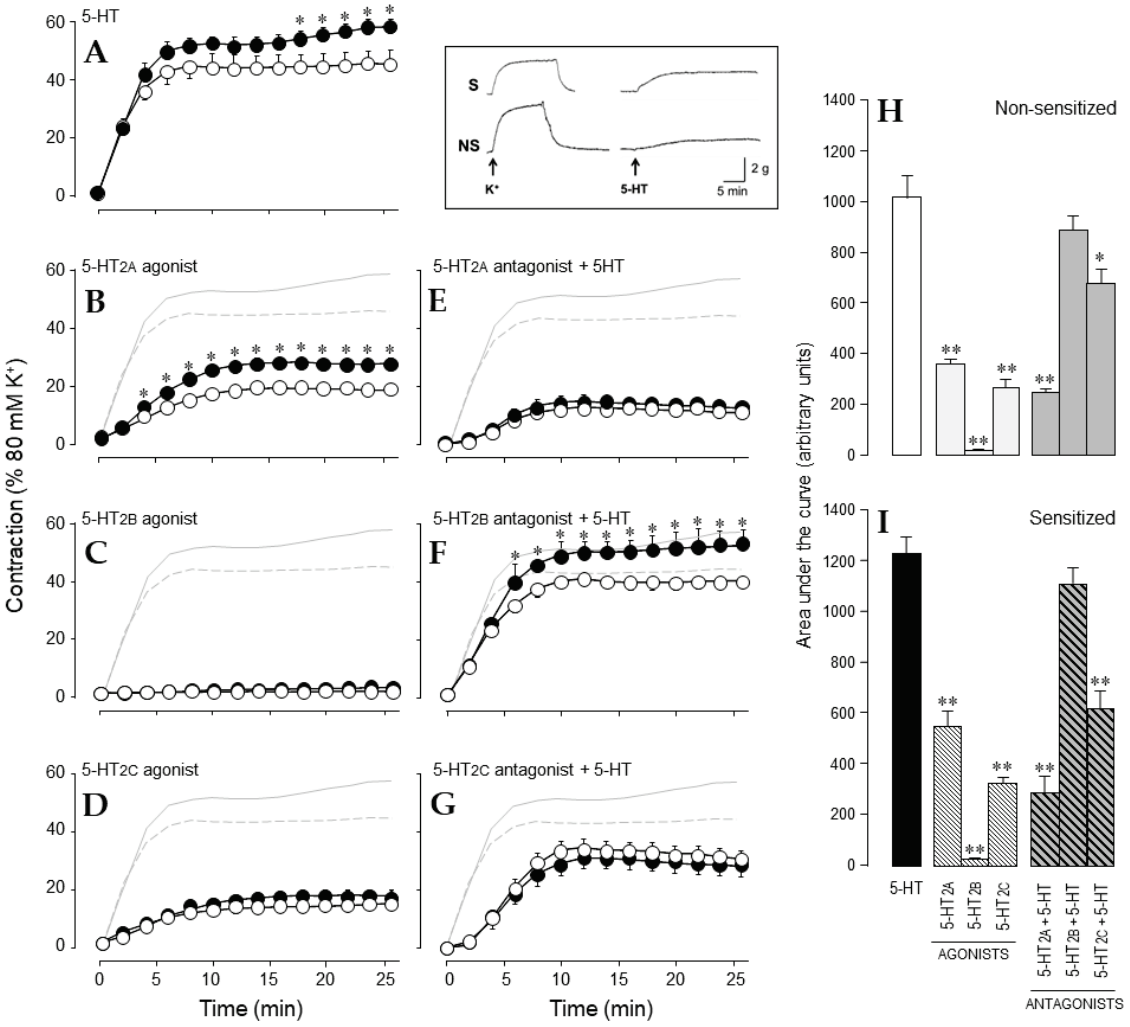

Fig. 1. Effect of sensitization and 5-HT$_2$ receptor family agonists and antagonists on contractile responses of guinea pig aortas. Inset in the upper right panel shows representative recordings of the 5-HT-induced contraction in sensitized (S) and non-sensitized (NS) aorta rings. (**A**) The response of sensitized (closed circles) and non-sensitized (open circles) aortas to 5-HT. (**B-D**) The responses to agonists of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors (TCB-2, BW723C86, and WAY161503, respectively, all at a concentration of 100 μM). (**E-G**) The effect of antagonists of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors (MDL 100907, RS-127445, and RS-102221, respectively, all at a concentration of 10 nM) added to the organ bath 20 min prior to 5-HT. (**H-I**) The already mentioned contractile responses measured as area under the curve. Symbols represent the mean ± standard error of n=8 experiments per group. * $p<0.05$ and ** $p<0.01$ after Student's *t*-test (A-G) or ANOVA followed by Student's *t*-test with Bonferroni correction (H, I).

and non-sensitized tissues (Fig. 1F). Finally, antagonism of the 5-HT$_{2C}$ receptors by RS-102221 caused a mild diminution of the contractile response to 5-HT in non-sensitized aortas, and sensitization did not further increase it (Fig. 1G).

In order to make comparisons among all of the above-mentioned responses, the area under the curve of each contractile response was calculated. As can be seen in Figure 1H, with this approach, in aortas from non-sensitized animals the 5-HT$_{2A}$ agonist produced a contraction that was 36 % that of 5-HT, whereas the 5-HT$_{2B}$ agonist almost had a null effect (2 %). Although it has been claimed that 5-HT$_{2C}$ receptor has not a preeminent role in the 5-HT-induced vascular smooth muscle contraction [9,10], we found that activation of 5-HT$_{2C}$ receptors by WAY161503 indeed caused aorta contractions almost as intense as that produced by TCB-2, reaching 27 % of the 5-HT-induced contraction. On the other hand, antagonism of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors diminished the 5-HT-induced contraction by 76 %, 13 %, and 33 %, respectively.

The above-mentioned patterns were essentially reproduced in tissues derived from sensitized animals (Fig. 1I). Thus, stimulation of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ receptors induced aorta contraction corresponding to 45 %, 2 %, and 26 % that of 5-HT, while antagonism of these receptors reduced the 5-HT responses by 76 %, 10 %, and 50 %, respectively.

As shown in Figure 2, in control non-sensitized aortas, the protein expression pattern of receptors was $5HT_{2B}$>$5\text{-}HT_{2A}$=$5\text{-}HT_{2C}$, which was essentially the same in sensitized aortas.

An increasing number of epidemiological studies provide convincing evidence that asthma is a risk factor for cardiovascular disorders, but potential mechanisms explaining this association are unexplored. The most significant 5-HT receptors contributing to the arterial contraction in normal and pathological conditions are $5\text{-}HT_{2A}$ and $5\text{-}HT_{1B}$ receptors [11], and at less extent $5\text{-}HT_4$ and $5\text{-}HT_7$ receptors [12]. In line with this concept, the $5\text{-}HT_{2A}$ agonist TCB-2 induced a contraction that was ~36 % that of an equimolar concentration of 5-HT, thus explaining a third of the 5-HT contractile response. Stimulation of $5\text{-}HT_{2C}$ receptors by WAY 161503 also induced a noteworthy contraction of aorta rings, slightly smaller than that produced by the $5\text{-}HT_{2A}$ agonist and corresponding to ~27 % that of 5-HT. This response to the $5\text{-}HT_{2C}$ agonist was unexpected because several studies have concluded that $5\text{-}HT_{2C}$ receptors are not present in vascular smooth muscle [9,10]. As far as we know, our study is the first to demonstrate that the $5\text{-}HT_{2C}$ receptors indeed participate in the serotonergic vascular contraction.

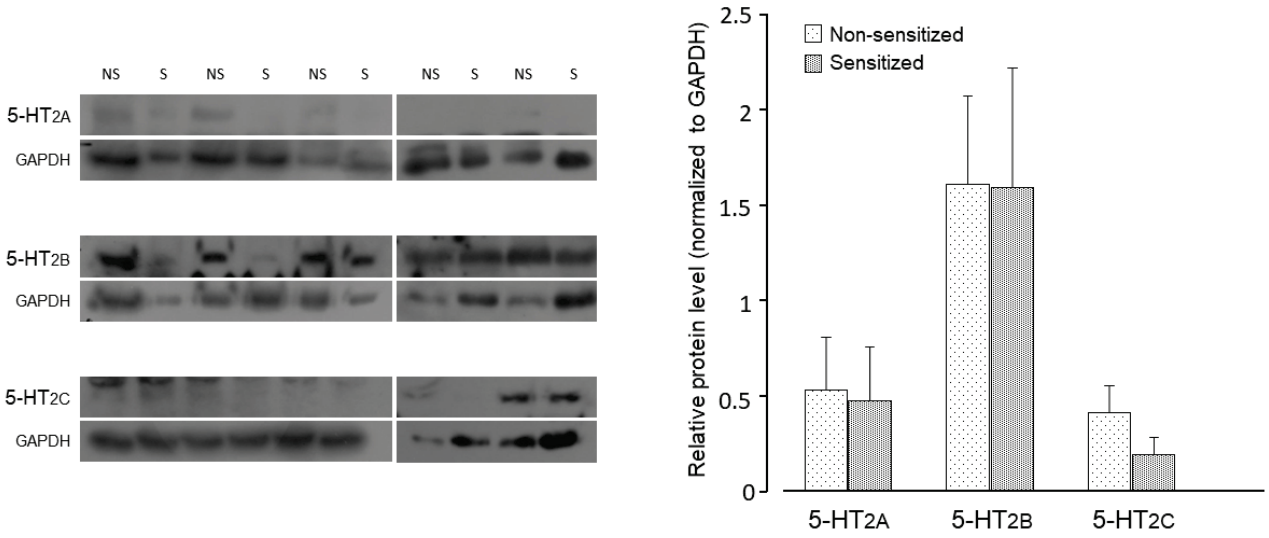

Fig. 2. Protein expression of $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, and $5\text{-}HT_{2C}$ receptors in aortas isolated from non-sensitized and sensitized guinea pigs. There are no differences in the expression of $5\text{-}HT_2$ receptor family in aortas measured by Western Blot analysis (ANOVA followed by Student's *t*-test with Bonferroni correction). Data are expressed by mean ± standard error (n=5 for each group). NS=non-sensitized, S=sensitized.

Concerning $5\text{-}HT_{2B}$ receptors, they appear to have no role in the 5-HT-induced aorta contraction, and their antagonism did not modify the allergy-induced enhanced contractile response to 5-HT. These results agree with previous studies showing that the $5\text{-}HT_{2B}$ receptor mainly causes endothelium-dependent relaxation [8], although it may produce a mild vascular smooth muscle contraction after elimination of the endothelial layer [13].

Sensitization caused a statistically significant increase in the contractile response to 5-HT, a finding that our research group has already reported [6]. In the present study, we could not find differences between aortas from control and sensitized animals regarding protein expression of the two major contraction-producing $5\text{-}HT_2$ receptors, i.e. $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ so an increased expression of these receptors after sensitization was discarded. Surprisingly, sensitization caused a differential effect on responses to $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ agonists. Thus, while sensitization induced increased responses to $5\text{-}HT_{2A}$ receptor stimulation, it did not modify responses to $5\text{-}HT_{2C}$ receptor stimulation. Considering that $5\text{-}HT_{2A}$ and $5\text{-}HT_{2C}$ receptors share the same transduction mechanism, i.e. Gq/G_{11} protein activation and IP_3 production, it would be expected that sensitization should produce the same effect on both receptors. However, signaling through G proteins is not always a linear pathway but has many non-canonical potential effectors and, on the other hand, 5-HT receptors may activate some mechanism independent of G proteins

[14,15]. Thus, this differential effect between 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor agonists may reflect allergy-induced changes in the downstream signaling pathways after initial Gq/G$_{11}$ activation. Other possibilities might be speculated. For example, the increased activation of platelets known to occur in allergy [16] might cause that platelets remaining adhered to the aorta ring endothelium to release higher amounts of vasoconstrictor mediators, such as TXA$_2$, through activation of their 5-HT$_{2A}$ receptors. On the other hand, if part of the 5-HT effect were accomplished by stimulating adrenergic nerves, then a higher release of catecholamines would occur after allergy-induced prejunctional M2 receptor dysfunction [17].

Our results corroborate that in this guinea pig asthma model, alteration of physiological responses goes beyond airways to include changes in vascular responsiveness. It is known that vasoconstriction, remodeling and lower vasodilator capacity of arterioles and small arteries have a major role in primary arterial hypertension [18]. Thus, if the heightened aorta contractile response to 5-HT induced by allergic sensitization were also present in the microcirculation vessels, this might constitute a potential mechanism partially explaining the increased cardiovascular risk in subjects with asthma. However, small vessels may not respond in the same way as large elastic arteries, so further studies in microcirculation vessels are needed to clarify the relevance of our findings.

In conclusion, we corroborated that allergic sensitization increased the aorta contractile responses to 5-HT. This increased contraction was partly mediated by enhanced responses of 5-HT$_{2A}$ receptors that were unrelated to changes in the expression of these receptors.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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