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Original

5-HT_{2A}, 5-$HT_{1B/D}$, $5HT_3$ and 5-HT_7 receptors as mediators of serotonin-induced direct contractile response of bovine airway smooth muscle

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Abstract

Background: Serotonin (5-hydroxytryptamine; 5-HT) performs a variety of functions in the body including the modulation of muscle tone in respiratory airways. Several studies indicate a possible role of 5-HT in the pathophysiology of bronchial hyperresponsiveness. However, the receptors and the molecular mechanisms by which 5-HT acts on airway smooth muscle (ASM) continue to be controversial. Most of the evidence suggests the participation of different subtypes of receptors in an indirect response. This study supports the proposal that 5-HT directly contracts ASM and characterizes pharmacologically the subtypes of serotonergic receptors involved. The characterization was carried out by using selective antagonists in an organ bath model allowing study of the smooth muscle of segments of bovine trachea. Results: The results obtained show that 5-HT_{2A} receptors are the main mediators of the direct contractile response of bovine ASM, with the cooperation of the 5-HT_7, 5-HT_3 and 5-$HT_{1B/D}$ receptors. Also, it was observed that the muscle response to serotonin is developed more slowly and to a lesser extent in comparison with the response to cholinergic stimulation. Conclusion: Overall, the receptors that mediate the direct serotonergic contraction of the smooth muscle of the bovine trachea are 5-HT_{2A}, 5-HT_7, 5-HT_3 and 5-$HT_{1B/D}$ receptors.

Key words: serotonin, smooth muscle, airway, contraction

Introduction

Serotonin (5-HT) has a variety of functions both in the nervous system and in the rest of the body. At the smooth muscle level, 5-HT contributes to the maintenance of blood pressure, peristalsis, and the modulation of airway smooth muscle (ASM) tone (1). On the airways, the 5-HT not only participates in tone control but also its accumulation promotes cell degranulation and has immunomodulatory effects that facilitate the contraction

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of this tissue (2). Consequently, different authors postulate that 5-HT could have a crucial role in the airway hyperresponsiveness (3–5). Nevertheless, the receptors and molecular mechanisms used by this amine to act on the ASM are not completely understood.

There is contradictory evidence found in animal models that associate one or more subtypes of serotonergic receptors with the contraction of ASM (4, 6–10). The main limitation of most of these studies is they were carried out in the presence of epithelium and other accompanying tissues, so the action described may be due to an indirect effect of 5-HT over the muscle by promoting the release of Acetylcholine (ACh), probably by acting on its receptors located in epithelial cells (11, 12) or cholinergic nerve endings (4, 8, 10, 13–15). Thus, it is Ach instead of 5-HT that directly contracts ASM.

The 5-HT effect on ASM has been evaluated in the absence of epithelium in only a limited number of studies. Most of them were performed in cell culture, where changes in the generation of phosphoinositols, intracellular variations in calcium levels or modifications in the activity of the muscle Na^+/K^+ pump were assessed (16–18), and not the contractile response per se. Nonetheless, Kummer et al. supports a direct contractile effect by 5-HT on ASM in rodents. They observed that 5-HT produces contraction of this muscle in knock-out mice for muscarinic receptors (M_2 and M_3), thus discarding a contraction mediated by ACh, and indicating instead a serotonergic direct activation of this tissue (19).

Likewise, there are clinical findings that support the role of 5-HT in ASM contraction. In 1996, Lechín et al. observed that an increase in the levels of free 5-HT in plasma correlates with the severity of the respiratory dysfunction present in the attacks of asthmatic patients (20). When the patients were treated with Tianeptine, a drug that helps platelets reuptake 5-HT through serotonin transporter (SERT) (21), there was a significant improvement in their respiratory capacity (22). Similarly, in 2012, Lau et al. demonstrated that elevated levels of 5-HT in plasma, which can be induced by cigarette smoke, increase the probability of suffering chronic obstructive pulmonary disease (COPD) (23). The principal source of 5-HT would be platelets and to a lesser extent mast cells, as both migrate toward airways due to the induction of an inflammatory response (24–26). In pathological processes such as asthma and COPD, this response is persistent, which leads to the accumulation of 5-HT in the tissue.

The establishment of 5-HT as an important element capable of directly contracting the airways would promote research and development of new therapeutic strategies that can modulate its action. These new approaches would enable the treatment of respiratory diseases like the aforementioned, particularly in those patients that do not respond to present therapies. Asthma, for example, gives a global burden of near 260 million people with an annual mortality of 461,000 patients. While COPD is currently the third leading cause of death worldwide, with a 50% mortality in the first five years and about three million deaths annually (27–29). For these reasons, it is urgent to find a new effective therapy for those patients resistant to current treatment.

The selection in this study of bovine tracheal smooth muscle as a model to evaluate the direct contractile effect of 5-HT on the airways is based on the fact that the muscle of the trachea from cattle do not normally produce action potentials (30). They have a contractile system without automatic and rhythmic activity (31). Instead, this muscle responds to stimulation of the Parasympathetic Nervous System (PNS) or to chemical mediators. This, together with the fact that cattle do not develop diseases such as asthma, makes it an ideal model where muscle contraction can be evaluated in a physiological state in the absence of interfering contractile responses. Additionally, the bovine model also has the advantage that huge amounts of muscle tissue can be obtained in comparison with mouse, rat, and guinea pig. It is also bioethically more convenient as the animal is sacrificed for human consumption and not exclusively for research. Finally, the cholinergic contractile responses have been widely studied in our lab using this model, which allows us to understand as a whole the

contractile responses of the airways.

In the present work, we have shown that serotonin directly induces the contraction of the ASM of the bovine trachea by activation of several specific receptors. Also, a model of the action mechanisms has been proposed by which the contractile response could be enhanced. This may play a role in the pathophysiology of respiratory diseases.

Methods

Isolation of the Bovine Tracheal Smooth Muscle (BTSM)

Fresh bovine tracheas were obtained from certified healthy animals recently sacrificed in the local slaughterhouse and keep cold during their transport to the laboratory.

The tracheas were washed with abundant water to remove blood and other contaminants. Then, the trachealis muscle was dissected, all the connective and epithelial tissue removed, and cut into longitudinal strips (following the plane of the muscle fibres) of 10 × 2 mm (approximately) attached to the cartilage to preserve muscle tone. Each strip was placed in Krebs-Ringer-Bicarbonate Buffer (KRB) with the following composition: 118.5 mM NaCl, 4.47 mM KCl, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$, 2.54 mM $CaCl_2$, 24.9 mM $NaHCO_3$ and 10 mM Glucose; pH 7,4, oxygenated with 95% O_2 and 5% CO_2 and maintained at Room Temperature (RT), making changes of the buffer every 30 min. The strips were used within a period no longer than 3 h after dissection.

Standardization of the contraction of the BTSM with carbachol

To evaluate the viability of using BTSM as a model and determine its maximum contractile capacity; the effect of Carbachol (CCh), an analogue of ACh, was evaluated. This was done by developing a cumulative concentration-response curve with CCh in concentrations from 10 nM to 10 mM.

Evaluation of the contractile effect of the 5-HT on BTSM

Once the viability of the model was determined, the effect of 5-HT on BTSM was evaluated in concentrations from 10 nM to 1 mM, both alone and in the presence of one of the specific antagonists against serotonergic receptors: 5-$HT_{1B/D}$ (GR127935), 5-HT_2 (Ritanserin), 5-HT_3 (Ondansetron), 5-HT_4 (RS 23597), 5-HT_7 (SB 258719), 5-HT_{2A} (Spiperone), 5-HT_{2B} (SB 204741), 5-HT_{2C} (N-Desmethylclozapine) or the non-selective antagonist of the muscarinic receptors, Atropine. The antagonists were added to the organ bath in a concentration of 1 μM, 5 min before the addition of 5-HT. Serotonin, carbachol, atropine and ondansetron were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). GR127935, ritanserin, spiperone, SB204741, N-Desmethylclozapine, RS23597 and SB258719 were purchased from Tocris Bioscience (Ellisville, MO, USA).

Concentration-response curves were constructed following the method described in Current Protocols in Pharmacology and modified by Guerra de González in 1995 (32). In summary, the BTSM was placed inside of an organ bath of 10 ml coupled to a polygraph (Polygraph 7400; Grass Instruments, Quincy, MA, USA) with a system of force displacement transducers (model FT03; Grass Instruments) and calibrated to 1 gram (g) of initial tension. The strips were attached to the transducers and then immersed in KRB at 37 °C and constantly bubbled with 95% O_2/5% CO_2. The tissue was equilibrated for 30–40 min (with washes every 10 min). After each concentration-response curve with 5-HT, the muscle was contracted with CCh 1 mM. This with three aims: 1) to obtain the maximum contraction response, 2) to verify the integrity of the contractile machinery is preserved, and 3) to normalize the contractile response induced by 5-HT, alone or in presence of the different

antagonists.

The experimental data are expressed as the mean ± standard error (SE). The results are analysed using a two-way ANOVA and the comparison between the antagonists with the control group (5-HT) is made using an unpaired Student's *t*-test with Welch's correction with a significance of $P<0.05$. Multiple comparison is made with the use of the Sidak test. The effective concentration 50 (EC_{50}) is estimated by non-linear regression with the Prisma 8.0.1 program. Prisma 8.0.1 (GraphPad Software, San Diego, CA, USA).

Results

The model in this study was validated through the contractile response generated by CCh, which showed its maximum effect (Emax) at a concentration of 1 mM with an EC_{50} of 3.11 ± 0.53 µM. While the contractile response induced by 5-HT started from 10 nM and reached similarly its Emax at 1 mM with an EC_{50} of 2.00 ± 0.57 µM, which is significantly lower than the EC_{50} of CCh ($P<0.05$). Additionally, the Emax-normalized of 5-HT on the BTSM is 72.02 ± 1.32% in relation to the Emax stimulated by CCh (Fig. 1).

The direct contractile response of the BTSM as a function of time of a single dose of 5-HT was determined and compared to CCh at a concentration of 1 mM. It was observed that the contraction of muscle by CCh began immediately and reached its Emax at 120.33 ± 2.55 sec, while with 5-HT the effect commenced around 2.5 sec after being administrated and its Emax obtained at 158.83 ± 5.53 sec, which represents a significantly longer time than CCh and evidence that the kinetic of both contractile responses are different (Fig. 2).

During the evaluation of the Atropine effect on the direct contraction of BTSM induced by 5-HT, it was observed that 5-HT 1 mM produced an Emax of 1.930 ± 0.050 g, which decreased to a value of 1.630 ± 0.087 g

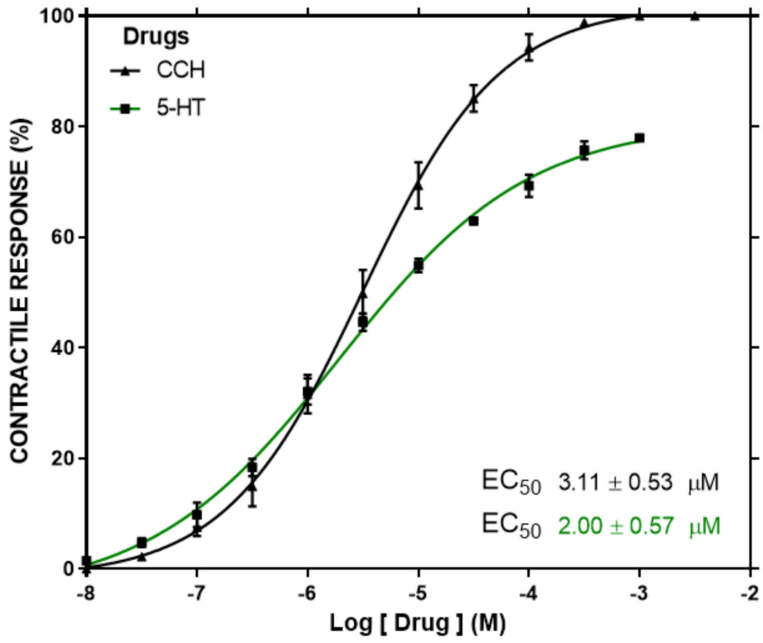

Fig. 1. Direct contractile response of cumulative 5-HT concentrations on BTSM compared to CCh. The contractile response was determined in an isolated organ system coupled to a GRASS polygraph (calibration: 1 g). 5-HT EC_{50} 2.00 ± 0.57 µM and an Emax-normalized of 72.02 ± 1.32% in comparison with CCh, with a EC_{50} 3.11 ± 0.53 µM. Each point represents the mean ± SE of 5 independent preparations ($n=5$), expressed in the case of 5-HT based on the percentage of the maximum response induced by 1 mM CCh.

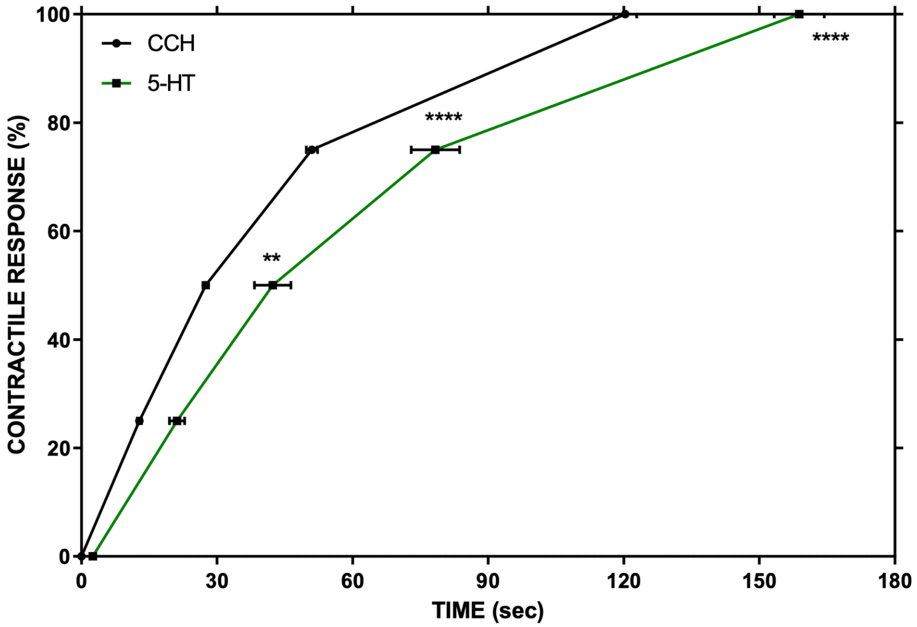

Fig. 2. Comparison of the response of BTSM to CCh and 5-HT as a function of time. The direct contractile response of the bovine tracheal smooth muscle was determined in an isolated organ system coupled to a GRASS polygraph (calibration: 1 g). CCh and 5-HT were added in a concentration of 1 mM. Each value represents the mean ± SE of 6 independent preparations (n=6). Significant difference with respect to CCh: **P<0.005; ****P<0.0001.

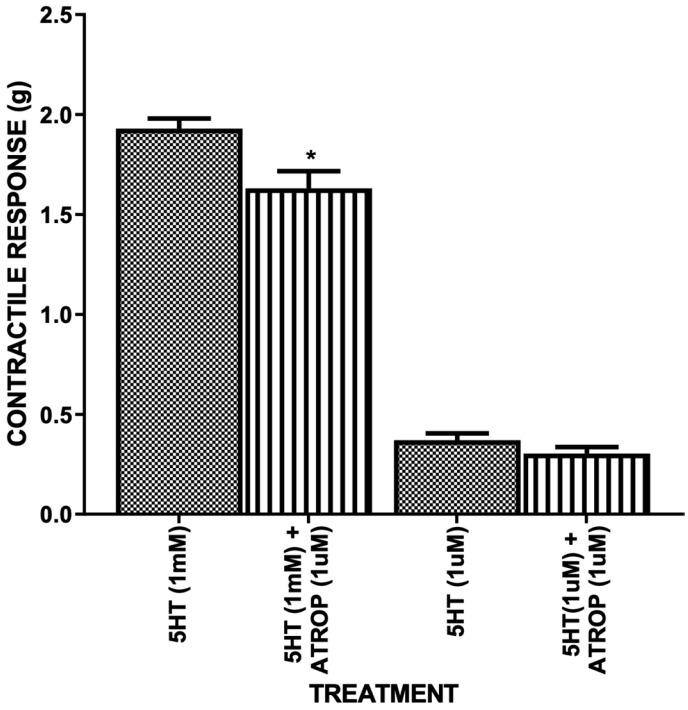

Fig. 3. Effect of Atropine on the contractile response induced by single doses of 5-HT on BTSM. The direct contractile response was determined in an isolated organ system coupled to a GRASS polygraph (calibration: 1 g). Maximum contractile response to single doses of 1 mM 5-HT: 1.930 ± 0.050 g; 1 mM 5-HT + 1 µM Atropine: 1.630 ± 0.087 g; 1 µM 5-HT: 0.370 ± 0.035 g; 1 µM 5-HT + 1 µM Atropine: 0.303 ± 0.033 g. Each point represents the mean ± SE of 5 independent preparations (n=5). Significant difference compared to 5-HT alone: *P<0.05.

in the presence of 1 µM of Atropine. This represents a 15.55% reduction in the Emax (P>0.05). On the other hand, 1 µM of 5-HT generated a contraction of 0.370 ± 0.035 g, which was not affected by Atropine at a concentration of 1 µM, 0.303 ± 0.033 g (Fig. 3).

In the pharmacological characterization of the serotonergic receptors that participate in the direct contractile effect of 5-HT on the ASM of bovine, 1 µM Ritanserin, a non-selective antagonist of 5-HT_2 receptors, significantly blocked the response (P>0.01). Ritanserin decreases the value of Emax-normalized of 5-HT to 19.51 ± 2.21%, which represents a reduction in the contraction of 73%, EC_{50} of 97.86 ± 56.52 µM (Fig. 4a). Spiperone 1 µM (a 5-HT_{2A} antagonist) produce a blockade of 95% of the response induced by 5-HT (P>0.001), with an Emax of 2.95 ± 0.46% and an EC_{50} of 0.09 ± 0.25 µM (Fig. 4b). Additionally, the Spiperone reduced the contractile basal tone of the BTSM by approximately 5%. Contrarily, the use of 1 µM of SB204741 (5-HT_{2B} antagonist) or N-Desmethylclozapine (5-HT_{2C} antagonist) did not inhibit the effect of 5-HT on the tissue. This was supported by finding an Emax of 85.10 ± 1.55%, with an EC_{50} of 19.27 ± 4.17 µM in the case of SB204741 (Fig. 4c), and an Emax of 74.55 ± 3.40%, with an EC_{50} of 62.31 ± 28.57 µM for N-Desmethylclozapine (Fig. 4d). Both responses have a variation in the adjustment of the curve in comparison with the concentration-response

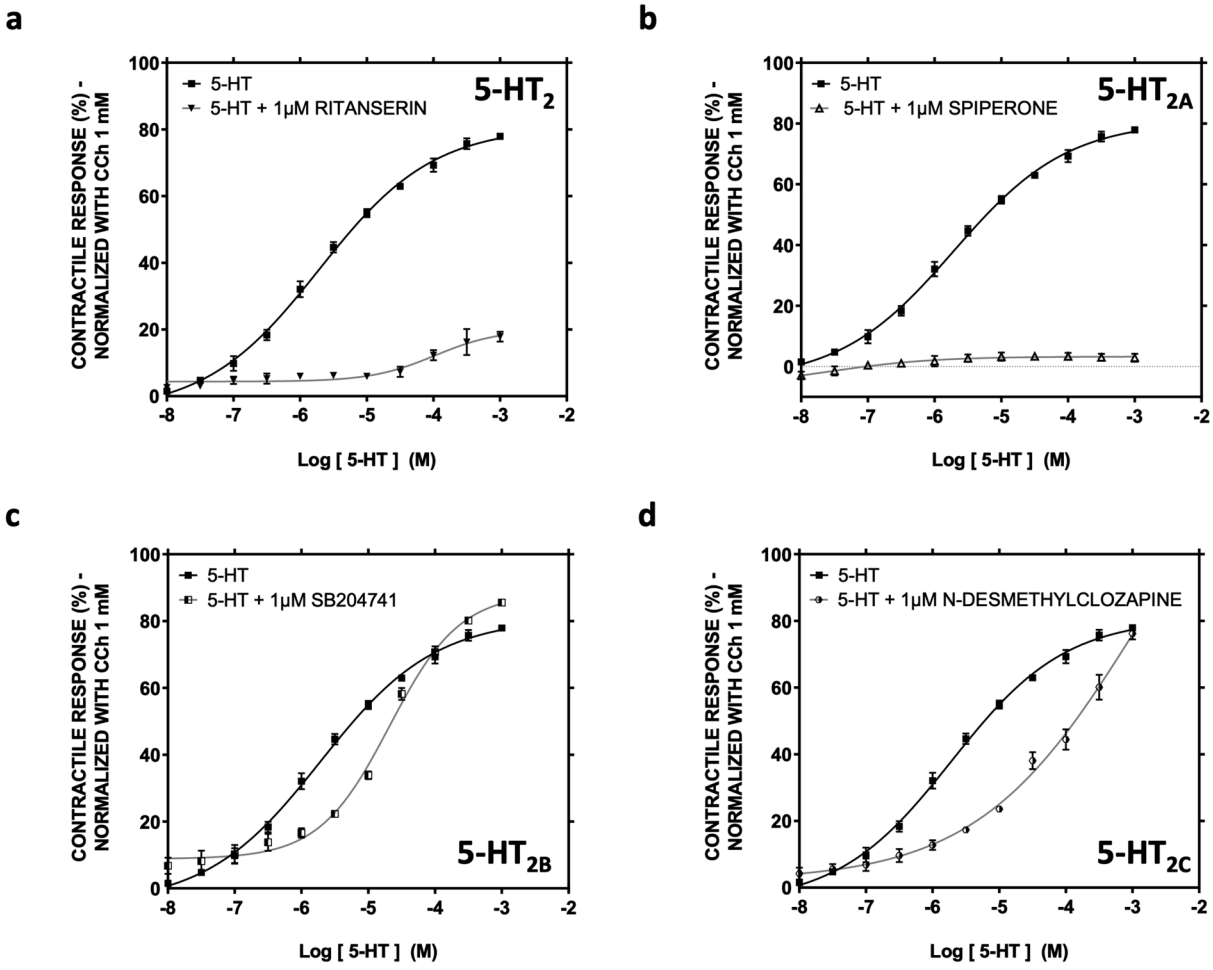

Fig. 4. Effect of 5-HT_2, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} antagonists on the contractile response induced by 5-HT. The direct contractile response of the bovine tracheal smooth muscle was determined in an isolated organ system coupled to a GRASS polygraph (calibration: 1 g). The receptor antagonists a) 5-HT_2 (Ritanserin), b) 5-HT_{2A} (Spiperone), c) 5-HT_{2B} (SB 204741) o d) 5-HT_{2C} (N-Desmethylclozapine) were added 5 min before the start of the curve. Each point represents the mean ± SE of 5 independent preparations (n=5), expressed as a percentage of the maximum response induced on BTSM with 1 mM CCh.

curve of 5-HT alone, without a significant difference when tested by an unpaired Student's t-test with Welch's correction or after an analysis of multiple comparisons.

The effects of 1 μM of GR127935 (5-$HT_{1B/D}$ antagonist), Ondansetron (5-HT_3 antagonist), RS23597 (5-HT_4 antagonist) or SB258719 (5-HT_7 antagonist), which have been described in the literature as possible inhibitors of the serotonin contractile response on the ASM, were evaluated. After an analysis of multiple comparisons of concentrations of the curves it was observed that GR127935 significantly reduced the contraction of BTSM by 5-HT at a concentration of 10 μM or higher in comparison with 5-HT alone (P>0.05), with an Emax of 60.26 ± 3.60% and an EC_{50} of 5.20 ± 1.87 μM (Fig. 5a). The inhibitory effect of Ondansetron (P>0.01) and SB258719 (P>0.01) began from a concentration of 1 μM of 5-HT. In the presence of Ondansetron, the 5-TH produces an Emax of 54.96 ± 1.86%, which represent a decrease of about 17% in the direct effect of 5-HT alone, EC_{50} of 15.75 ± 4.25 μM (Fig. 5b). While with SB258719, we saw a reduction of nearly 23% of the response, reaching an Emax of 48.73 ± 1.08%, and an EC_{50} of 3.57 ± 1.21 μM (Fig. 5c). Finally, RS23597 does not produce inhibition of the effect of 5-HT on the muscle, Emax. 77.84 ± 2.66% and EC_{50} of 12.44 ± 4.65 μM (P=0.8407) (Fig. 5d).

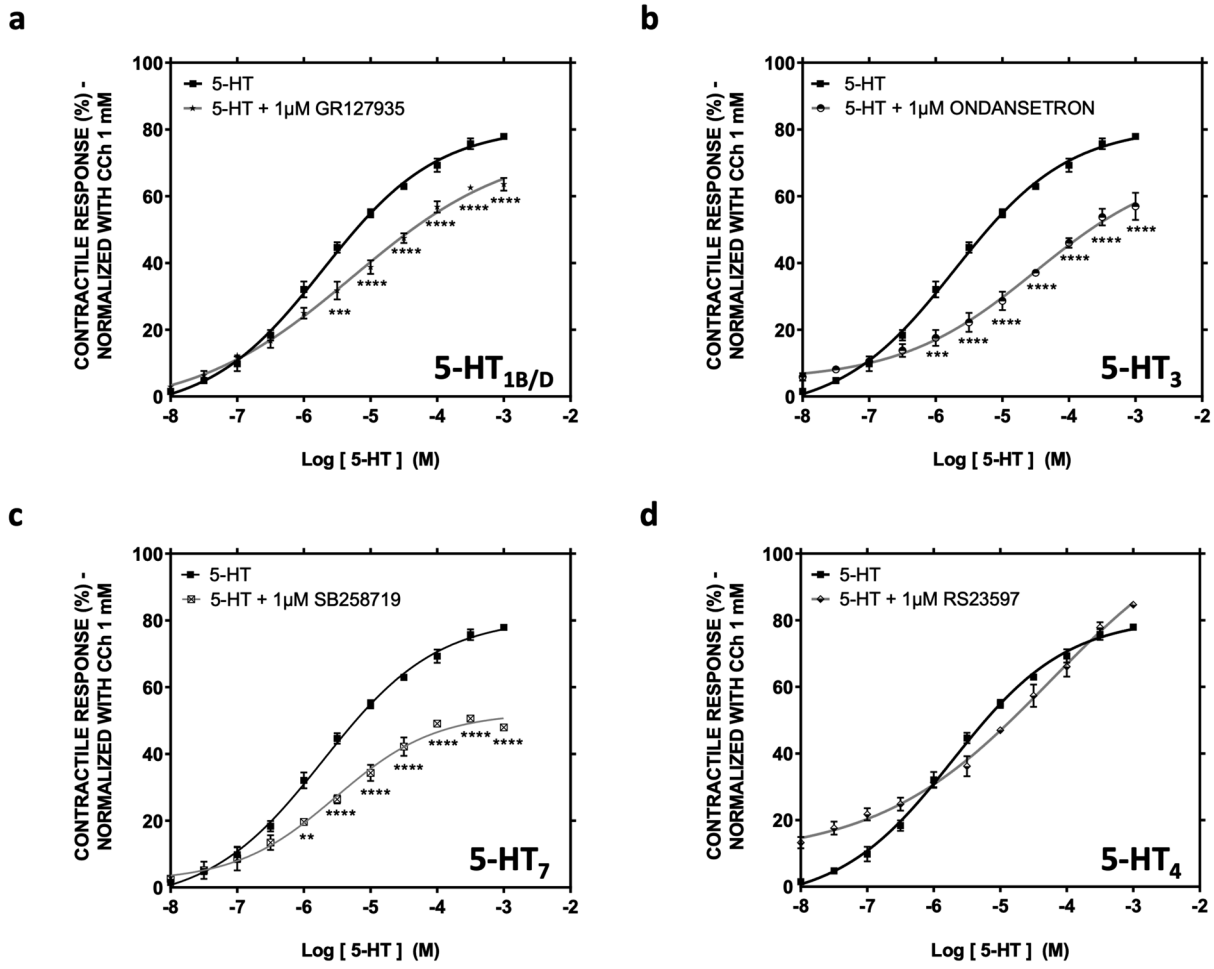

Fig. 5. Effect of 5-$HT_{1B/D}$, 5-HT_3, 5-HT_4 and 5-HT_7 antagonists on the contractile response induced by 5-HT. The contractile response of the bovine tracheal smooth muscle was determined in an isolated organ system coupled to a GRASS polygraph (calibration: 1g). The receptor antagonists a) 5-HT_1 (GR127935), b) 5-HT_3 (Ondansetron), c) 5-HT_7 (SB 258719) or d) 5-HT_4 (RS 23597) were added 5 min before the start of the curve. Each point represents the mean ± SE of 5 independent preparations (n=5), expressed as a percentage of the maximum response induced on BTSM with 1 mM CCh. Significant difference in comparison to the 5-HT curve alone: **P<0.01, ***P<0.001; ****P<0.0001.

Discussion

The present work is the first to characterize pharmacologically the different subtypes of serotonergic receptors that mediate the direct contractile response of the smooth muscle of the bovine trachea induced by 5-HT in the absence of the epithelium and connective tissue.

Initially, the validation of the contractile model was carried out by determining the concentration-response curve to CCh, which presented an equivalent pattern to that obtained by Guerra de González et al., in 1999 (33). The Emax of CCh was reached at a concentration of 1 mM, agreeing with the value observed by Barnes et al. for the cholinergic activation of the bovine ASM in absence of epithelium (34). In addition, the elimination of the epithelium and the accompanying connective tissue in the experiments significantly increased the contractile sensitivity of the tissue to both cholinergic and serotonergic stimulation, as previously described by Spicuzza et al. (35).

The pharmacological parameters obtained with 5-HT in the concentration-response curve are comparable with the findings of Barnes et al. (34), but differ from those values observed by Cadieux et al. (36), Baumgartner et al. (13) and Spicuzza et al. (35). However, such difference could be explained by the presence of epithelial and connective tissue in their preparations. In these cases, serotonin would induce the contractile response indirectly by stimulating the release of several chemical mediators stored mainly in the epithelium, such as Ach, histamine and catecholamines, which will contract the ASM (10, 15). Likewise, 5-HT shown to have an Emax close to 30% lower than the cholinergic agonist on the BTSM, a value that is similar to the results described in the ASM by Pérez & Sanderson in mice and by Szarek et al. in rats (15, 37). A response that is important to highlight, are the consequences of the interaction of 5-HT with all of its different subtypes of receptors present in the ASM.

Antagonists of serotonergic receptors were used at a concentration of 1 µM to identify the subtypes involved in the direct contractile response of BTSM to 5-HT. This concentration has shown to be effective, and it is equivalent to more than 100 times that of the reported inhibition constant (Ki) for each one of them (9, 38–41).

Ritanserin, a non-selective antagonist of 5-HT_2 receptors (42), inhibited by 73% the contractile response induced by 5-HT on the BTSM, which is significant. This result is in accord with that described by Selig et al. in 1988 (41), who found that Ritanserin blocked the increase in the intra-tracheal pressure induced by 5-HT in the ASM of guinea pig and corroborated the importance of 5-HT_2 receptors in ASM cited in previous works (13, 14, 43, 44).

Different specific antagonists of the three subtypes of 5-HT_2 receptors (5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}) were used to discern which subtype or subtypes of 5-HT_2 receptors mediate the response (38, 45). In our study was found that 1 µM of Spiperone (5-HT_{2A} antagonist) blocked by nearly 95% the direct contractile response of BTSM to 5-HT, even when it is used at a concentration of 1 mM of the amine, which is a concentration three orders of magnitude higher. Also, we observed that Spiperone reduced the muscle tone by 5% in the absence of 5-HT. Based on the literature reviewed, these results represent an original pharmacological finding and suggest a future therapeutic utility.

Regarding the subtype receptors 5-HT_{2B} and 5-HT_{2C}, when the antagonists SB204741 or N-Desmethylclozapine were used, no significant change was seen in their concentration-response curves, respectively, when compared with that for 5-HT alone (46, 47). These results are in agreement with the findings of Rhoden et al. in guinea pigs (18), Adner et al. in mice (48), and more recently by Sommer et al. in the bovine, where they associated the response induced by 5-HT on ASM mainly to the 5-HT_{2A} subtype (49).

GR127935, an antagonist of the 5-$HT_{1B/D}$ receptor (50, 51), produced a reduction of about 10% in the Emax in comparison with 5-HT alone. This effect was only significant at high concentrations of 5-HT, suggesting that these receptors are not the principal mediators of the response. Additionally, it is possible that at least partially the effect observed is due to a non-selective binding of GR127935 to 5-HT_{2A} receptors, which have previously been described by De Vries et al. in 1997 (51), who demonstrated that GR127935 can block the contraction mediated by 5-HT_{2A} receptors of smooth muscle in the blood vessel of rat when it was used in high concentrations.

In this study, the use of Ondansetron (an antagonist of 5-HT_3 receptor) blocked by about 17% the direct contractile response induced by 5-HT on BTSM in the absence of the epithelium and connective tissue. This observation together with the findings of Takahashi et al. (52), Rizzo et al. (7) and Dupont et al. (8), where they found that 5-HT_3 receptors can stimulate the contraction of ASM by an indirect mechanism, indicating that these receptors contract ASM both directly and indirectly. In addition, the inhibition of 15.5% produced by Atropine over the contractile response induced by 5-HT on BTSM is similar to the Ondansetron response, which could be explained by a non-selective binding of Atropine to 5-HT_3 receptors. This possibility is based on the structural similarity between Atropine and the antagonists of these receptors, which has been described in the literature (53).

RS23597, an antagonist of 5-HT_4 receptors (54), did not modify the contractile response induced by 5-HT. This agrees with the work of Lucchelli et al. in 1994 (9), who point out that 5-HT_4 antagonists are unable to inhibit the contraction stimulated by 5-HT on the trachea of guinea pigs. However, it is postulated that these receptors could play a role in the modulation of the relaxation process of the muscle (55).

SB258719, a 5-HT_7 antagonist (56), produced a reduction in the contractile response of the BTSM induced by 5-HT. These receptors are associated mainly with a G_s protein capable of activating adenylate cyclase (AC) and thereby produced an increase in the generation of the cyclic adenosine monophosphate (cAMP) inside cells of the ASM (57, 58), which would cause relaxation of the muscle (59–61). Nevertheless, recent studies established that these receptors can activate another less known signalling pathway mediated by G_{12} protein (57, 62). Through this pathway the 5-HT_7 receptors block the activation of the myosin light chain phosphatase (MLCP) and produce the phosphorylation of the myosin light chain. The mechanism consists in a protein cascade involving the guanine nucleotide exchange factors (GEFs), RhoA and RhoKinase. The effect is the enhancement of the contractile process of the muscle (57, 62, 63). The possibility of a dual response for 5-HT_7 receptors in conjunction with the fact of these receptors exist in three different isoforms due to alternative splicing and their ability to form both homodimers and heterodimers, as shown in the literature (57, 64, 65), would help to explain the complex and paradoxical effect observed with these receptors, which has also been described in another type of tissue (66).

Conclusion

In conclusion, our data show that together with the 5-HT_{2A} receptors, which are the principal mediators of the direct contractile response induced by 5-HT in the ASM, the receptor subtypes 5-HT_3, 5-$HT_{1B/D}$ and particularly 5-HT_7, also play an important role in the contraction of this tissue, particularly when 5-HT is present in high concentrations. Therefore, we propose a model described in Figs. 6 and 7, where we depict the possible mechanisms used by serotonin to contract and enhance its effect in this muscle. This model describes how 5-HT, which comes mainly from platelets and in a smaller proportion from mast cells, accumulates in the airways during the inflammatory response and induces a direct contractile response of this tissue by activating its receptors located on the muscle. For 5-HT_{2A}, 5-$HT_{1B\,/\,D}$ and 5-HT_3 receptors, the mechanisms of

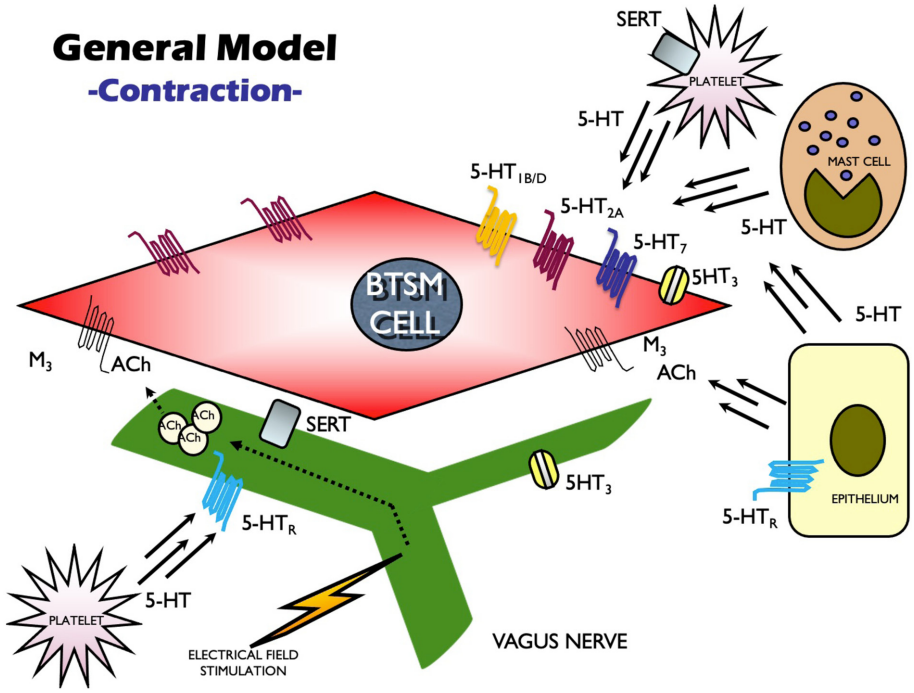

Fig. 6. Proposed model of receptors that participate in the direct contractile response of 5-HT on ASM.

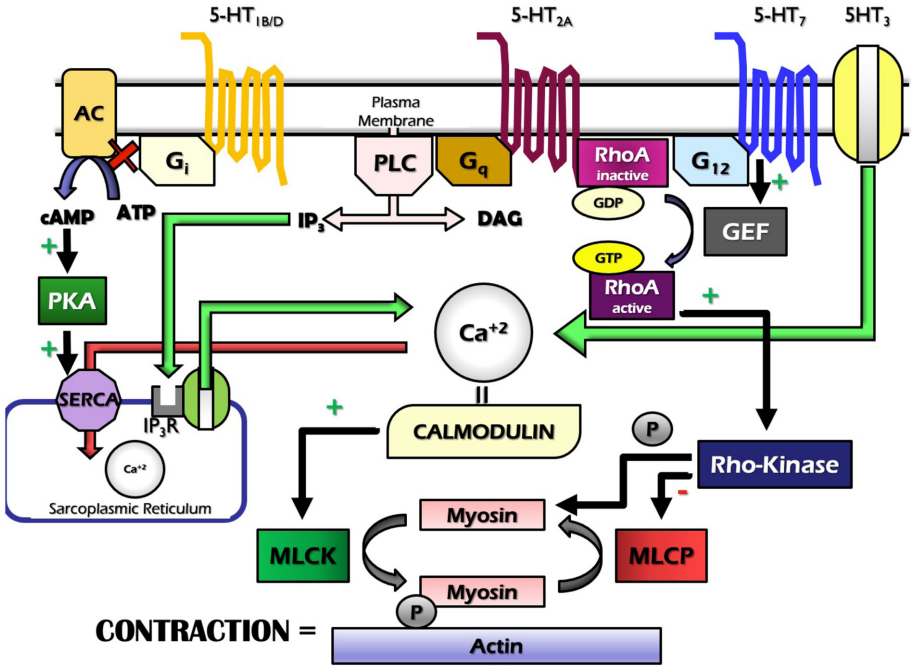

Fig. 7. Proposed model for the mechanisms of action of 5-HT on ASM.

action activated are those traditionally described for each of these receptor subtypes. However, 5-HT_7 receptors would participate in the direct contractile response through an alternative pathway, which is mediated by Rho-Kinase. Additionally, our model also represents the ability of 5-HT to indirectly contract this muscle by promoting the release of Ach from its neuronal and epithelial stores. Together, both paths allow us to appreciate the key role of serotonin in the contractile response of the airways. Of course, future validation of the signal-

ling pathways associated with the contraction of the ASM by 5-HT must be performed, in special those related with 5-HT_7 receptors, G_{12}, RhoA and Rho Kinase, as well as the possible formation of heterodimers between 5-HT_7 and other subtypes of serotonergic receptors, in special the 5-HT_{2A} receptors.

Ethics Standards

Not applicable.

Availability of Data and Materials

Data and materials are available upon request.

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Conflicts of Interest

The authors declare that they have no competing interests.

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