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Airway smooth muscle relaxation induced by 5-HT_{2A} receptors: Role of Na^+/K^+-ATPase pump and Ca^{2+}-activated K^+ channels

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ABSTRACT

Aims: Although 5-hydroxytryptamine (5-HT) contracts airway smooth muscle in many mammalian species, in guinea pig and human airways 5-HT causes a contraction followed by relaxation. This study explored potential mechanisms involved in the relaxation induced by 5-HT.
Main methods: Using organ baths, patch clamp, and intracellular Ca^{2+} measurement techniques, the effect of 5-HT on guinea pig airway smooth muscle was studied.
Key findings: A wide range of 5-HT concentrations caused a biphasic response of tracheal rings. Response to 32 μM 5-HT was notably reduced by either tropisetron or methiothepin, and almost abolished by their combination. Incubation with 10 nM ketanserin significantly prevented the relaxing phase. Likewise, incubation with 100 nM charybdotoxin or 320 nM iberiotoxin and at less extent with 10 μM ouabain caused a significant reduction of the relaxing phase induced by 5-HT. Propranolol, L-NAME and 5-HT_{1A}, 5-HT_{1B}/5-HT_{1D} and 5-HT_{2B} receptors antagonist did not modify this relaxation. Tracheas from sensitized animals displayed reduced relaxation as compared with controls. In tracheas precontracted with histamine, a concentration response curve to 5-HT (32, 100 and 320 μM) induced relaxation and this effect was abolished by charybdotoxin, iberiotoxin or ketanserin. In single myocytes, 5-HT in the presence of 3 mM 4-AP notably increased the K^+ currents ($I_{K(Ca)}$), and they were completely abolished by charybdotoxin, iberiotoxin or ketanserin.
Significance: During the relaxation induced by 5-HT two major mechanisms seem to be involved: stimulation of the Na^+/K^+-ATPase pump, and increasing activity of the high-conductance Ca^{2+}-activated K^+ channels, probably via 5-HT_{2A} receptors.

Introduction

5-Hydroxytryptamine (5-HT or serotonin) is an important neurotransmitter of the central nervous system and the digestive tract, and has a major role in some conditions such as migraine and inflammatory bowel disease. Serotonergic fibers have not been described in the respiratory system, but in different mammalian species, including humans, there are non-neuronal sources of 5-HT such as mast cells and neuroendocrine cells (Joos et al., 1997; Lauweryns et al., 1974; Fu et al., 2002). Moreover, in pulmonary tissue, 5-HT levels are directly proportional to their plasmatic concentrations, being the platelets as its main source (Cazzola and Matera 2000).

The role of 5-HT in asthma has been controversial, but there are some clues indicating its potential involvement in this disease. Plasma concentration of free 5-HT notably increases during an asthmatic exacerbation and this increment is related to asthma severity (Lechin et al., 1996). In addition, it has been reported that tianeptine (a drug that lowers plasma 5-HT by enhancing the 5-HT re-uptake) improves pulmonary function in asthmatic children (Lechin et al., 1998). Therefore, it is important to better understand the physiologic effect of 5-HT on the airway smooth muscle.

5-HT induces a direct sustained contraction of airway smooth muscle from bovine, dog, equine or mouse (Goldie et al., 1982; Lemoine and Kaumann 1986; Doucett et al., 1990; Buckner et al., 1991; Baron et al., 1993; Adner et al., 2002), but in other preparations such as guinea pig trachea or human bronchi the responses elicited by 5-HT

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are biphasic (contraction followed by relaxation) in nature (Goldie et al., 1982; Baumgartner et al., 1990; Ben-Harari et al., 1994). The relaxing phase of this biphasic response has been proposed to occur only at 5-HT concentrations ≥10 μM (Baumgartner et al., 1990), and it has been postulated that 5-HT_2 receptor is the main serotonergic receptor involved in this effect. Further characterization of the mechanisms involved in the 5-HT-induced relaxation has been scarcely investigated. Baumgartner et al. (1990) described that the relaxation caused by high 5-HT concentrations in guinea pig tracheal strips was coincident with a decrease of IP_3 production, and they postulated that an increase in the cAMP might be involved. By evaluating the ouabain-sensitive $^{86}Rb^+$ uptake in cultured guinea pig tracheal smooth muscle cells, Rhoden et al. (2000) found that 5-HT stimulated the activity of Na^+/K^+-ATPase via 5-HT_{2A} receptors, but these authors did not explore the physiological consequences of such stimulation. Finally, Ben-Harari et al. (1994) postulated that the relaxation phase induced by a single concentration of 5-HT was related to a receptor-dependent desensitization.

The present work was aimed to investigate the potential role of several relaxing mechanisms triggered by 5-HT in guinea pig airway smooth muscle, including the role of Na^+/K^+-ATPase and high-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels.

Materials and methods

Animals

Male Hartley guinea pigs (500–600 g) bred in conventional conditions in our institutional animal facilities (filtered conditioned air, 21±1 °C, 50–70% humidity, sterilized bed) and fed with Harlan® pellets and sterilized water were used. The protocol was approved by the Scientific and Bioethics Committees of the Instituto Nacional de Enfermedades Respiratorias. The experiments were conducted in accordance with the published Guiding Principles in the Care and Use of Animals, approved by the American Physiological Society.

Sensitization procedure and antigenic challenge

Guinea pigs were sensitized at day 0 by intraperitoneal administration of 60 mg ovalbumin (OA) and 1 mg $Al(OH)_3$ in 0.5 ml of saline (0.9% NaCl). At day 8, the animals were nebulized with 3 mg·ml^{-1} OA in saline for 5 min, delivered by a ultrasonic nebulizer (model US-1, Puritan Bennett, Carlsbad, CA). Guinea pigs were nebulized again on day 15 with 0.5 mg·ml^{-1} OA in saline for 1 min, and they were studied at day 21–25.

Organ baths

Animals were deeply anesthetized with pentobarbital sodium (35 mg·kg^{-1}, i.p.) and exsanguinated. Major airways were dissected and cleaned of connective tissue; four rings were obtained from the middle of the trachea (each ring was submitted to different experimental conditions) and hung in a 5 ml organ bath filled with Krebs solution with 1 μM indomethacin, as previously described (Campos-Bedolla et al., 2006).

Tissues were stimulated three times with KCl (60 mM), and then temporal course of the responses to 5-HT was evaluated by adding single concentrations of this drug (1, 3.2, 10, 32, 100 and 320 μM) to different tracheal rings. Some of these tissues were preincubated with one of the following drugs during 15 min before addition of a selected concentration of 5-HT (32 μM): tropisetron, methiothepin, ketanserin, WAY-100135, GR 127935, SB 204741, propranolol, L-NAME, ouabain, charybdotoxin and iberiotoxin. Concentrations and descriptions of these drugs are shown in Table 1. None of these drugs modified the basal tone. All responses were expressed as percentage of the third KCl response. We corroborated that the concentration used of ketanserin caused 84% inhibition of the contractile response to a specific 5-HT_{2A} agonist (α-methyl-5-HT, 32 μM) and completely abolished the intracellular Ca^{2+} peak induced by 100 μM α-methyl-5-HT (data not shown).

In order to evaluate the relaxing effect of 5-HT, tracheal rings were precontracted with 10 μM histamine, and then a cumulative concentration–response curve to 5-HT (32, 100 and 320 μM) was done. In some of these tissues, 100 nM charybdotoxin, 32, 100 and 320 nM iberiotoxin or 10 nM ketanserine was added 10 min before histamine administration.

In a separate set of experiments, tracheal rings from guinea pigs sensitized to OA were used to evaluate the temporal course of the response to 32 μM 5-HT, which were compared with control (non-sensitized) tissues.

Patch clamp recordings

Isolated myocytes from guinea pig trachea were obtained as follows. Tracheal airway smooth muscle freed from any residual connective tissue was placed in 5 ml Hanks solution containing 2 mg cysteine and 0.05 U·ml^{-1} papaine, and incubated for 10 min at 37 °C. The tissue was washed with Leibovitz's solution to remove the enzyme excess, and then placed in Hanks solution containing 1 mg·ml^{-1} collagenase type I and 4 mg·ml^{-1} dispase II (neutral protease) during ~20 min at 37 °C. The tissue was gently dispersed by mechanical agitation until detached cells were observed. Enzymatic activity was stopped by adding Leibovitz's solution, the cells were centrifuged at 800 rpm at 20 °C during 5 min and the supernatant was discarded. This last step was repeated once.

For myocytes culture, the cell pellet was resuspended in minimum essential medium containing 5% guinea pig serum, 2 mM L-glutamine, 10 U·ml^{-1} penicillin, 10 μg·ml^{-1} streptomycin and 15 mM glucose, and plated on rounded coverslips coated with sterile rat tail collagen. Cell culture was performed at 37 °C in a 5% CO_2 in oxygen during 24–48 h.

Airway smooth muscle cells were allowed to settle down in the bottom of a 0.7 ml coverglass submerged in a perfusion chamber. The chamber was perfused by gravity (~1.5–2.0 ml·min^{-1}) with external solution (mM): NaCl 130, KCl 5, $CaCl_2$ 1, HEPES 10, glucose 10, $MgCl_2$ 0.5, $NaHCO_3$ 3, KH_2PO_4 1.2, and niflumic acid 0.1 (pH 7.4, adjusted

Table 1
Drugs used in the experimental protocols

Drug	Description	Concentration	References
Tropisetron	5-HT_3/5-HT_4 antagonist	1 μM	Pype et al. (1994); Dupont et al. (1996)
Methiothepin	5-HT_1/5-HT_2/5-HT_5/5-HT_6/5-HT_7 antagonist	1 μM	Gerhardt and Van Heerikhuizen (1997); Kitazawa et al. (2006)
Ketanserin	5-HT_{2A} receptor antagonist	10 nM	Hoyer et al. (2002)
WAY-100135	5-HT_{1A} receptor antagonist	100 nM	Hoyer et al. (2002)
GR 127935	5-HT_{1B}/5-HT_{1D} receptor antagonist	10 nM	Germonpré et al. (1998)
SB 204741	5-HT_{2B} receptor antagonist	32, 100 nM	Hoyer et al. (2002)
Ouabain	Na^+/K^+-ATPase pump inhibitor	10 μM	Rhoden et al. (2000)
Charybdotoxin	Ca^{2+}-activated K^+ channel blocker	100 nM	Miller et al. (1985)
Iberiotoxin	High-conductance Ca^{2+}-activated K^+ channel selective blocker	32, 100 or 320 nM	Liu et al. (2007)
Propranolol	β-Adrenoceptor antagonist	100 nM	Campos-Bedolla et al. (2006)
L-NAME	Inhibitor of NO synthase	10 μM	Jing et al. (1995)