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The effect of serotonin on airway transepithelial sodium ion pathways

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Abstract

The action of serotonin on ionic transport processes in the airways was revealed in a study of the isolated rabbit tracheal wall mounted in a Ussing apparatus. It was shown that the transepithelial electrical potential difference of the tissue was hyperpolarized transiently after mechanical stimulation by means of gentle rinsing of its mucosal side. This hyperpolarization was greatly influenced by the addition of serotonin and/or ambroxol to the stimulation (rinsing) fluid. By means of selective blocking of ion transport transepithelial pathways with amiloride and/or bumetanide, the effects of serotonin and/or ambroxol on the hyperpolarization after mechanical stimulation could be explained as changes of sodium ion currents. The importance of stimulated ionic currents for airway clearance and for the efficacy of drugs acting on airways is postulated.

Keywords: Airway; Ambroxol; Electrical potential difference; 5-HT (5-hydroxytryptamine, serotonin); Na^+ transport, transepithelial

1. Introduction

The action of serotonin in the respiratory system has been carefully studied. The localization of serotonin-producing cells (Scheuermann et al., 1992; Cazzola et al., 1995), the mechanism of its action on the airways (Barnes, 1992; Rizzo et al., 1993; Szarek et al., 1993; Lucchelli et al., 1994), the interaction with neuropeptides liberated from C-fibers (Pype et al., 1994; Joos et al., 1997; Germonpre et al., 1998) and also its effects on ion transport (Tamaoki et al., 1996, 1997) have been described. It has also been shown that gentle mechanical stimulation of the mucosal side of the airways hyperpolarizes the tissue (Tyrakowski et al., 1997a,b, 1998a,b,c). This effect was explained as the opening of ionic channels activated by neuropeptides from a C-fiber ending (calcitonin gene-related peptide, neurokinin A, substance P, and others; Tyrakowski et al., 1997b, 1998a). The aim of the present study was to evaluate the influence of serotonin on the changes in airway ion transport which were evoked by mechanical stimulation, with concomitant comparison of the effect of ambroxol on these reactions.

Although the study was based on an electrophysiological method, as an isolated rabbit tracheal wall was studied in a Ussing chamber, it was possible to identify the transepithelial transport pathways involved in the reactions by incubating the tissue with selective inhibitors of Na^+ or Cl^- transport—amiloride or bumetanide, respectively, as suggested by Boucher (1994a,b).

The rationale behind the studies was to search for a local mechanism able to inhibit the activation of a C-fiber ending (a cough receptor), which might be a prospective cough treatment (Stone et al., 1993; Higenbottam, 1984).

2. Materials and methods

The experiments were performed on isolated rabbit tracheas. New Zealand rabbits of either sex, (3500–4000 g) were killed by CO_2 asphyxiation and the tracheas were excised, placed in room-temperature Ringer solution and trimmed of fat and connective tissue. The tracheas were cut along the membranous part, divided into several pieces (15–20 mm long), and incubated in Ringer solution without or with the addition of inhibitors—amiloride and/or bumetanide.

After about 60 min of incubation, the tissues were mounted in the Ussing chamber. The area of tissue under study was 1.0 cm^2. The tissues were bathed on each side with 10 ml of Ringer solution without or with the addition

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Table 1
The influence of the Na^+ and/or Cl^- transepithelial transport inhibitors amiloride and bumetanide on the electrophysiological properties of the isolated tracheal wall under control conditions and after mechanical stimulation

Experimental conditions (*n*)	PD (mV)	*r* (Ω cm)	dPD (mV)
RH/RH (21)	−3.1 ± 0.4	121.0 ± 7.6	−0.9 ± 0.2
AMI/RH (29)	−4.4 ± 0.4	157.2 ± 12.8	−1.8 ± 0.3[a]
AMI/AMI (25)	−4.5 ± 0.4[b]	138.2 ± 8.9	−0.5 ± 0.1[b]
BUME/RH (20)	−4.0 ± 0.7	167.5 ± 23.0	−0.3 ± 0.1[b]
BUME/BUME (30)	−4.5 ± 0.5[b]	165.3 ± 18.9	−0.3 ± 0.1[b]
AMI + BUME (10)	−0.84 ± 0.2[a]	235.0 ± 21.0[a]	−0.04 ± 0.01[a]

PD—transepithelial electrical potential difference, *r*—transepithelial electrical resistance, dPD—the difference between the maximum stimulated value and control value of PD.
The tissues were incubated before being mounted, then studied in a bathing fluid in a Ussing apparatus and were also stimulated by rinsing from a nozzle connected to a peristaltic pump. The experimental conditions were as follows:

RH/RH—incubation, bathing and stimulation solutions were Ringer solution,
AMI/RH—incubation in the presence of amiloride, bathing and stimulation fluids were Ringer solution,
AMI/AMI—incubation, bathing and stimulation fluids with the addition of amiloride,
AMI/AMI (RH)—incubation and the bathing fluids with amiloride, the stimulation (rinsing) fluid was Ringer solution,
BUME/RH—incubation with the addition of bumetanide, bathing and stimulation fluids were Ringer solution,
BUME/BUME—incubation, bathing and stimulation fluids with the addition of bumetanide,
BUME/BUME (RH)—incubation and bathing fluid with bumetanide, the stimulation (rinsing) fluid was Ringer solution,
AMI + BUME—incubation, bathing and stimulation (rinsing) fluids with the addition of amiloride and bumetanide.

Number of experiments given as (*n*).
Means and S.E.M. are shown.
[a]Different from group RH/RH and AMI/AMI at $a = 0.05$.
[b]Different from RH/RH group at $a = 0.05$.

Table 2
The influence of serotonin and ambroxol on hyperpolarization (dPD in mV) of the isolated tracheal wall after mechanical stimulation by gentle rinsing

Experimental conditions	Control (*n*)	5-HT (*n*)	ABX (*n*)
RH/RH	−0.9 ± 0.1 (21)	−0.3 ± 0.1[a] (22)	−0.6 ± 0.1[b] (21)
AMI/AMI	−0.5 ± 0.1 (25)	−0.2 ± 0.0 (13)	−0.2 ± 0.1 (14)
AMI/AMI (RH)	−0.9 ± 0.1 (24)	−0.3 ± 0.1[b] (11)	−0.5 ± 0.1[b] (16)
AMI/RH	−1.8 ± 0.3 (29)	−1.0 ± 0.2[b] (16)	−1.5 ± 0.3 (15)
BUME/BUME	−0.3 ± 0.1 (20)	−0.5 ± 0.1 (23)	−0.4 ± 0.2 (23)
BUME/BUME (RH)	−0.3 ± 0.1 (20)	−0.3 ± 0.2 (10)	−0.5 ± 0.1 (10)
BUME/RH	−0.3 ± 0.1 (20)	−0.3 ± 0.1 (10)	−0.5 ± 0.1 (10)

Explanations, see Table 1.
5-HT—stimulation (rinsing) fluid with the addition of serotonin.
ABX—stimulation (rinsing) fluid with the addition of ambroxol.
[a]Different from "control" and "ABX" groups at $a = 0.05$.
[b]Different from "control" group at $a = 0.05$.

of the inhibitors as described in the legends to the figures and tables.

The changes in electrical potential difference, under control conditions and after mechanical stimulation of the mucosal sensory receptors by gentle rinsing of the isolated tracheal wall, were tracked by means of the Ussing method as previously described (Koefoed-Johnsen and Ussing, 1958; Tyrakowski et al., 1997a,b). The transepithelial electrical resistance was calculated according to Ohm's law from voltage deflections after the ±10-μA current was passed before and after all experimental maneuvers.

A modified Ussing apparatus was applied. First, the tissue was mounted horizontally and secondly, a nozzle was fixed to the wall of the chamber. The diameter of the nozzle was approximately 1.2 mm and it was fixed some 12 mm away from the tissue. The jets from the nozzle, produced with a peristaltic pump, directed on to the tissue, were applied as short mechanical stimuli. The standard stimulus was five jets, with a total volume of 1.2 ml and 15-s duration. The stimulation fluid was Ringer solution without or with the inhibitors, serotonin and ambroxol, as described in the legends to the figures and tables.

The EVC 4000 voltage/current clamp apparatus (manufactured by WPI, USA), connected to a BD111 recorder (Kipp and Zonen, Holland) was used to measure voltage and resistance.

The measuring equipment was connected to the solutions bathing the tissue by means of Ag/AgCl electrodes

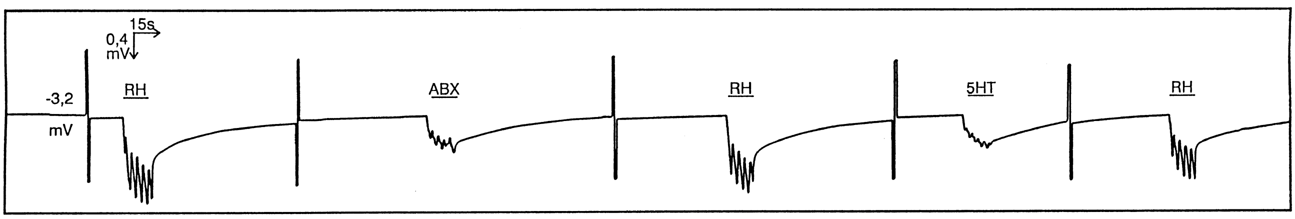

Fig. 1. Serotonin or ambroxol inhibited the hyperpolarization of the airway wall after mechanical stimulation. The drugs were added to the stimulation (rinsing) fluid. A single experiment is shown. The horizontal lines denote the period of mechanical stimulation (gentle rinsing) with the Ringer solution—RH, with RH plus serotonin—5-HT, and with RH plus ambroxol—ABX. The vertical lines denote the measurement of electrical resistance. The tissue was incubated and bathed in solutions with the addition of amiloride.

Table 3
The influence of serotonin and ambroxol on Na^+ and Cl^- transepithelial pathways as evaluated under the influence of amiloride

Stimulation fluid	Experimental conditions	
	AMI/AMI (*n*)	AMI/RH (*n*)
AMI + 5-HT	+0.1 ± 0.0 (14)	−0.08 ± 0.1 (15)
RH + 5-HT	+0.6 ± 0.1[a] (10)	+0.6 ± 0.1[a] (15)
AMI + ABX	+0.1 ± 0.0 (14)	+0.06 ± 0.01[a] (15)
RH + ABX	+0.4 ± 0.1[a] (15)	+0.03 ± 0.2 (13)
AMI + 5-HT + ABX	+0.2 ± 0.2 (10)	−0.03 ± 0.0 (14)
RH + 5-HT + ABX	+0.5 ± 0.1[a] (15)	+0.5 ± 0.1[a] (15)

The data are presented as the difference between two consecutive hyperpolarizations after mechanical stimulation: the first without and the second with the drug (serotonin—5-HT, ambroxol—ABX or both). Augmented hyperpolarization is marked with the sign "−", and diminished hyperpolarization with "+".
For further explanations see Table 1.
[a] Significantly different from zero at *a* = 0.05.

and agar bridges. The ground was the antilumenal side and the lumen was negative.

The solutions used throughout the experiments were (concentrations in mM): Ringer's solution without any additions (Na 147.2, K 4.0, Ca 4.4, Cl 155.6, HEPES 10.0) and with additions as given in the descriptions of the experiments (end concentrations given in mM): ambroxol (0.005), amiloride (0.1), bumetanide (0.1), serotonin (0.005) (all supplied by Sigma-Aldrich, Poland).

The values of all variables are given as means ± S.E.M. The data were statistically evaluated using Student's *t*-test (onesam or twosam procedures from the Statgraphics computer program). Differences were reported as significant at the *a* value of 0.05.

3. Results

A total of 131 tracheal specimens from 49 animals were studied. The electrophysiological characteristics of the tissues under various experimental conditions are described in Table 1.

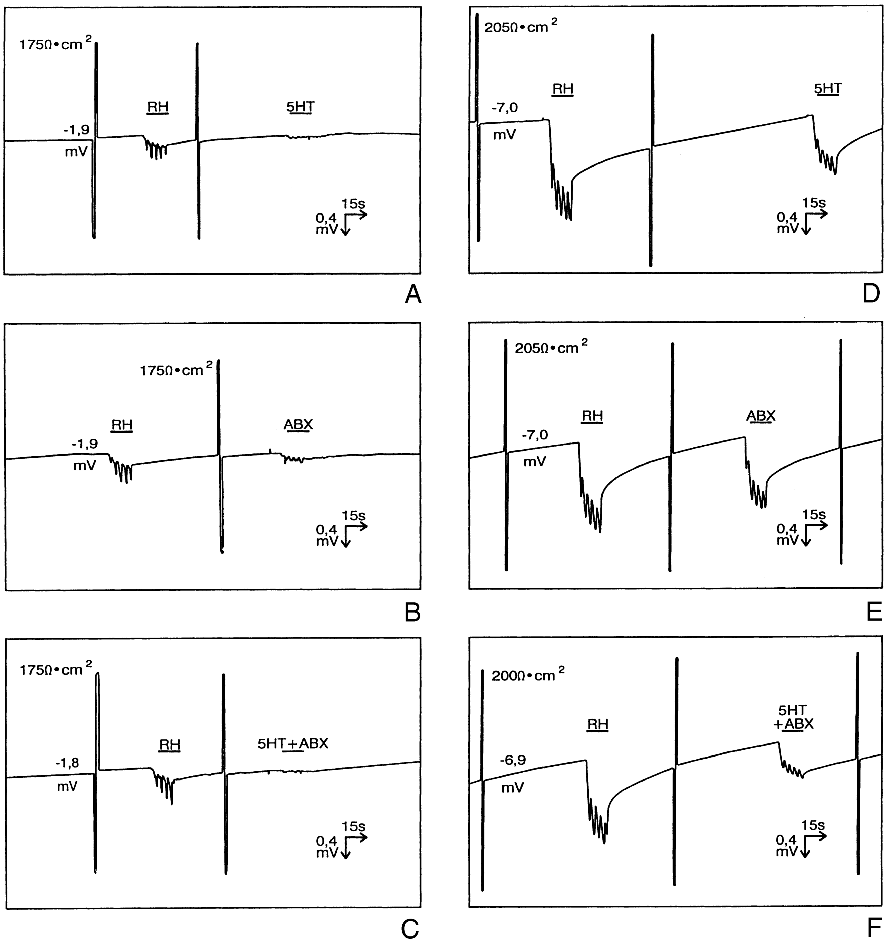

Fig. 2. The serotonin and ambroxol effects on the hyperpolarizing Na^+ currents elicited by the mechanical stimulation (gentle rinsing) of the mucosal side of the tracheal wall under the influence of amiloride. Single experiments are shown. The tissue was incubated and bathed in the presence of amiloride, stimulated (rinsed) with the Ringer solution—RH, or RH with the addition of serotonin—5-HT (A), ambroxol—ABX (B), or both test substances—5-HT + ABX (C), or the tissue was incubated in the presence of amiloride, bathed in Ringer solution—RH and stimulated with RH without or with the addition of serotonin—5-HT (D), ambroxol—ABX (E) or both test substance (F). In experiments (A), (B), and (C), amiloride was rinsed out of the system during stimulation, but when the stimulus (rinsing) stopped, the action of the inhibitor was restored, because it was present in the bathing solution.

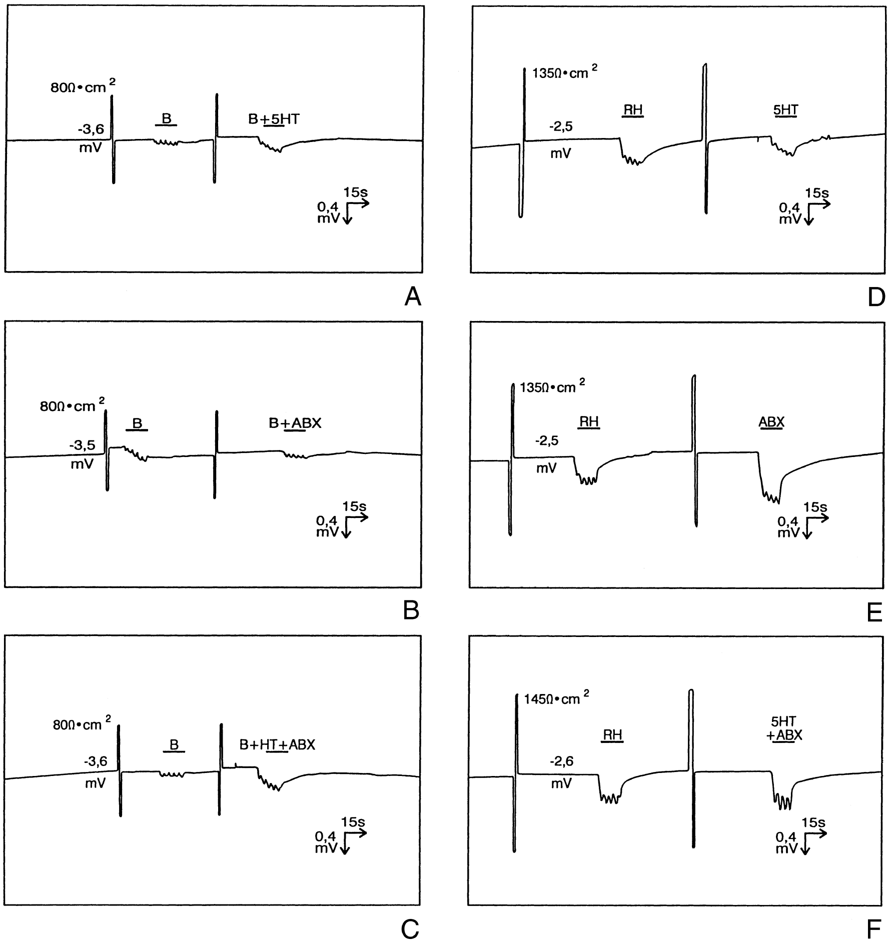

Fig. 3. The serotonin and ambroxol effects on the hyperpolarizing Na^+ currents elicited by the mechanical stimulation (a gentle rinsing) of the mucosal side of the tracheal wall under the influence of bumetanide. Single experiments are shown. The tissue was incubated, bathed and stimulated (by a gentle rinsing) in the presence of bumetanide—B, without and with the addition of serotonin—5-HT (A), ambroxol—ABX (B) or both test substances—5-HT + ABX (C), or the tissue was incubated and bathed in the presence of bumetanide and stimulated with Ringer solution—RH without or with serotonin—5-HT (D), ambroxol—ABX (E) and both test substances—5-HT + ABX (F).

Mechanical stimulation of the mucosal side of the tracheas, by means of gentle rinsing, almost invariably caused hyperpolarization of the tissues and the reactions were influenced by such experimental maneuvers as the addition of serotonin or ambroxol to the stimulation fluid (Fig. 1).

The potential difference value before stimulation and the hyperpolarization after mechanical stimulation of the tracheal wall were only slightly influenced after 1-h incubation and the presence of amiloride or bumetanide in the experimental system but the potential difference diminished greatly and the reaction disappeared if both inhibitors were present simultaneously (Table 1). The analysis of the effects of serotonin and ambroxol as seen in Fig. 1 is presented in Table 2. The addition of serotonin or ambroxol modified the hyperpolarization reaction differently under different experimental conditions.

With amiloride in the stimulation fluid, no influence on the hyperpolarization after mechanical stimulation was seen after the addition of serotonin and/or ambroxol to the stimulation fluid (Table 3). When the stimulation fluid was Ringer solution, as in Fig. 2 and Table 3, both serotonin and ambroxol clearly diminished the hyperpolarization.

With bumetanide in the stimulation fluid, the addition of serotonin and ambroxol augmented the hyperpolarization in some experimental groups although the effect dis-

Table 4
The influence of serotonin and ambroxol on Na^+ and Cl^- transepithelial pathways as evaluated in the presence of bumetanide

Stimulation fluid	Experimental conditions	
	BUME/BUME (n)	BUME/RH (n)
BUME + 5-HT	-0.2 ± 0.1 (23)	-0.2 ± 0.2 (10)
RH + 5-HT	-0.03 ± 0.1 (19)	$+0.02 \pm 0.1$ (10)
BUME + ABX	-0.1 ± 0.1 (24)	-0.2 ± 0.1 (10)
RH + ABX	-0.4 ± 0.1^a (19)	-0.2 ± 0.1 (10)
BUME + 5-HT + ABX	-0.7 ± 0.1^a (13)	-0.3 ± 0.2 (10)
RH + 5-HT + ABX	-0.5 ± 0.3 (9)	-0.1 ± 0.1 (9)

For explanations see Tables 1 and 3.
[a] Significantly different from zero at $a = 0.05$.

appeared when bumetanide was washed out with Ringer solution stimulation fluid (Fig. 3, Table 4).

4. Discussion

This study of the isolated tracheal wall showed that gentle mechanical stimulation of the mucosal face of the tissue caused a transient hyperpolarization, which could be modified by a hormonal or a pharmacological action (Fig. 1).

Before relating these results to airway pharmacology, an evaluation of the experimental model and some general considerations about the regulation of epithelial functions seem to be reasonable.

4.1. The evaluation of the experimental model

The experimental model we used was the isolated tracheal wall mounted in a Ussing apparatus (Koefoed-Johnsen and Ussing, 1958). The Ussing chamber was modified in this laboratory by fixing to its wall a nozzle connected to a peristaltic pump which temporarily produced a jet flux and acted as a stimulation device (Tyrakowski et al., 1997a,b).

The particulars of the method, the detailed procedure for standardization of the stimulation and the evaluation of the stability of the isolated tracheal wall have already been published (Tyrakowski et al., 1998a,b). This modification enables us to study the electrogenic ion transport of isolated epithelial tissues after stimulation of the intrawall regulatory mechanisms.

The ion currents in the airways in this study were analyzed under control and stimulated conditions and also after the addition of amiloride and/or bumetanide to the experimental system.

After 60-min incubation and the single addition of amiloride or bumetanide to the bathing fluid, the unstimulated potential difference value was augmented, but the hyperpolarization after mechanical stimulation was slightly diminished (Table 1). The immediate effects of the addition of amiloride or bumetanide (not shown), as opposed to the effects after 60-min incubation, were the variable diminution of the potential difference (Boucher, 1994a,b; Welsh, 1987) and the hyperpolarization after mechanical stimulation (Tyrakowski et al., 1998b). The difference between these experimental conditions results from the action of an intrawall regulatory system which efficiently compensates for the inhibition of one (Na^+ or Cl^-) transepithelial pathway by augmented activity of the other.

During the washout of amiloride from the system, the potential difference of tracheas and the hyperpolarization after mechanical stimulation were both augmented (Table 1, second row) as a result of the addition of the sodium ion current to the chloride ion current.

The combined addition of amiloride and bumetanide to the experimental system almost completely blocked both transepithelial pathways (for Na^+ and Cl^-) and the result was that both the unstimulated potential difference and the hyperpolarization were not different from zero (Table 1, last row).

On the basis of these experiments, the participation of both transepithelial ion transport pathways (for Na^+ and Cl^-) in the electrical potential difference under both control and stimulated conditions in this experimental system of the isolated tracheal wall was inferred.

The different experimental procedures (groups) were chosen from the wider array of pilot experiments to show the hyperpolarization after mechanical stimulation and also to identify the type of ionic current involved.

4.2. The regulation of epithelial functions

An intrawall regulatory system has been observed in many neurophysiological studies related to mucosal membranes and, particularly, it was shown that gentle mechanical stimulation was able to stimulate the afferent receptors of a local neural network, the most abundant of which are the C-fibers (Lee et al., 1992; Sengupta and Gebhart, 1994).

The stimulation evokes afferent impulses in nerve fibers (which form the afferent part of a neural reflex—in the airways cough reflex; Karlsson et al., 1988; Lalloo et al., 1996) and also liberates neuropeptides from sensory endings (which triggered a local reaction such as mucus secretion, the augmentation of blood flow and smooth muscle contraction; Barnes, 1992, 1996; Barnes et al., 1991a,b; Maggi, 1995; Maggi et al., 1995).

These local reactions in various epithelia include changes in ion transport and/or in transepithelial potential difference (Boucher, 1994a,b; Frizzell, 1988; Knowles et al., 1981; Lippe et al., 1994; Tyrakowski et al., 1997a,b, 1998c).

The influence of serotonin on the C-fibers and other intrawall neural elements is well documented (Barnes, 1992; Douglas and Ritchie, 1957; Neto, 1978; Christian et al., 1989; Kay and Armstrong, 1991). In the light of the above mentioned studies, it seems probable that the transepithelial hyperpolarization observed after mechanical stimulation was the result of transepithelial ion transport changes triggered and modulated by the mediators of the local regulatory system localized in the airway walls.

4.3. Serotonin diminishes the transepithelial potential difference

The actions of serotonin and ambroxol on stimulated transepithelial ion transport in airways were studied using the addition of these drugs (singly or combined) to the stimulation fluid. Both drugs were able to inhibit the sodium currents in an experiment in which amiloride was washed out of the experimental system,[1] and the net effect was a diminished hyperpolarization after mechanical stimulation of the tissue (shown in Figs. 1 and 2, and Table 3).

Similar studies showing that ambroxol acts on the unstimulated (Tamaoki et al., 1991) and stimulated (Tyrakowski et al., 1997a, 1998c) ion transport in airways were published recently.

In experiments aimed to show the action of serotonin on Na^+ and Cl^- transport in an airway epithelium (Jung et al., 1997; Tamaoki et al., 1996, 1997), the drug was added to the bathing solution and caused inhibition of the unstimulated Na^+ transport, but also augmented the unstimulated Cl^- transport.

The present study gave a picture of the inhibitory action of serotonin on the stimulated currents, which hyperpolarizes the transepithelial electrical potential difference of airways after mechanical stimulation. The addition of ambroxol to serotonin under these experimental conditions did not change the reaction (Fig. 2A–C, Table 3).

The inhibitory effects of serotonin, ambroxol and the combination of both were also seen in experiments without inhibitors (Fig. 2D–F, Table 3), but the ionic currents involved could not be identified in those experiments.

4.4. Serotonin augmented the transepithelial potential difference

Under conditions of blocked Cl^- transport (as in the experiments with bumetanide, Fig. 3A–C, Table 4), serotonin is able to augment the hyperpolarization after mechanical stimulation, and that is probably the result of additional Na^+ currents. In the presence of ambroxol, the effect of serotonin was augmented. The positive influence of ambroxol on the reaction had been observed earlier (Tyrakowski et al., 1998c). After complete washout of bumetanide from the Ussing chamber, the augmented hyperpolarization in the presence of serotonin, ambroxol or both was not maintained or preserved for the whole experimental group (Table 4), although in some preparations the effect could be shown repeatedly (e.g. Fig. 3D–F).

The action of serotonin on the hyperpolarization of airway wall after mechanical stimulation was clearly bi-directional. In one series of experiments, it was possible to show the diminution, and in another, the augmentation of Na^+ transport. It is hypothesized that serotonin interacts with membrane receptors as a partial agonist which inhibits maximum ion transport function but stimulates it if it is too weak. The action of ambroxol was indeed very similar, so it is, therefore, conceivable that both substances bind to the same or to closely related membrane receptors in the airway neuroendocrine control system.

4.5. Stimulated chloride current

In some experimental settings (60 min of incubation with the drug), the application of amiloride as a single inhibitor was not able to block the potential difference and the hyperpolarization after mechanical stimulation. Thus, the values of both these parameters in the presence of amiloride represented the chloride ion transport in the airway epithelium in these experiments. After mechanical stimulation in the presence of amiloride, the hyperpolarization of -0.5 ± 0.1 mV could result from a stimulated chloride current (Table 1). After the stimulation fluid was modified by the addition of serotonin and/or ambroxol, the hyperpolarization was not changed (Tables 2 and 3). It was not possible to show the influence of serotonin and ambroxol on stimulated chloride transport in these experiments.

In general, this study significantly broadened the understanding of the integrated airway reaction on the action of serotonin and also of ambroxol, as it added to such pathophysiological variables as mucus production, blood flow and the contraction of smooth muscle, a new one—the versatile changes in ionic currents and/or in transepithelial potential difference.

In summary, the study produced evidence that:

(1) Under conditions of withdrawal of the inhibition of transepithelial sodium ion transport (washing out the amiloride), serotonin diminished the hyperpolarization after the mechanical stimulation of the airways, so serotonin is able to diminish or inhibit Na^+ currents in the airway epithelium.

(2) Under conditions of inhibited transepithelial chloride transport (in the presence of bumetanide), serotonin augmented the hyperpolarization after stimulation; serotonin can thus augment Na^+ currents in the airway epithelium.

(3) During hyperpolarization after mechanical stimulation of the airway epithelium, there is a clear-cut augmentation of the chloride currents, but this phenomenon is not influenced by serotonin or ambroxol.

[1] When amiloride is in a bathing fluid but the stimulation fluid from the peristaltic pump is free of the drug, the hyperpolarization reaction after mechanical stimulation (rinsing by a series of jets) is composed of a series of needlelike fluctuations above the surface of the hyperpolarization as in the first record in Fig. 1. The fluctuations can be explained as being caused by a temporary opening of sodium channels by washout of the amiloride with jets of a rinsing (stimulation) fluid, then, because the amiloride is in a bathing fluid, when rinsing stops, the channels are closed by amiloride. The surface of the hyperpolarization under the fluctuations in these experimental conditions represents the chloride ion currents (Tyrakowski et al., 1998b).

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References

Barnes, P.J., 1992. Modulation of neurotransmission in airways. Physiol. Rev. 72, 699–729.

Barnes, P.J., 1996. Neuroeffector mechanisms: the interface between inflammation and neuronal responses. J. Allergy Clin. Immunol. 98, 73–83.

Barnes, P.J., Baraniuk, J.N., Belvisi, M.G., 1991a. Neuropeptides in the respiratory tract. Part One. Am. Rev. Respir. Dis. 144, 1187–1198.

Barnes, P.J., Baraniuk, J.N., Belvisi, M.G., 1991b. Neuropeptides in the respiratory tract. Part Two. Am. Rev. Respir. Dis. 144, 1391–1399.

Boucher, R.C., 1994a. Human airway ion transport. Part One. Am. J. Respir. Crit. Care Med. 150, 271–281.

Boucher, R.C., 1994b. Human airway ion transport. Part Two. Am. J. Respir. Crit. Care Med. 150, 581–593.

Cazzola, M., Matera, M.G., D'Amato, G., Rossi, F., 1995. Effects of serotonin on airways: recent developments. Allergy 50, 1–10.

Christian, E.P., Taylor, G.E., Weinreich, D., 1989. Serotonin increases excitability of rabbit C fiber neurons by two distinct mechanisms. J. Appl. Physiol. 667, 584–591.

Douglas, W.W., Ritchie, J.M., 1957. On the excitation of non-medullated afferent fibers in the vagus and aortic nerves by pharmacological agents. J. Physiol. (London) 138, 31–43.

Frizzell, R.A., 1988. Role of absorptive and secretory processes in hydration of the airway surface. Am. Rev. Respir. Dis. 38, 3–6.

Higenbottam, T., 1984. Cough induced by changes of ionic composition of airway surface liquid. Bull. Eur. Physiopathol. Respir. 20, 553–562.

Germonpre, P.R., Joos, G.F., Pauwels, R.A., 1998. Modulation by 5-HT_{1A} receptors of the 5-HT_2 receptor-mediated tachykinin-induced contraction of the rat trachea in vitro. Br. J. Pharmacol. 123, 1571–1578.

Joos, G.F., Lefebvre, R.A., Bullock, G.R., Pauwels, R.A., 1997. Role of 5-hydroxytryptamine and mast cells in the tachykinin-induced contraction of rat trachea in vitro. Eur. J. Pharmacol. 338, 259–268.

Jung, J.S., Oh, S.O., Kim, M.G., Kang, D.S., Lee, S.H., 1997. Cl-secretion induced by 5-hydroxytryptamine and calcitonin gene related peptide (CGRP) in rat tracheal epithelia. Pfluegers Arch. 435, 20–27.

Karlsson, J.A., Sant'Ambrogio, G., Widdicombe, J., 1988. Afferent neural pathways in cough and reflex bronchconstriction. J. Appl. Physiol. 65, 1007–1023.

Kay, I.S., Armstrong, D.J., 1991. MDL 72222 (a selective 5-HT_3 receptor antagonist) prevents stimulation of intrapulmonary C fibres by pulmonary embolization in anaesthetized rabbits. Exp. Physiol. 76, 213–218.

Knowles, M.R., Gatzy, J., Boucher, R., 1981. Increased bioelectric potential difference across respiratory epithelia in cystic fibrosis. N. Engl. J. Med. 305, 1489–1495.

Koefoed-Johnsen, V., Ussing, H.H., 1958. The nature of the frog skin potential. Acta Physiol. Scand. 42, 298–308.

Lalloo, U.G., Barnes, P.J., Fan Chung, K., 1996. Pathophysiology and clinical presentations of cough. J. Allergy Clin. Immunol. 98, 91–97.

Lee, B.P., Sant'Ambrogio, G., Sant'Ambrogio, F.B., 1992. Afferent innervation and receptors of the canine extrathoracic trachea. Respir. Physiol. 90, 55–65.

Lippe, C., Bellantuono, V., Castronuovo, G., Ardizzone, C., Casano, G., 1994. Action of capsaicin and related peptides on the ionic transport across the skin of Rana esculenta. Arch. Int. Physiol. Biochem. Biophys. 102, 51–54.

Lucchelli, A., Santagostino-Barbone, M.G., Barbieri, A., Tonini, M., 1994. A pharmacological analysis of receptors mediating the excitatory response to 5-hydroxytryptamine in the guinea pig isolated trachea. Br. J. Pharmacol. 112, 763–768.

Maggi, C.A., 1995. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. Prog. Neurobiol. 45, 1–98.

Maggi, C.A., Giahetii, A., Dey, R.D., Said, S.I., 1995. Neuropeptides as regulators of airway function: vasoactive intestinal peptide and the tachykinins. Physiol. Rev. 75, 277–322.

Neto, F.R., 1978. The depolarizing action of 5-HT on mammalian non-myelinated nerve fibres. Eur. J. Pharmacol. 49, 351–356.

Pype, J.L., Verleden, G.M., Demedts, M.G., 1994. 5-HT modulates noncholinergic contraction in guinea pig airways in vitro by prejunctional 5-HT_1-like receptor. J. Appl. Physiol. 77, 1135–1141.

Rizzo, C.A., Kreutner, W., Chapman, R.W., 1993. 5-HT_3 receptors augment neuronal, cholinergic contractions in guinea pig trachea. Eur. J. Pharmacol. 234, 109–112.

Scheuermann, D.W., Adriaensen, D., Timmermans, J.P., De Groodt-Lasseel, M.H.A., 1992. Comparative histological overview of the chemical coding of the pulmonary neuroepithelial endocrine system in health and disease. Eur. J. Morphol. 30, 101–112.

Sengupta, J.N., Gebhart, G.F., 1994. Gastrointestinal afferent fibers and sensation. In: Johnson, L.R. (Ed.), Physiology of the Gastrointestinal Tract. Raven Press, New York, NY, pp. 483–519.

Stone, R.A., Worsdell, Y.M., Fuller, R.W., Barnes, P.J., 1993. Effects of 5-hydroxytryptamine and 5-hydroxytryptophan infusion on the human cough reflex. J. Appl. Physiol. 74, 396–401.

Szarek, J.L., Zhang, J.Z., Gruetter, C.A., 1993. 5-HT_2 receptors augment cholinergic nerve-mediated contraction of rat bronchi. Eur. J. Pharmacol. 231, 339–346.

Tamaoki, J., Chiyotani, A., Yamauchi, F., Takeuchi, S., Takizawa, T., 1991. Ambroxol inhibits Na^+ absorption by canine airway epithelial cells in culture. J. Pharm. Pharmacol. 43, 841–843.

Tamaoki, J., Chiyotani, A., Takemura, H., Konno, K., 1996. Effect of 5-hydroxytryptamine on bioelectric properties of airway epithelial cells in culture. Res. Commun. Mol. Pathol. Pharmacol. 92, 285–298.

Tamaoki, J., Chiyotani, A., Takemura, H., Konno, K., 1997. 5-Hydroxytryptamine inhibits Na^+ absorption and stimulates Cl^- secretion across canine tracheal epithelial sheets. Clin. Exp. Allergy 27, 972–977.

Tyrakowski, T., Sedlaczek, A., Greczko, I., Bartlomowicz, M., Wojciechowska, M., 1997a. Ambroxol effect on transepithelial electrical potential difference of isolated tracheal wall. Pol. J. Pharmacol. 49, 53–58.

Tyrakowski, T., Wojciechowska, I., Banach, B., Moscibroda, A., Greczko, I., 1997b. Electrophysiological study of local ion transport changes of tracheal wall in vitro. Ann. Acad. Med. Stetin. 43, 99–111.

Tyrakowski, T., Banach, B., Greczko, I., Bartlomowicz, M., Wojciechowska, M., 1998a. Electrophysiological study of the interaction between epithelium and the airway fluid lining. Int. Rev. Allergol. Clin. Immunol. 4, 59–65.

Tyrakowski, T., Banach, B., Moscibroda, A., Bartlomowicz, M., Wojciechowska, M., 1998b. Reappraisal of amiloride action on transepithelial electrical potential difference of isolated tracheal wall. Arch. Immunol. Ther. Exp. 46, 45–50.

Tyrakowski, T., Greczko, I., Sedlaczek, A., Banach, B., Bartlomowicz, M., Wojciechowska, M., Binkowska, I., Chojnacka, H., 1998c. Electrophysiological investigation of the effects of ambroxol on the transepithelial Na^+ ion transport pathway in airways. Pol. J. Pharmacol. 50, 31–38.

Welsh, M.J., 1987. Electrolyte transport by airway epithelia. Physiol. Rev. 67, 1143–1184.