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# The short-term rinsing of airways by N-acetylcysteine helps expectoration: The mechanism of sodium and chloride transport

## Pozytywny wpływ krótkotrwałego opłukiwania dróg oddechowych roztworem N-acetylocysteiny na odkrztuszanie – udział mechanizmu przezbłonowego transportu jonów sodowych i chlorkowych

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**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

**Summary:**

N-acetyl-L-cysteine (NAC) mucolytic and antioxidant role is well known, but the effect on epithelial ion transport has not been yet described. The aim of the study was to evaluate the short-term and prolonged influence of NAC on ion transport in the epithelium.

The experiment was performed on 108 fragments of rabbit tracheae. Fragments were divided into four groups: inhibited sodium (I) and chloride (II) transport, NAC with inhibited sodium (III) and NAC with inhibited chloride (IV) transport. The changes in electrophysiological parameters were measured in stationary conditions and during mechanical-chemical stimulation after immediate (15 s) and prolonged (60 min) N-acetylcysteine administration on the tissue.

Each 15-second stimulation caused repeatable changes in the electric potential of the tissue. In trachea fragments with blocked chloride ion transport, significantly lower ($P < 0.0001$) values of electric potential following prolonged NAC effect were observed when compared to short-term NAC-stimulation. The values of resistance were constant during experiments, which reflects the vitality of the tissue.

Short-term NAC administration influences sodium ion transport, which is not observed in a prolonged stimulation. The use of the NAC solution to rinse the airways is of great clinical importance due to the short and intense contact with the epithelium.

**Keywords:** amiloride, bumetanide, N-acetylcysteine, transepithelial potential

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**Abbreviations:** **A** – amiloride solution (0.1 mM), used as blocker of transepithelial sodium transport, **ASL** – airway surface liquid, **B** – bumetanide solution (0.1 mM), used as blocker of transepithelial chloride transport, **CFTR** – cystic fibrosis transmembrane regulator, **dPD** – hyperpolarization of transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (mV), **ENaC** – epithelial sodium channel, **NAC** – N-acetyl-L-cysteine, **PD** – transepithelial potential difference of epithelial tracheal surface (mV), **PDmin** – minimal transepithelial potental difference during 15 s stimulation of epithelial tracheal surface (mV), **R** – transepithelial resistance ($\Omega/cm^2$).

## INTRODUCTION

N-acetylcysteine (N-acetyl-L-cysteine, NAC) is a thiol (sulfhydryl-containing) antioxidant compound which exerts its action through the reduction of extracellular cystine to cysteine, promoting glutathione biosynthesis, therefore diminishing the levels of free radicals and their active metabolites [3, 4, 7, 15, 18, 19, 28, 29, 30]. In addition to its antioxidant function, NAC also influences mucus production [19, 39]. Through its interaction with the mucin disulfide bridges, active sulfhydryl groups of NAC induce thinning of the mucus and facilitate formation of hydrophilic complexes [16, 19, 21, 29, 30, 34, 39, 41]. Furthermore, at low concentrations, NAC stimulates activity of the cilia, whereas at higher concentrations presents an opposite effect [5, 32].

Both changes in airway surface liquid (ASL) and ciliary activity are regulated by epithelial ion and water transport. Continuous flow of the fluid and changes in its quantity and viscosity inhibits the adhesion and growth of microorganisms colonizing the respiratory epithelium [21, 37]. The activity of sodium-potassium pump, chloride ion secretion through cystic fibrosis conductance regulator (CFTR), and sodium ion absorption through epithelial sodium channel (ENaC), create an electronegative charge of the respiratory epithelial surface [24, 36]. The electronegative charge affects the proteins building cilia and creates a space for cilia movement by pushing negatively charged proteins (e.g. mucins) [2, 13]. Changes in sodium and chloride ion transport are associated with the activation of cough reflex together with ASL concentration, which leads to difficulties with its expectoration, such as these observed in cystic fibrosis, asthma and chronic obstructive pulmonary disease (COPD) [2, 11, 13, 16, 17, 21, 26, 34, 35, 38, 39]. Thus, studies on ion transport in airways seem to be of great importance for determining the pathobiochemical mechanisms of diseases associated with cough and inflammatory responses, particularly cystic fibrosis, asthma and COPD [2, 11, 13, 17, 21, 22, 26, 41].

Previous research suggested that NAC influences ion flow regulation in airways, although the types of ions and ion pathways influenced by NAC have not been specified [3, 33]. The aim of the presented study was to evaluate the short-term and prolonged influence of NAC on ion transport of the epithelium. To specify whether chloride or sodium ion transport is affected, we conducted the experiments in the presence of sodium transport blocker (amiloride) to assess the effect of NAC on chloride ion transport, and chloride transport blocker (bumetanide) to assess the effect of NAC on sodium ion transport. Due to NAC's high biochemical activity [18, 29], we evaluated the way and conditions in which this compound influences changes of electrical potential on the surface of respiratory epithelial cells. We hypothesize this phenomenon is important for the abruption of mucus plugs from tracheal walls during the onset of the cough reflex. In addition, the administration of infusions into the respiratory tract stimulates the movement along the epithelial surface, causing the intensification of ion transport, and induces the cough reflex; NAC administration can minimize this effect.

## MATERIAL AND METHODS

The experiments were performed on 108 fragments of tracheae from 36 rabbits. Adult New Zealand albino rabbits of both sexes, weighing between 3.5 and 5.0 kg, three to four months old, were the study subjects. The rabbits were maintained on a 12/12 light/dark cycle, with food and water available ad libitum and were asphyxiated with isoflurane and a high concentration of $CO_2$ (c. 60% of the inhaled air) [35]. The tracheae were excised and immediately placed in Ringer solution, then cut along the membranous part and divided into 2 to 3 fragments. The specimens prepared in this way were proven to contain an intact epithelium and nerves fibres [38]. The fragments were transferred to the incubation solutions according to the experimental schedule and then mounted in an Ussing apparatus. The Local Committee for Ethical Animal Experiments approved the experimental schedules (permit no. 16/21012, June 21, 2012).

## EXPERIMENTAL PROCEDURE

After 30 min of incubation in a proper solution (A, A-NAC, B, B-NAC, respectively), a piece of tissue was positioned in the adapter. Then, the adapter was mounted in the Ussing

apparatus for measuring the electrophysiological parameters and was filled with the appropriate liquid (fig. 1).

In the experiment, the modified Ussing apparatus, fitted with a nozzle connected to a peristaltic pump, was used. The specimens of tissue were mounted horizontally. The nozzle was placed 0.2 mm away from the tracheal surface and stimulation (washing) fluid jets from the peristaltic pump were directed in a direction perpendicular to the tissue sample. Mechanical-chemical stimulus was applied to the mucosal side of the tissue, which is associated with the localization of receptor systems [11, 35, 38]. Stimulation was performed using proper solutions, with fluid discharged from the nozzle at a volume of 0.05 ml/s (0.75 ml/15 s) at a temperature of 25°C. Each stimulation lasted 15 s, which was sufficient to induce repeatable and significant changes in transepithelial electrical potential difference (PD). The composition of washing (stimulation) fluids is described in fig. 1.

The electrodes Ag/AgCl and agar bridges provided a connection of the chamber with the measuring equipment. Electrophysiological parameters were assessed continuously. The measurement of PD (mV) in stationary conditions reflects the constant ion transport in the tissues after the application of incubation fluid and was recorded continuously. Changes of the electric potential (dPD, mV) and minimal potential (PDmin, mV) were measured during the stimulation of the trachea fragments with the solution provided in the experimental design (fig. 1). Electrical resistance of tissue (R, Ω/cm$^2$) was determined by applying a current intensity of ±10 μA to the tissue stimulus and by measuring the corresponding voltage change according to Ohm's law. The experimental procedure for a single tissue specimen lasted approximately 60 minutes at a temperature of 25 ±2°C.

## STUDY DESIGN

The scheme of study design is presented in fig. 1. The following steps of the schedule were performed:

1. Immediate NAC-action (15 s)

To estimate the immediate effects of NAC on sodium and chloride transport, 54 trachea fragments were divided into two groups:

- Group 1 – Inhibited sodium transport (A) – fragments incubated in Ringer solution with the sodium transport blocker: amiloride, N = 26. Trachea was stimulated by solution A for 15 s (Stimulation I), then by A-NAC for 15 s (Stimulation II).
- Group 2 – Inhibited chloride transport (B) – fragments incubated in Ringer solution with the chloride transport blocker: bumetanide, N = 28. Trachea was stimulated by B solution for 15 s (Stimulation I), then by B-NAC for 15 s (Stimulation II).

2. Prolonged NAC-action (60 min)

To estimate the prolonged effects of NAC on sodium and chloride transport, another 54 trachea fragments were divided into two groups:

- Group 3 – Long-term incubation by NAC with inhibited sodium transport (A-NAC) – fragments incubated in Ringer solution with amiloride and NAC (A-NAC), N = 26. Trachea was stimulated by A-NAC for 15 s (Stimulation I) solution, then by A for 15 s (Stimulation II).
- Group 4 – Long-term incubation by NAC with inhibited chloride transport (B-NAC) – fragments incubated in Ringer fluid with amiloride and NAC (B-NAC) N = 28. Trachea was stimulated by B-NAC solution for 15 s (Stimulation I), then by B for 15 s (Stimulation II).

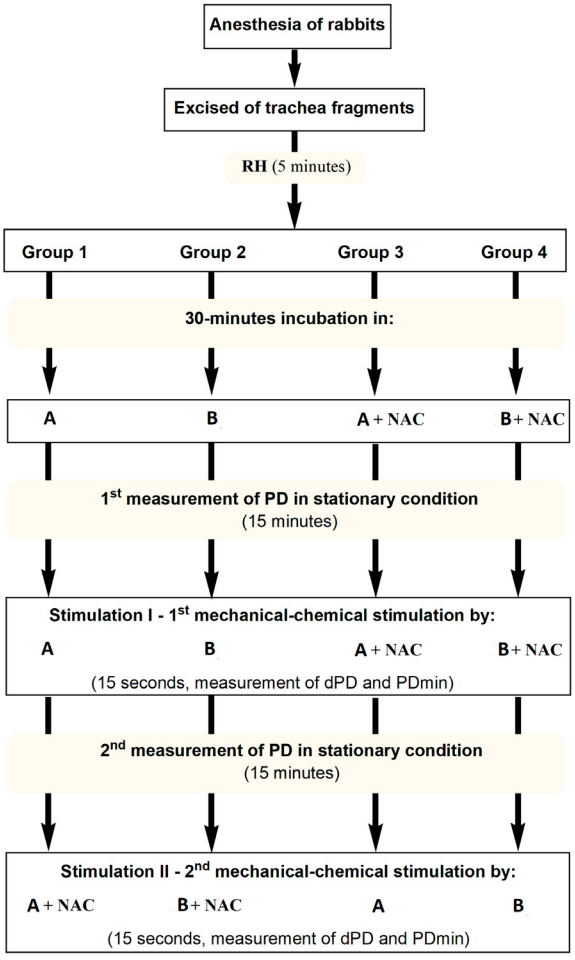



**Fig. 1.** Study design with the composition of stimulation fluids; : A – inhibited sodium transport by amiloride (0.1 mM), used as blocker of transepithelial sodium transport, B – inhibited chloride transport by bumetanide (0.1 mM), used as blocker of transepithelial chloride transport, A-NAC – N-acetylcysteine (0.1 mM) in A solution, B-NAC – N-acetylcysteine (0.1 mM) in B solution, PD – transepithelial potential difference of epithelial tracheal surface (mV), PDmin – minimal transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (mV), dPD – hyperpolarization of transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (mV)

## CHEMICALS

During the experiments the following reagents were used:

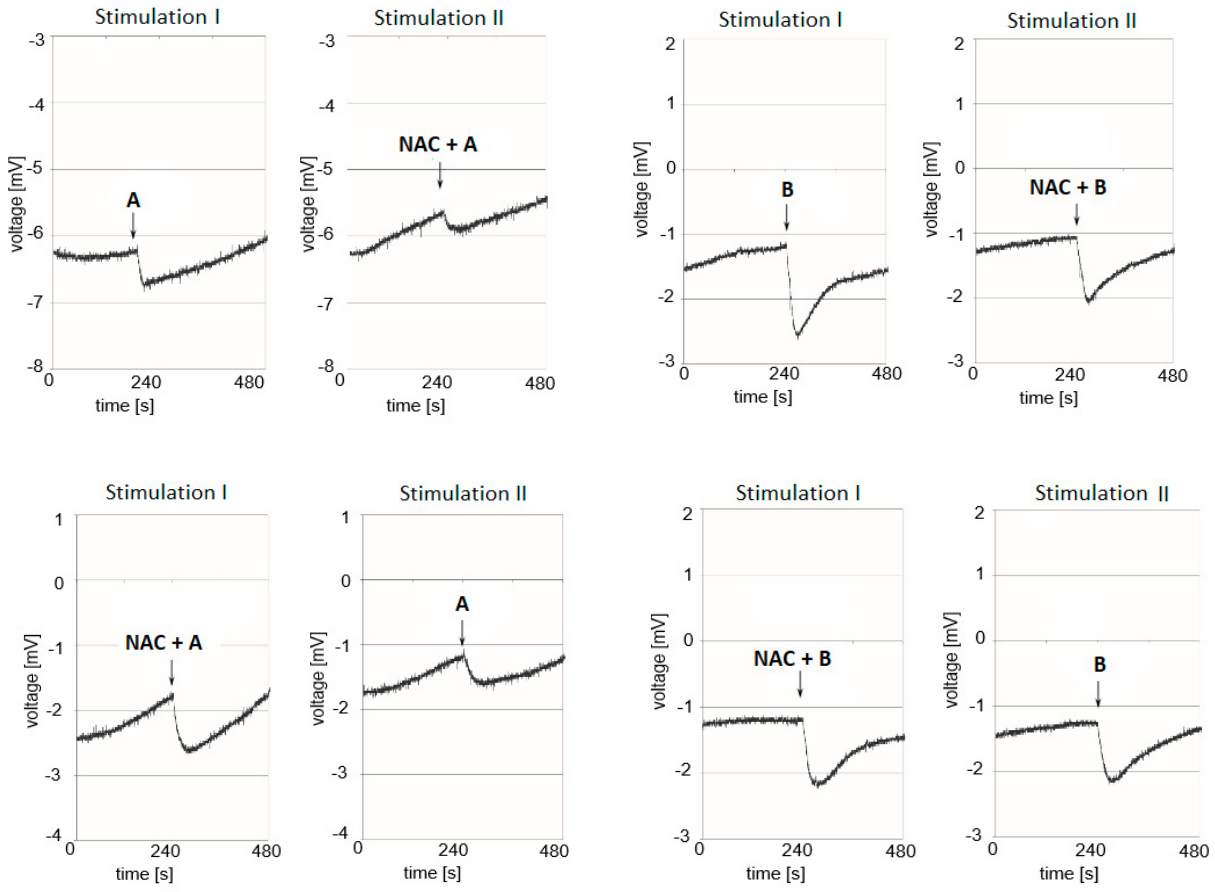



**Fig. 2.** An exemplary voltage of tissue sample during short-term (15 s) and long-term (1 h) N-acetylcysteine (NAC) stimulation with the inhibitor of sodium and chloride epithelial transport of tracheal wall. Time-course of single experiment with stimulation I and II is shown. Each chart represents a sample reaction to mechanical-chemical stimulation (15 s) from a series of reaction in consistence with the experimental protocol. The arrows indicate the start of mechanical-chemical stimulation; A) Short-term (15 s) N-acetylcysteine (NAC) stimulation with the inhibitor of sodium epithelial transport. B) Short-term (15 s) N-acetylcysteine (NAC) stimulation with the inhibitor of chloride epithelial transport. C) Long-term (1 h) N-acetylcysteine (NAC) action with the inhibitor of sodium epithelial transport. D) Long-term (1 h) N-acetylcysteine (NAC) stimulation with the inhibitor of chloride epithelial transport : A – inhibited sodium transport by amiloride (0.1 mM), used as blocker of transepithelial sodium transport, A-NAC – N-acetylcysteine (0.1 mM) in A solution, B – inhibited chloride transport by bumetanide (0.1 mM), used as blocker of transepithelial chloride transport, B-NAC – N-acetylcysteine (0.1 mM) in B solution

- RH – Ringer solution: K+ 4.0 mM; Na+ 147.2 mM; Ca2+ 2.2 mM; Mg2+ 2.6 mM; Cl- 160.8 mM; Hepes (4-(2-hydroxyethyl)piperazine-1-ethanosulfonic acid) 10.0 mM, adjusted to pH 7.4 under the control of a pH-meter; basic solution with iso-osmotic properties.
- Amiloride hydrochloride hydrat (A, 0.1 mM) – amidynoamid acid, 3,5-diamino-6-chloro-2-carboxylic acid; 266.09 g/mol (Sigma-Aldrich, USA), dissolved and diluted in RH, used as blocker of transepithelial sodium transport.
- Bumetanide (B, 0.1 mM) – 3-butylamino-4-phenoxy--5-sulfamoylbenzoic acid; 364.42 g/mol (Sigma--Aldrich, USA), dissolved in DMSO (dimethyl sulfoxide) and diluted in RH, final concentration DMSO 0.1%, used as blocker of transepithelial chloride transport.
- N-acetylcysteine, (NAC, A-NAC, B-NAC, 0.1 mM) – N-acetyl-L-cysteine; 163.19 g/mol (Sigma-Aldrich, USA), dissolved and diluted in A or B solutions.
- Mineral compounds: KCl, NaCl, CaCl2, MgCl2 (POCH, Poland).

## DATA ANALYSIS

Data was recorded by an experimental protocol EVC 4000 (WPI, USA) apparatus to measure voltage resistance, connected to an experimental computer data acquisition system MP 150 (Biopac, USA), and translated using the data acquisition program, AcqKnowledge 3.8.1 (WPI, USA). Statistical analysis was performed using STATISTICA 13.1 (Statsoft, Poland). ANOVA mixed models were used for immediate and prolonged NAC stimulation independently, using TIME (stimulation I, stimulation II) as a within-subject factor and GROUP (A vs. B for short-term NAC stimulation and A-NAC vs. B-NAC for long-term NAC stimulation) as a between-subject factor. Post-hoc Bonferroni test was used to compare differences between groups. To analyze the prevalence of the overshoot (binary outcome) between the four groups, Fisher Exact test 4 x 2 contingency table was

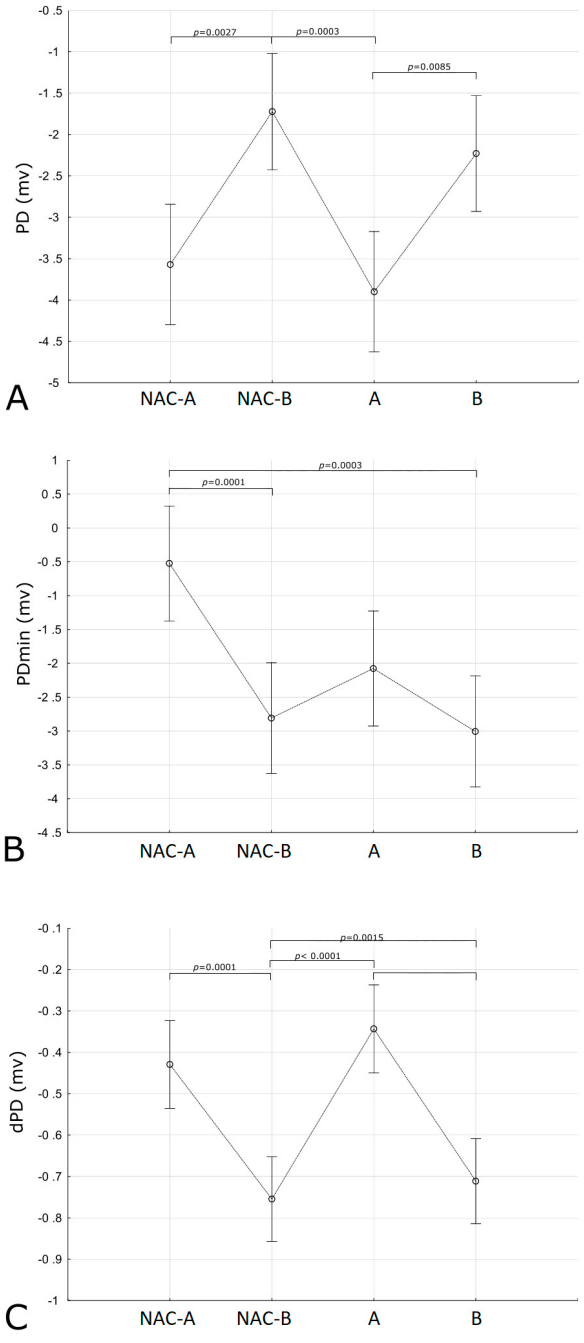



**Fig. 3.** The comparison of electrophysiological parameters for stimulation I of short-term (A and B) and long-term (A-NAC, B-NAC) N-acetylcysteine action: A) transepithelial potential difference of epithelial tracheal surface measured in stationary conditions (PD, mV), B) minimal transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (PDmin, mV), C) hyperpolarization of transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (dPD, mV) : A – inhibited sodium transport by amiloride (0.1 mM), used as blocker of transepithelial sodium transport, B – inhibited chloride transport by bumetanide (0.1 mM), used as blocker of transepithelial chloride transport, A-NAC – N-acetylcysteine (0.1 mM) in A solution, B-NAC – N-acetylcysteine (0.1 mM) in B solution

performed. The results were considered as significant at $p < 0.05$.

## RESULTS

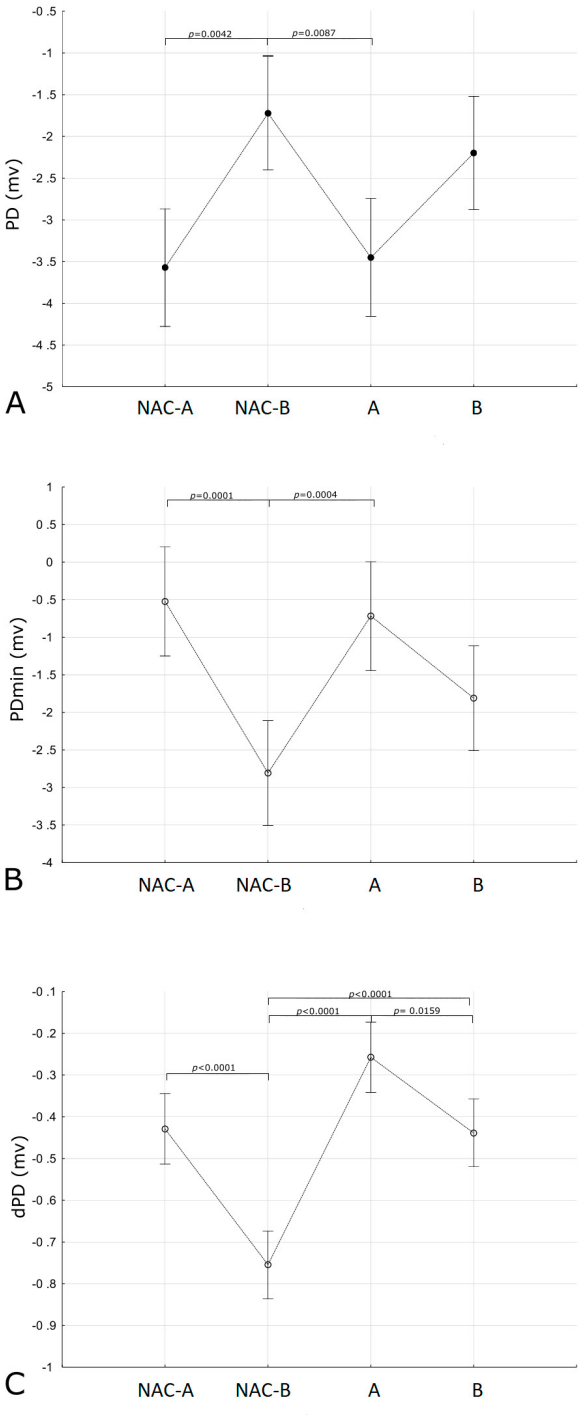
The measured stationary PD values were significantly different from the dPD and PDmin measured during stimulation, regardless of sodium/chloride ion transport inhibition. Each mechanical-chemical stimulation induced hyperpolarization, i.e. repetitive changes in ion transport were reflected in changes of the PD (fig. 2A-D).

During NAC stimulation under conditions of inhibited sodium chloride transport at steady-state stationary PD, we observed repetitive hyperpolarization reactions in all analyzed fragments (dPD, PDmin).

Tissue fragments incubated in the amiloride solution presented a stationary PD of –3.33 mV at the beginning of the experiment and –3.21 mV after 15 s stimulation with NAC at a concentration of 0.1 mM. In the bumetanide solution, the PD ranged from –2.00 mV to –1.85 mV at the end of the experiment. Long-term use of the solution of NAC and amiloride solution induced a PD change from –2.93 mV to –3.33 mV, and incubation in NAC and bumetanide from –2.05 mV to 1.85 mV.

The PD measured during the experiment did not change significantly in each of the studied groups. However, the PDs compared between the groups were significantly different under the conditions of Na (A) and Cl (B) inhibition relative to the analogous conditions after NAC, A-NAC, B-NAC (fig. 3A). An especially important observation is the statistically lower differences of PD following prolonged NAC effect with blocked chloride ion transport when compared to the short-term NAC stimulation. In addition, the PDmin measured during stimulation under the conditions of inhibition of sodium and chloride ion transport was significantly different after administration of NAC in the same conditions. There was also a change in PDmin after administration of amiloride and NAC (A-NAC) against administration of bumetanide (B) (fig. 3B). The change in dPD measured during stimulation was also significantly different in the Na (A) and Cl (B) ion inhibition conditions relative to the analogous conditions after A-NAC, B-NAC. Also, dPD after administration of ion transport inhibitors and NAC were significantly different (A vs. A-NAC and B vs B-NAC). (fig. 3C).

The measured dPD of tissue incubated with NAC solution showed a statistically significant change both under the conditions of inhibited sodium and chloride ion transport ($p < 0.05$). Additionally, a significant change in dPD values was observed under conditions of prolonged NAC effect on the tissue (fig. 4). Particular attention should be paid to the result of measured dPD obtained, which indicated that short term NAC-B administration influences sodium ion transport ($p < 0.001$), it is not observed in a prolonged stimulation.




**Ryc. 4.** The comparison of electrophysiological parameters for stimulation II of short-term (A and B) and long-term (A-NAC, B-NAC) N-acetylcysteine action: A) transepithelial potential difference of epithelial tracheal surface measured in stationary conditions (PD, mV), B) minimal transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (PDmin, mV), C) hyperpolarization of transepithelial potential difference during 15 s stimulation of epithelial tracheal surface (dPD, mV): A – inhibited sodium transport by amiloride (0.1 mM), used as blocker of transepithelial sodium transport, B – inhibited chloride transport by bumetanide (0.1 mM), used as blocker of transepithelial chloride transport, A-NAC – N-acetylcysteine (0.1 mM)

The PDmin measured during stimulation showed no significant changes, regardless of the experimental conditions.

A phenomenon observed during incubation in the NAC and bumetanide was an occurrence of the overshoot reaction, i.e. the spontaneous reaction of the tissue after the end of the mechanical and chemical stimulation. The average duration of this reaction was 27 s. The potential difference (dPD) after cessation of the hyperpolarization reaction was –0.86 mV (median). In the case of incubation in the NAC and amiloride solution, the overshoot was recorded in 57% of tissue fragments, which was significantly greater number than in other conditions, with an average time of 40 s and a dPD of –1.10 mV.

The measured value of resistance during the experiments was maintained at a constant level, regardless of the experimental conditions, which reflects the vitality of the tissue. Resistance values ranged from 128 to 155 Ω/cm$^2$ (median) and presented no statistically significant differences.

## DISCUSSION

The use of electrophysiological methods provides an opportunity to confirm the role of the physiological mechanisms regulating processes of electrogenic ion segregation for the production/generation and movement of airways surface liquid along the lining of bronchi and trachea [2, 11, 13, 14, 17, 22, 25, 26, 33, 35, 38]. Results of experiments presented in this paper confirm the possibility of transepithelial ion transport modification by applying NAC.

The chamber used to perform these experiments enabled the structure and thickness of the tracheal wall to be maintained unchanged during the measurement of its electrophysiological parameters [2, 11, 13, 35, 38]. The nerve endings of analyzed fragments were preserved. The fragments of examined tissues were reactive and the observed reactions were repetitive (fig. 2A-D). It is worth emphasizing that the used procedure of killing experimental animals with a mixture of isoflurane and carbon dioxide has been described by Smuszkiewicz and co-authors [35], where it was shown that the applied mixture has no effect on the electrophysiological parameters measured.

The measured values of electrical resistance were comparable to those previously described in literature [11, 35, 38], which reflects the integrity and preserved function of epithelial tissue [25]. The measured values of electrical resistance were not affected by the experimental condition, the form of administration of NAC and blockers of chloride and sodium transport. It has been proven that the tracheal fragments were alive and had the active endings of nerve fibers, and NAC did not act on the integrity of the respiratory epithelium and did not change its structure [7, 19, 39]. This model refers to the mechanical rinsing of the mucous wall of the trachea, which "mimics" mechanical stimulation under physiological conditions during a cough [1, 11, 38]. It also mimics the airway flow of inhaled substances, like tobacco smoke and small foreign bodies, including

powders containing psychoactive substances, glass particles, and potato starch [10, 23].

The analyzed PD values measured in stationary conditions and during mechanical and chemical stimulation (PDmin, dPD) correspond to the ion segregation through the mechanism of chloride ion secretion by CFTR and/or absorption of sodium ions by ENaC [11, 38]. Therefore, we can assume that NAC induce changes in ion transport measured in the conditions of preserved chloride ion secretion and blocked sodium ion absorption as well as in the conditions of preserved sodium ion and inhibited chloride transport.

The effect of 15 s NAC stimulation on tracheal ion transport has been followed by a change in stationary PD (fig. 4). Short-term NAC stimulation induced PD increase, which can be explained by the influence of NAC on the receptor proteins responsible for ion transport regulation [23]. Furthermore, NAC can interact with mucins [6, 30, 31, 40], which can induce changes in ASL hydration and transepithelial ion transport [12, 24, 36]. Lack of difference in stationary potential measured under the conditions of inhibited sodium and chloride ion transport (fig. 4) can be explained by the adaptation of the tracheal tissue [1, 14, 35]. Rapid changes in the hydration of liquid lining on the surface of the respiratory epithelium could be caused by changes in the transport of sodium and chloride ions [24, 36]. Administration of NAC during 15 s mechanical-chemical stimulation affects the surface of the airways (fig. 2A-D). Measured reactions were repeatable and were modified by using sodium or chloride ion transport blockers [11, 38].

The processes associated with chloride ion secretion and sodium ion reabsorption on the apical surface of respiratory epithelium influence the electrophysiological parameters of isolated tracheal walls [2, 11, 26, 38]. The secretion of chloride implies hydration of ASL, whereas sodium absorption causes it to be dense [2, 13]. Local thickening of the ASL causes the additional irritation of the epithelium and initiates a cough reflex [1, 36]. This process is necessary to clear the airways and the ability to open/close the ion channels rapidly supports the cough [11, 36]. The opening of the sodium channels in the nerve endings, supported by NAC, initiates the reaction of the cough reflex, which helps to remove the ASL.

NAC did not change the stationary potential during amiloride stimulation which proves the lack of influence on chloride ions secretion. Under conditions of inhibited chloride ion transport, NAC reduces the reactivity of the tissue, thereby interfering with the absorption of sodium ions. The ENaC channel activity determines the proper operation of cilia [12, 13, 14]. The interaction of CFTR, ENaC and aquaporin channels as well as the appropriate movement of the cilia are necessary for proper/better hydration and for the transport of ASL fluid [2, 12, 14, 22, 24, 27]. Electric potential measurement under stationary conditions and during stimulation reflects local change in ion transport induced by chloride secretion through CFTR and /or sodium absorption by ENaC. The effect of NAC on the transport of sodium ions seems to be related not only to the change in hydration, but also to the movement of cilia [32]. Sodium ion transport is extremely important to maintain the airway patency, the proper cilia movements and ASL density and viscosity. In addition, the sodium ion transport is necessary to initiate the cough reflex, hence the administration of NAC influencing sodium ion transport may be important for patients suffering from respiratory diseases.

NAC may interact with the sodium channels presented on the surface of the respiratory epithelium [20]. However, this is a short-term process: after 30 min of tissue incubation in NAC there was no difference in the electrophysiological potential when compared to the baseline. The tissue seems to be saturated with NAC, which can be explained by the impossibility of further intervention in the process of sodium absorption from the surface of epithelial tissue.

However, short-term contact with the NAC solution caused an overshoot: a hyperpolarization of the tissue after administration of the mechanical-chemical stimulation with a solution of NAC and bumetanide. Then, the clinical effect of using the NAC solution for rinsing the airways seems to be caused by its short and intense contact with the epithelium [8, 22, 31, 41]. These rapid changes in the transport of sodium ions are relevant in the treatment of diseases with thick and viscous ASL [8, 12, 13, 23, 24, 25, 26, 27, 41]. Short-term contact with NAC may affect immediate hydration of the secretion, whereas oral NAC administration may strengthen the process by initiating the breakdown of the disulfide bridges [15, 40].

The administration of NAC solutions directly into the respiratory tract, in combination with oral administration, seems to be particularly important for patients with residual secretion and impaired cough reflex [21, 31, 41]. It seems that long-term oral NAC administration may additionally facilitate expectoration by liquefaction of secretions, dissolution of bipartite mucins and support of ciliary functions [32]. Furthermore, the regulation of oxidative-antioxidant status by NAC may be beneficial in the course of inflammatory diseases [8, 18, 40]. What is more, rinsing the airways with the NAC solution may also be important for dissolving and/or removing foreign bodies from the airways, including powders, and glass and metal particles [10].

Transport of sodium ions may support the cough reflex, which is an additional element supporting airway clearance in the case of sudden hydration and local thickening of the ASL [12, 13]. Changes in the transport of sodium ions are connected with neuropeptide release from nerve endings that are associated with the development of hypersensitivity and inflammatory reactions [24, 36]. In addition, the production of dense and viscous ASL induces an influx of immunocompetent cells, cytokine ejection and activation of the inflammation [1, 8, 13, 36].

The search for the molecular mechanisms that could explain the relationship between the different transepithelial

transport pathways in the reaction of hyperpolarization after mechanical stimulation has revealed the role of the CFTR protein. Undisturbed functioning of the CFTR channel affects the sodium, chloride, and potassium channels' activity, which are important for the production and maintenance of the electrical potential of epithelial cells [11, 14, 22, 23]. All molecular mechanisms of CFTR action on the biochemical processes in the cell and on the functioning of other ion channels remain unknown [14, 24]. Transepithelial potential difference can be regulated by numerous factors, including CFTR, mechanoreceptors on fibres of submucous glands, the tachykinin system, mediators of neuroepithelial and neuroendocrine cells, and the immune system [11, 35, 38].

The use of NAC, especially combined with a surfactant, effectively supports the treatment of inflammatory diseases of the respiratory tract and alleviates their effects [4, 7, 8, 15, 17, 29, 39]. In addition, due to its antioxidant properties, NAC may prevent colonization with biofilm-forming bacteria of a dense discharge [8, 9, 21].

Further research is needed to evaluate the effects of NAC on changes in the respiratory tract caused by disturbed ion secretion, cough, and inflammation. Particularly the efficient combination of NAC with other drugs should be investigated to prepare the most effective treatment plans [8, 9, 18, 21, 23, 27, 39, 41].

In summary, short-term contact of a NAC solution with the surface of respiratory epithelium, e.g. during rinsing of the airway, may support the process of treatment of diseases causing mucus gland discharge in the airways, through the influence of local changes in sodium ion transport, thereby supporting the cough reflex and expectoration. The long-term administration of NAC abolishes its effect on sodium ion transport by hydrating the ASL, changing the charge on the airway epithelium and detaching mucin particles.

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