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The Role of Afferent C-Fibers in Muscle Contraction of Trachea and Bronchi in Rats

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ABSTRACT

The present experimental study on some basal neurophysiological systems aimed to evaluate the effect of the non-adrenergic non-cholinergic system on muscle contraction of the trachea and bronchi. Moreover, the study was targeted toward the investigation of the effect of the local intramural ganglion. The obtained results indicated that C-fibers, which represent the excitatory non-adrenergic non-cholinergic system, caused smooth muscle contraction by the realization of reflex through local intramural ganglia. Furthermore, it was observed that C-fibers affected the muscle by releasing tachykinins for constricting effect. The constricting influence of fibers was greater in the case of involving local reflex through the ganglion, and less significant in the case of activating of the humeral mechanism related to tachykinins. This finding became apparent as a result of the comparison of contractile muscle responses in Krebs-Henseleit's solution with atropine, and with activating C-fiber capsaicin applications. It was also observed that in the rat trachea and bronchus the elimination of NO-ergic mechanisms led to an increase in the contraction, and the dilatation effect of nitric oxide was associated with preganglionic and postganglionic nerve structures of the intramural ganglia. In conclusion, it is identified that afferent C-fibers increase the contractions of the smooth muscle of the trachea and bronchi of the rat mainly with the involvement of ganglion neurons. The humoral mechanism of C-fibers performs a minor and additional role in muscle contraction by the release of tachykinins. The obtained data can contribute to the study of the interaction of the autonomous nervous system and non-adrenergic non-cholinergic system.

Keywords: Bronchi, Intramural ganglion, Non-adrenergic non-cholinergic system, Tachykinins

INTRODUCTION

The smooth muscle of the trachea and bronchi is controlled by sympathetic, parasympathetic and non-adrenergic non-cholinergic innervation (Stretton, 1991). Sympathetic innervation has the least effect on the development of smooth muscle contraction of the lower respiratory tract (Stretton, 1991). Mediators of sympathetic nerve endings (noradrenaline) influence on beta-2-adrenoreceptors, mediating the muscle relaxation (Sergio and Angelina, 2010). Parasympathetic innervation has the most significant influence on the development of smooth muscle contractile response. The mediator of parasympathetic nerve fibers (acetylcholine) excites muscarinic receptors - M3R - and leads to an increase in the contractile response (Carlson and Kraus, 2020). However, in certain conditions, acetylcholine can influence on muscarinic receptors M2R, which leads to inhibit the subsequent release of acetylcholine (Soukup et al., 2017). In such conditions, contractile muscle responses are reduced. The role of non-adrenergic non-cholinergic system (NANC) in the overall muscle contraction or relaxation has not been studied enough. Therefore, considerable attention in modern physiology is paid to the role of NANC system (Fedin et al., 1997). An important area of attention is C-fibers involved in the mechanism of hyperresponsiveness and pathogenesis of chronic obstructive pulmonary disease. C-fibers are afferent fibers of NANC, performing functions - generation of nervous excitation and the release of biologically active substances. This question still remains to be clarified: which mechanism of C-fibers, namely the release of tachykinins or realization of afferent transmission through local intramural ganglion, has a leading role in the realization of muscle contraction of respiratory tract (Kryukova et al., 2001). Bronchoconstriction and bronchodilation of the respiratory passages are mediated by different divisions of nervous system (Figure 1). The cholinergic and excitatory are responsible for bronchoconstriction, while the adrenergic and inhibitory (NANC) are responsible for bronchodilation. One musclular cell can receive impulses from different neurons and both excitatory and inhibitory potentials can occur there (Fedin et al., 1997). The NANC afferent fibers extend from the neurons of the jugular and nodose ganglia (Jacoby, 2003). In addition, the lungs innervated by fibers coming from the dorsal root ganglia and they are similar to fibers of the jugular ganglia (Undem and Kollarik, 2005). These are capsaicin sensitive C-fibers connected with epithelium and neurons of intramural ganglia of the respiratory passages. Tachykinins (neurokinin A) released from C-fibers cause airway constriction due to a direct effect on smooth muscle via neurokinin receptors of the second type (NK2 receptors) (Kwong et al., 2001) through increased activity of ganglionic neurons which have receptors for substance P (SP). Substance P is a neuropeptide from the family of tachykinins produced by C-fiber structures.

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Substance P influences on the neurokinin receptors of the first type (NK1 receptors) and leads to an increase in contraction of the smooth muscle. Capsaicin-sensitive C-fibers form a plexus right beneath the epithelium and cause bronchoconstriction in reaction to thermal stimuli, cold, mechanical or chemical influence. They may also react to substances released as a result of tissue damage or inflammation (Undem and Kollarik, 2005; Vanhoutte, 2013). The bronchopulmonary C-fibers of the NANC system constitute the majority of afferent fibers innervating the respiratory tract. These fibers primarily react to chemical stimuli and present low-threshold, slowly adapting receptors (Kubin, 2016). Almost all C-fibers are capsaicin-sensitive and they tend to have a double function: the release of biologically active substances and participation in the form of afferent pathway in the local reflex with the involvement of intramural ganglion (Elekes et al., 2007). C-fibers are activated by small doses of capsaicin and produce tachykinins causing constriction (through neurokinin A, SP) or relaxation (as a result of vasoactive intestinal peptide (VIP); nitric oxide (NO) effects (Mazzone and Undem, 2016). C-fibers are found around capillaries in the lung parenchyma (Undem and Kollarik, 2005). The main inhibitory mediators of the neurons of the functional module are nitric oxide and vasoactive intestinal peptide. These mediators modulate cholinergic neurotransmission at the level of the smooth muscle or through presynaptic inhibition of acetylcholine release. The density of NO-containing fibers in the smooth muscle of a human decreases from the trachea to the periphery and is completely absent in the bronchioles. In ganglia, on the contrary, the number of NO-containing neurons increases from 57% (in the trachea) to 83% (in small bronchi). Furthermore, different mediators produce bronchodilation through nitric oxide synthesized by epithelial cells (Hennel et al., 2018). Vasoactive intestinal peptide also modulates the cholinergic transmission reducing the SP-induced release of acetylcholine. In chronic bronchitis, the level of VIP is reduced and it correlates negatively with the severity of bronchial obstruction. This mediator has an inhibitory effect on smooth muscle at low concentrations in a presynaptic way and at high concentrations in a postsynaptic one. The frequent co-localization of NO and VIP in neurons was marked. Considering the fact that, C-fibers are anatomically and physiologically connected with NO-structures, the question of NO-mediated contraction of the smooth muscle of the trachea and bronchi is interesting. The role of C-fibers in NO-mediated contraction is not clarified. The question is not fully investigated - with which structures (nerve or muscle) is the NO-mediated effect predominantly associated (Zhu and Dey, 2001; Hennel et al., 2018). The purpose of this study is to determine the participation of C-fibers in excitatory and inhibitory mechanisms of the non-adrenergic non-cholinergic system in the tracheal and bronchial smooth musculature contraction in the rat. Also, the goal was to study the role of C-fibers in NO-mediated muscle contraction and in establishing the structures with which the dilatation effect of nitric oxide is mainly associated.

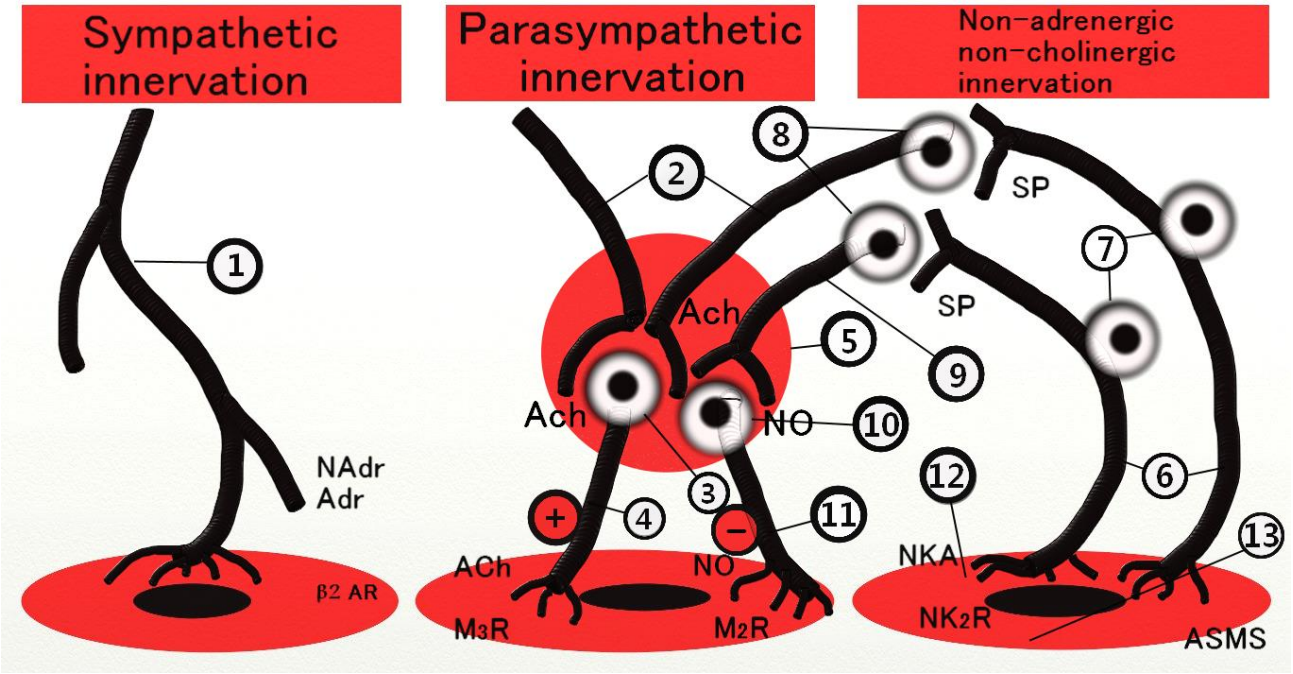

Figure 1. Scheme of innervation of smooth muscle of trachea and bronchi in mammals: sympathetic innervation, parasympathetic innervation, non-adrenergic non-cholinergic innervation (participation of C-fiber in the trachea and bronchi smooth muscle innervation). ASMS: airway smooth muscle cells. NAdr: noradrenaline. Adr: adrenaline. B2AR: beta-2-adrenoreceptor. Ach: acetylcholine. M3R, M2R: muscarinic receptors. NO: nitric oxide. NKA: neurokinin A. NK2R: neurokinin receptor. SP: substance P. « + »: nervous pathway leading to smooth muscle contraction. « ─ »: nervous pathway leading to the relaxation of smooth muscle. 1: sympathetic fiber, 2: preganglionic parasympathetic fibers, 3: cholinergic neuron, 4: postganglionic parasympathetic fiber, 5: ganglion, 6: C-fiber, 7: body of sensory neuron of the jugular ganglia, 8: body of neuron of the brain ganglia, 9: preganglionic NO-ergic fiber, 10: NO-ergic neuron, 11: postganglionic NO-ergic fiber, 12: focus of ejection of local tachykinins, 13: body of the muscle cell.

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MATRIALS AND METHODS

Animals

Twenty five wister rats of both sexes with average body weight of 230 g were used. There were no differences in the contractile responses of the muscles of males and females, because females were taken for experiments in the period of diestrus (not in estrus). Animals were kept in vivarium, which owned all the requirements for animals. Diet of animals was standard formulated rodent diets, with *ad libitum* daily access. Anesthesia procedure was carried out by placing the animal in a chamber with 4-5% chloroform. The narcotic effect causes a fairly rapid loss of consciousness. After the animal lost consciousness, decapitation was applied. This approach provided quick and painless euthanasia of the animal (Recommendations for euthanasia of experimental animals, European commission) (Close et al., 1997). The animal was fixed on the dissection table. After that, the thorax was opened and then the operation was performed with the extraction of the respiratory tract of the animal. The parenchymal tissue of the lungs was removed mechanically with a wooden spatula (Hatziefthimiou et al., 2005). The respiratory tract was washed in the Krebs-Henseleit solution, and then the trachea and bronchi preparations were made, according to Kolahian et al. (2010). The preparation was 0.4 – 0.6 cm long and 0.5 – 0.7 cm wide. The incision line of the trachea and bronchi passed through the cartilaginous half-rings. The smooth muscle remained intact. The preparations of the trachea and bronchi were placed in a chamber with Krebs-Henseleit solution, where one edge of the preparation was fixed by needles and the second edge of the preparation was mounted by hooks-holders attached to an electromechanical sensor which registered the magnitude of the contractile response (measured in mN) (Kolahian et al. 2010; Noller et al., 2019).

Ethical Approval

All of process was done in acoording to ethical regulations of Ministry of Education and Science of the Russian Federation within the framework of the applied scientific research and experimental development on "Research and development of cross-cutting technology of production of functional food products to ensure food security of the Northern territories of the Russian Federation" (Ethical certificate; project ID – RFMEFI57717X0264).

Equipment

In the experiments, a physiological complex was used, including special chambers for the trachea and bronchi, an ultratermostat, aerator, peristaltic pump (ML0146/C-V, Multi Chamber Organ Baths, Panlab, Germany), electromechanical sensors (Grass FT-03 force displacement transducer, Astro Med, West Warwick, RI, USA), an electrostimulator (direct-current stimulator, Grass S44, Quincy, MA, USA), a personal computer, special software (Chart v4.2 software, Power Lab, AD Instruments, Colorado Springs, CO, USA).

Electrophysiological experiments

The electrical field stimulation was applied in all experiments. For this purpose, two silver electrodes were put into the dishes with the preparations. During the work the electric stimulation of preganglionic nerve fibers (frequency of stimuli:8 Hz, duration : 0.5 ms, amplitude : 20 V, duration of stimulation: 10 s), postganglionic nerve fibers (frequency of stimuli : 30 Hz, duration : 0.5 ms", amplitude: 20 V, duration of stimulation: 10 s) and muscle (frequency of stimuli : 30 Hz, duration : 2 ms, amplitude : 20 V, duration of stimulation: 10 s) was applied. Electrical stimulation simulated the natural conduction of electrical impulses through the preganglionic and postganglionic link of reflex chain or muscle. The contractile response of smooth musculature of the trachea and bronchi of the rat was studied in the case of using electrical stimulation and pharmacological agents. First, electrical stimulation was given to the chambers with trachea and bronchi. Then the contractile muscle responses were recorded. These answers were taken as basal level (or 100%). After this, pharmacological substances were added and contractile muscle responses were recorded. Thus, the responses of the tracheal and bronchial muscles were recorded taking into account electrical stimulation and pharmacological preparations. The magnitude of the contractile responses to the drug usage largely depended on the original tonus of smooth muscle as well as the control contractile responses in the case of application of electrical stimulation in connection with the physiological saline. Despite the fact that all animals were the same age and the sampling was homogeneous, the variability of the original tonus and control responses (measured in mN) of such organs as the trachea and bronchi was rather high, and this fact determined the accounting of contraction in percentage (counted in percentage of the basal level of activity taken at 100 %). The methods of electrical stimulation of preganglionic, postganglionic nerves and muscles are taken from the research methods proposed by Fedin et al. (1997).

Statistical analysis

Statistical analysis was carried out with the Statistical Package for the Social Sciences (SPSS Inc., Chicago, Illinois, USA, version 17.0.2). A comparison between the groups of control and experimental results was performed with

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the use of independent sample t-test. Data were expressed as mean ± standard deviation. The value of $P<0.05$ was considered statistically significant.

Pharmacological procedure

Perfusion was performed with Krebs-Henseleit solution of the following composition: sodium chloride (118 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); potassium chloride (4.8 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); magnesium sulphate (1.18 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); potassium dihydrophosphate (1,2 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); calcium chloride (2.5 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); sodium bicarbonate (25.0 mmol/l in the form of perfusion, Sigma-Aldrich, Germany); glucose (5.5 mmol/l in the form of perfusion, Sigma-Aldrich, Germany). The necessary level of oxygen, temperature (37^0C) and pH (6.9 – 7.1) was maintained in the dishes with the preparations, (Kryukova et al., 2001). The flow of fresh Krebs-Henseleit solution was provided regularly as well as the outflow used in Fedin et al. (1997) study. During the experiments, the following substances were exogenously administered: capsaicin (3 nmol in the form of application with volume 0.2 ml, the duration of capsaicin application 1 minute, Sigma-Aldrich, Germany) for the activation of C-fibers, capsaicin (3 nmol in the form of perfusion during 30 minutes, Sigma-Aldrich, Germany) for the inactivation of C-fibers, atropine (0.2 nmol in the form of perfusion during 30 minutes, Sigma-Aldrich, Germany). Activation of C-fibers or their inactivation depended on the time of exposure to capsaicin. With a short exposure time, the fibers were excited, and with prolonged exposure, their blockade occurred. Also, in the study, we used atropine (0.2 nmol in the form of perfusion) for the interruption of neuromuscular transmission, a drug inhibitor of NO-synthase L-NAME (0.4 nmol, in the form of perfusion, Sigma-Aldrich, Germany). The study needs to eliminate the influence of the epithelium so its physiological effects were inhibited by indomethacin (3 nmol, in the form of perfusion during 30 minutes, Sigma-Aldrich, Germany) in all experiments. Indomethacin had no effect on the smooth muscle contractions of the trachea and bronchi of rats. This finding was tested previously, before proceeding with the main series of experiments (Saeideh et al., 2019). Moreover nitric oxide induces relaxation of airway smooth muscle through COX-2-derived PGI2 (COX-2/PGI2) pathway (Dorris and Peebles, 2012). Doses of drugs were selected on the basis of preliminary experiments (Vanhoutte, 2013).

RESULTS

The following results were obtained during the study to define the physiological significance of afferent C-fibers on the contractile activity of smooth-muscle system. This series of experiments involved the study of the contractile responses of smooth muscle in conditions of C-fibers activation by low doses of capsaicin (in the form of applications) and their inactivation due to long-term capsaicin perfusion which led to their long-term depletion and loss of ability of afferent transmission and the release of tachykinins. The excitation of C-fibers led to the realization of local reflex. It is the cause for an increase in the frequency of contractile responses after the application of capsaicin (Figure 2). The magnitude of the contractile responses of tracheal smooth muscle during the excitation of C-fibers increased to 130.11 % ± 3.53 ($n = 8$, significant differences in comparison with response in the Krebs-Henseleit solution, $P = 0.01$). The responses of the bronchial muscle were also increased and reached 121.12 % ± 3.01 ($n = 8$, significant differences in comparison with response in the Krebs-Henseleit solution, $P = 0.02$). With the inactivation of C-fibers, the contractile responses of the tracheal muscles were 95.31 % ± 2.11 ($n = 8$, no significant differences) and the responses of the bronchial muscle – 84.02 % ± 1.92 ($n = 8$, significant differences in comparison with response in the Krebs-Henseleit solution, $P = 0.04$) (Figure 2 and Table 1).

Due to the reason that the C-fiber of the non-adrenergic/non-cholinergic system are capable to be active without the participation of metasympathetic intramural ganglia, an important stage of the research will be experiments to define the role of activated C-fibers in isolation of the ganglion by interrupting atropine neuromuscular transmission. In such experimental conditions, smooth muscle responses are associated mainly with the tachykinins released from the fiber and their local effect on neuromuscular structure. Compared to the smooth muscle responses recorded in Krebs-Henseleit solution, the responses with blocked cholinergic receptors were strongly decreased (Table 2). The magnitude of the contractile responses of tracheal muscle decreased to 15.61 % ± 2.23 ($n = 8$) and bronchial muscle to 18.44 % ± 1.87 ($n = 8$). Further, capsaicin in the form of applications was placed in the dishes with the preparations and there was an increase of contractile responses of the trachea to 27.33 % ± 1.67 ($n = 8$, significant differences in comparison with atropine, $P = 0.04$) and bronchi to 32.29 % ± 2.46 ($n = 8$, significant differences in comparison with atropine, $P = 0.04$).

We examined the effects of NO-ergic system on the smooth muscle of the rat by applying the inhibitor of NO-synthase L-NAME. Different types of electrical stimulation of preganglionic and postganglionic nerve fibers as well as muscles were used during this series of experiments. The highest value of contraction of preparations was noted during the stimulation of preganglionic and postganglionic nerve fibers. During the stimulation of preganglionic fibers, the

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tracheal responses were 123.82 % ± 5.11 (n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.02). The contraction responses of the bronchi were 121.92% ± 4.82 (n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.02) (Table 3).

With electrical stimulation of postganglionic nerves, the contractile responses of the trachea were 113.01 % ± 5.01 (n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.04). The bronchial muscle responses were 117.02 % ± 4.71 (n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.03) (Table 4).

During electric stimulation of the muscle the tracheal responses were 87.83 % ± 4.51 (n = 9, no significant differences) and for bronchus up to 79.22 % ± 4.01 (n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.02) (Table 5).

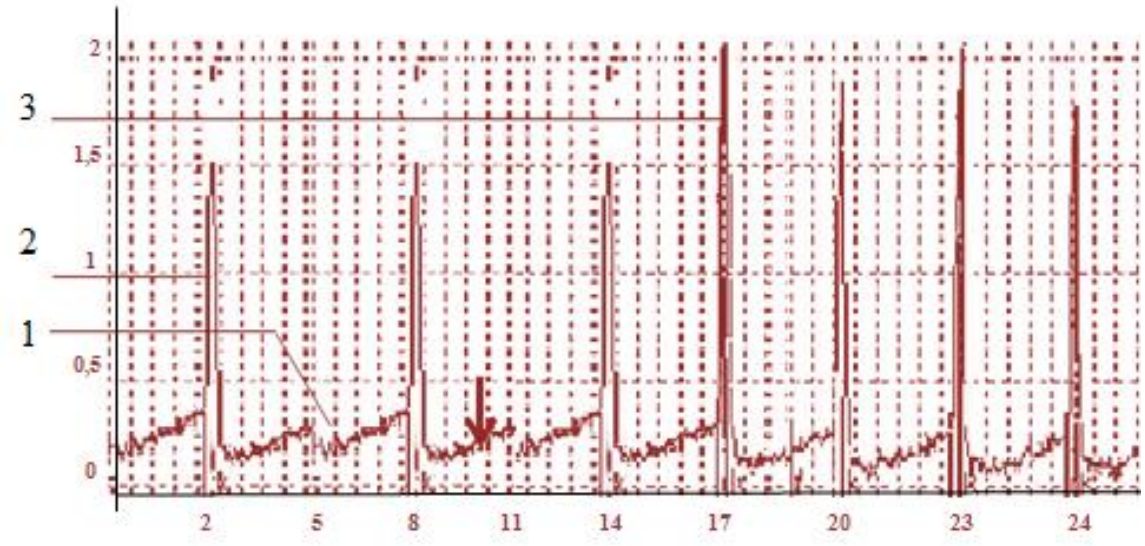

Figure 2. Sample of representative recording of the contractile response of the trachea in control (left part illustration) and after capsaicin application (right part illustration); by the abscissa axis the time is indicated (in seconds). By the ordinate axis the changes of smooth muscle responses are indicated (in mN). The brown arrow indicates the introduction of a drug into the chamber with a trachea. 1: basal level of responses (natural muscle tone); 2: control contractile responses to electrical stimulation of postganglionic nerve fibers; 3 : contractile response after capsaicin application.

Table 1. The magnitude of the tracheal and bronchial muscle contraction in millinewton and percentage.

Drug application	Trachea		Bronchi	
	Magnitude (mN)	Magnitude (%)	Magnitude (mN)	Magnitude (%)
Krebs-Henseleit solution perfusion	1.52 mN ± 0.02	100.00 % ± 1.32	1.48 mN ± 0.05	100.00 % ± 3.39
Capsaicin (activation). 3 nmol. application	1.98 mN ± 0.07	130.11 % ± 3.53	1.81 mN ± 0.05	121.12 % ± 3.01
Capsaicin (inactivation). 3 nmol. perfusion	1.45 mN ± 0.03	95.31 % ± 2.11	1.24 mN ± 0.03	84.02 % ± 1.92

Table 2. The magnitude of the contraction of tracheal and bronchial muscles in millinewton and percentage.

Drug application	Trachea		Bronchi	
	Magnitude (mN)	Magnitude (%)	Magnitude (mN)	Magnitude (%)
Krebs-Henseleit solution, perfusion	1.49 mN ± 0.03	100.00 % ± 2.01	1.46 mN ± 0.03	100.00 % ± 2.05
Atropine, 0.2 nmol, perfusion	0.23 mN ± 0.01	15.61 % ± 2.23	0.27 mN ± 0.01	18.44 % ± 1.87
Atropine, 0.2 nmol, perfusion + Capsaicin(activation), 3 nmol, application	0.41 mN ± 0.01	27.33 % ± 1.67	0.47 mN ± 0.01	32.29 % ± 2.46

Table 3. The magnitude of the contraction of the tracheal and bronchial muscles in millinewton and percentage during stimulation of preganglionic nerve fibers

Drug application	Trachea		Bronchi	
	Magnitude (mN)	Magnitude (%)	Magnitude (mN)	Magnitude (%)
Krebs-Henseleit solution, perfusion	1.54 mN ± 0.03	100.00 % ± 1.95	1.51 mN ± 0.03	100.00 % ± 1.99
L-NAME, 0,4 nmol, perfusion	1.91 mN ± 0.09	123.82 % ± 5.11	1.84 mN ± 0.08	121.92 % ± 4.82

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Table 4. The magnitude of the contraction of the tracheal and bronchial muscles in millinewton and percentage during stimulation of postganglionic nerve fibers

Drug application	Trachea		Bronchi	
	Magnitude (mN)	Magnitude (%)	Magnitude (mN)	Magnitude (%)
Krebs-Henseleit solution, perfusion	1.54 mN ± 0.03	100 % ±1.95	1.51 mN ± 0,03	100.00 % ± 1.99

By the abscissa axis used preparations are indicated. By the ordinate axis the changes of smooth muscle responses are indicated in %. *: significant differences (n = 9, P = 0.04) of the tracheal contractile response in comparison with a control response. *: significant differences (n = 9, P = 0.03) of the bronchial contractile response in comparison with a control response. After administration of the drug, the responses were recorded for 5 minutes.

Table 5. The magnitude of the contraction of the tracheal and bronchial muscles in millinewton and percentage during stimulation of muscles

Drug application	Trachea		Bronchi	
	Magnitude (mN)	Magnitude (%)	Magnitude(mN)	Magnitude (%)
Krebs-Henseleit solution, perfusion	1.54 mN ± 0.03	100.00 % ± 1.95	1.51 mN ± 0.03	100.00 % ± 1.99

By the abscissa axis used preparations are indicated. By the ordinate axis the changes of smooth muscle responses are indicated in %. *: significant differences (n = 9, P = 0.02) of the bronchial contractile response in comparison with a control response. After administration of the drug, the responses were recorded for 5 minutes.

DISCUSSION

Having analyzed the results of experiments, which had provided the contractile responses of the smooth muscles of the trachea and bronchi in the physiological solution, during activation and inactivation of C-fibers (tables 1 and 2), a constricting role of C-fibers of the non-adrenergic non-cholinergic system became apparent. During their excitation by application of capsaicin, the contractile responses of the trachea and bronchi increased. There was no significant difference between the responses of the trachea (130.11 % ± 3.53; n = 8) and bronchi (121.12 % ± 3.01; n = 8). The P value was 0.06 which is not significant. During inactivation of C-fibers affected by prolonged capsaicin perfusion, the decrease of contractile responses was occurred. This decrease was more apparent in bronchi (84.02 % ± 1.92; n = 8, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.04) than in the preparations of the trachea (95.31 % ± 2.11; n = 8, no significant differences). Perhaps in the bronchi the constrictor effect of C-fibers is more pronounced.

Reynolds and Doherty (2008) conducted similar experiments on the effect of capsaicin on the tracheal muscle of guinea pigs. In their experiments, prolonged perfusion with capsaicin reduced contractile responses of the tracheal muscle (Reynolds et al., 2008). Our experiments, in contrast to the studies of Reynolds et al. (2008), were conducted on rats and allowed us to evaluate the effect of capsaicin not only on the trachea, but also on the bronchi. The novelty of our study is that in the inactivation of C-fibers, reduction of contractile responses from the control value was more pronounced in the bronchi than in the trachea. Probably, the constrictor effect of C-fibers is more pronounced in the bronchi than in the trachea. This may be due to the higher density of C-fibers in the bronchi compared to the trachea or large release of excitatory tachykinins in the bronchi unlike the trachea. At the moment, this assumption has not yet been confirmed by other research methods and it should be clarified in more details. The present assumption is currently based on a study of the contractile effects of the muscle. To identify the role of the metasympathetic intramural ganglion in contractile activity of the smooth musculature of the trachea and bronchi mediated by C-fibers, the experiments were carried out with the interruption of atropine neuromuscular transmission (Figure 3). Once atropine, an anticholinergic drug, was placed in the dishes with the preparations, the contractile responses of the trachea and bronchi reduced to the minimal value. The responses started to increase as soon as capsaicin was put into the dishes and C-fibers were activated. The contraction responses of the trachea increased to 27.33 % ± 1.67 (n = 8, significant differences in comparison with responses in the atropine, P = 0.04) and bronchi to 32.29 % ± 2.46 (n = 8, significant differences in comparison with responses in the atropine, P = 0.04). Activation of C-fibers which leads to muscle contraction, is represented by two mechanisms in the respiratory tract of rats. The first mechanism leading to muscle contraction is a local reflex through the ganglia and the second mechanism is the release of tachykinins. In the present experiments, atropine blocked cholinergic transmission is the main pathway leading to muscle contraction through the ganglia. Blocking cholinergic transmission to the muscle leads to such experimental conditions, when the influence of ganglia is eliminated. In such experimental conditions at excitation of C-fibers can be assessed humoral mechanism of activation of muscle contraction through the release of tachykinins. Taking into account the fact that after cholinergic blockade the activation of C-fibers led to an increase in muscle responses, it can be assumed that C-fibers release primarily excitatory tachykinin, leading to increased contraction of the muscles. It is possible to assume that excitatory tachykinins can activate muscle cells

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bypassing the intramural ganglion. Information about excitatory influence of tachykinins of C-fibers (in particular about neurokinin A) on neurokinin receptor with the subsequent development of muscle construction is confirmed in the works by Kwong et al. (2001), Elekes et al. (2007), and Majkowska-Pilip et al. (2019). However, in these works and other similar studies the experiments were conducted without using any postganglionic stimulation, which increases the importance of the present results obtained under conditions closest to natural ones. The comparison of the muscle responses during activation of the C-fibers under the influence of the intramural ganglion (figure 2) and elimination of the effects by atropine (figure 3) allows monitoring the fact that activated C-fibers in the presence of the ganglion cause stronger contractile responses. This result may indicate that the mechanism of muscle contraction mediated by activation of C-fibers occurs mainly with the involvement of intramural ganglion and to a lesser extent with the involvement of excitatory tachykinins. Establishing the contribution of intramural ganglion and C-fibers to the local reflex of smooth muscle contraction is the novelty of this study.Nitric oxide is one of the main inhibiting mediators of the non-adrenergic non-cholinergic system (Aleksandrov et al., 2015). Maarsingh et al. (2005) provides report on the role of nitric oxide in regulating the activity of tracheal smooth muscle in a guinea pig, however most studies have been conducted without the use of electrical stimulation of nerve fibers. Its synthesis in the non-adrenergic non-cholinergic system of the respiratory tract is carried out by specific intramural ganglion neurons. The inhibition of the NANC-induced generation of nitric oxide leads to a decrease in relaxation of smooth muscle in the mouse trachea (Elekes et al., 2007). The highest value of contractions of the preparations were noted during the stimulation of preganglionic (123.82 % ± 5.11 for tracheal responses and 121.92 % ± 4.82 for bronchial responses; n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.02 for the trachea and bronchi, figure 4) and postganglionic nerve fibers (113.01 % ± 5.01 for tracheal responses and 117.02 % ± 4.71 for bronchial responses; n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.04 and P = 0.03 respectively, for the trachea and bronchi, Figure 5), which indicates a significant role of NO-synthesizing neurons of the intramural ganglia in the mediation of the relaxation effect on smooth muscle. Probably the relaxation effect of nitric oxide is associated with preganglionic and postganglionic nerve structures of the intramural ganglia. As a result of experiments with L-NAME, it was found that in the lower respiratory tract of the rat the dilating role of the NO-ergic system is related to nervous structures and is not associated with muscle structures. It was found out that in the rat trachea and bronchus the elimination of NO-ergic mechanisms leads to an increase in the contraction. NO-ergic mechanisms are associated with preganglionic and postganglionic nerve fibers. Information on the association of NO-mediated contraction with neural, but not muscular structures represents the novelty of our study. However, at the moment, this assumption has not yet been confirmed by other research methods and it has yet to be clarified in more details. Also, this study contains some new information on the role of C-fibers in the contraction of the muscle of the trachea and bronchi, taking into account the physiological effect of the ganglion. Vanhoutte (2013) confirms in his research that nitric oxide can be produced not only from the epithelial cells but also from the intermediate cells in airway smooth muscle, and nitric oxide production from the intermediate cells is stimulated by acetylcholine. Based on the data of Vanhoutte (2013) and the present investigation, we can make an assumption about some reciprocal effect on the smooth muscle from the neuro-humoral system. On the one hand, acetylcholine is produced for neuromuscular transmission, and on the other hand, in response to acetylcholine production, nitric oxide is released as a compensatory mechanism. Electrical stimulation of preganglionic fibers always excites C-fibers (Noller et al., 2019). In present studies, when preganglionic fibers were stimulated, contractile responses of the tracheal muscles against the background of L-NAME were higher than those of the trachea against the background of the same preparation, but under conditions of stimulation of postganglionic nerves (123.82 % ± 5.11 - tracheal responses in terms of electrical stimulation of the pregaglionic nerves and 113.01 % ± 5.01 - tracheal responses in terms of electrical stimulation of the postgaglionic nerves; n = 9, significant differences, P = 0.04; Fig. 4 and Fig. 5). Taking this fact into account, it can be concluded again about the exciting role of C-fibers and assume their contribution to the NO-mediated contractile reactions. It can be assumed that excitation of C-fibers and NO-mediated relaxation are two opposite mechanisms that, under the conditions of the physiological norm, form a certain balance between constriction and dilatation effects on the tracheal smooth muscle. Probably in this balance there is a definite relationship between the effects of C-fibers and NO-influences on the muscle.Under conditions of electrical muscle stimulation inhibition of NO-synthase resulted in reduction of bronchial contractile responses (79.22 % ± 4.01 bronchial responses; n = 9, significant differences in comparison with responses in the Krebs-Henseleit solution, P = 0.02, figure 6). This may indicate a constrictor effect of nitric oxide in the conditions of electrical stimulation of bronchial muscles. However, these results have not yet been confirmed by other studies and require further study. Different responses of the bronchial muscles to the blockade of nitric oxide under various types of stimulation can be explained by the fact that the dilative function of nitric oxide is associated mainly with neural structures and constrictor-effect associated with inhibition of NO-synthase, is related to muscle structure of the bronchi of the rat.

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CONCLUSION

As a result of the conducted studies it was found that the leading role in the contraction of the muscle of the trachea and bronchi is associated with the ganglion. Afferent C-fibers play a smaller role in contraction compared to ganglia. So, it is necessary to conclude what is the novelty of this study. The constricting influence of C-fibers is far greater in the case of involving local reflex through the ganglion, and far less in the case of activating of the humoral mechanism related to tachykinins. It was also established that relaxation effect of nitric oxide is associated with preganglionic and postganglionic nerve structures of the intramural ganglia, but not with the smooth muscle structures of the trachea and bronchi of the rat. We found that in the bronchi of the rat, compared with the trachea, there is a more pronounced excitatory (constrictor) effect of C-fibers.

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Authors' contributions

Authors have similar role in all process of study and writing of manuscript. The manuscript was approved by all authors.

Competing interests

The authors declare that they have no competing interests.

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