



Study of the expression of receptors to IL4, IL4R genes in trachea and the role of IL4 in contraction of tracheal muscle among rats

Sleep apnea; beyond cardiovascular disease

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Abstract

This article examines the effect of IL-4 on the contraction of the muscles of the trachea and bronchi of rats, demonstrates the results of studies of the levels of expression of the IL4R gene and the levels of expression of molecular IL4R in the trachea of control and sensitized rats. The choice of this interleukin and its receptors is due to the great importance of these structures in the sensitization and pathogenesis of allergic bronchial asthma.

The trachea of 20 Wistar rats were studied using real-time PCR, immunohistochemical method, and mechanography using electrical stimulation of nerve fibers.

As a result of the studies, it was found that under sensitization conditions in the rat trachea tissues there is a pronounced expression of IL4R genes, a significant expression of the molecular receptor for interleukin-4, and an increase in the constrictor effect on smooth muscle due to the influence of the cytokine interleukin-4 on it. In tracheal regions containing intramural ganglia (bifurcation region), the level of expression of IL4R genes, the level of expression of the molecular receptor IL4R, and the magnitude of muscle contractile responses in response to interleukin-4 administration were significantly higher than in trachea samples without ganglia.

Keywords: proinflammatory cytokine, sensitization, intramural ganglion, smooth muscle.

Resumen

Este artículo examina el efecto de IL-4 sobre la contracción de los músculos de la tráquea y bronquios de ratas, demuestra los resultados de estudios de los niveles de expresión del gen IL4R y los niveles de expresión de IL4R molecular en la tráquea de control y ratas sensibilizadas. La elección de esta interleucina y sus receptores se debe a la gran importancia de estas estructuras en la sensibilización y patogénesis del asma bronquial alérgica. Se estudió la tráquea de 20 ratas Wistar mediante PCR en tiempo real, método inmunohistoquímico y mecanografía mediante estimulación eléctrica de fibras nerviosas. Como resultado de los estudios, se encontró que en condiciones de sensibilización en los tejidos de la tráquea de la rata, existe una expresión pronunciada de genes IL4R, una expresión significativa del receptor molecular para interleucina-4 y un aumento en el efecto constrictor sobre lisos. músculo debido a la influencia de la citocina interleucina-4 sobre él. En las regiones traqueales que contienen ganglios intramurales (región de bifurcación), el nivel de expresión de los genes IL4R, el nivel de expresión del receptor molecular IL4R y la magnitud de las respuestas contráctiles del músculo en respuesta a la administración de interleucina-4 fueron significativamente más altos que en las muestras de tráquea sin ganglios.

Palabras clave: citoquina proinflamatoria, sensibilización, ganglio intramural, músculo liso.

This article examines the effect of IL4 on the contraction of the muscles of the trachea and bronchi of rats, demonstrates the results of studies of the levels of expression of the IL4R gene and the levels of expression of molecular IL4R in the trachea of control and sensitized rats. The choice of this interleukin and its receptors is due to the great importance of these structures in the sensitization and pathogenesis of allergic bronchial asthma.

CheXiao-wen and ZhangYing point to a significant role of the proinflammatory cytokine, IL4, in the pathogenesis of asthma in the early stages. Scientists report that a beneficial effect in the treatment of bronchial asthma can be achieved by suppressing the synthesis of this interleukin. Also, IL4 increases airway hyperresponsiveness in patients with asthma¹.

Liu XJ, Xin ZL. in their studies have shown that the level of IL4 in the blood and the bronchoalveolar fluid in patients with asthma significantly exceeds normal values and correlates with the level of IgE².

IL4 stimulates the production of IgE, which binds to the Fcε receptors of mast cells, leads to their activation with the release of pro-inflammatory mediators (histamine, leukotrienes, prostaglandins, and cytokines, which are known triggers of asthma exacerbation), which contribute to the development of pronounced contraction of smooth muscles of the trachea and bronchi³.

According to A.G. Chuchalin, IL4 is a therapeutic target for monoclonal antibodies in the treatment of bronchial asthma. Biological inhibition of IL4 by monoclonal antibodies leads to a decrease in the level of IgE in the blood serum and a decrease in the contractile activity of the tracheobronchial muscles⁴. In sensitized rats, exposure to ovalbumin caused a significant increase in the expression of IL-4^{5,6,7}.

IL4 signaling is via a specific receptor. The IL-4 receptor is a heterodimeric complex, in which the IL4Pα chain is necessarily present, which has a high affinity for IL4⁸.

The receptor for human IL-4 is a transmembrane protein and exists in membrane-bound and soluble forms. Signal transduction occurs via STAT-6, which are critical molecules for IL4 signal transduction⁹.

In the absence of a cytokine, both subunits of the receptor are dissociated in the cell membrane, and their association occurs after IL4 binding^{10,8}. After assembly of the complex, signal transduction into the cell is carried out by activation of STAT6 (Signaltransducerandactivatoroftranscription)¹⁰.

The effect of IL4 on gene expression is mediated through the transcription factor STAT6, which is phosphorylated at

the Tyr641 residue and dimerized in the cytoplasm, after which it is translocated into the nucleus¹¹. In the nucleus, the STAT6 dimer binds to its specific site TTC-GAA, separated by 4 nucleotides¹².

IL4 initiates signal transduction into the cell through the membrane receptor complex, which can be of two types. The receptor for IL4 (IL4R) type I consists of an α-chain (IL4Rα) and a γ-chain. This type of receptor is located on the surface of immune cells and binds to IL4 with high specificity. IL4R type II consists of two subunits, IL4Rα and IL13Rα1, which are expressed by hematopoietic cells and can bind both IL4 and IL13. IL4 acts by triggering the Januskinase/SignalTransducerandActivatorofTranscription (JAK/STAT) signaling pathway, the main pathway for intracellular signal transmission from the cytokine receptor^{13,14}. Type I and II receptors are activated by STAT6¹⁰.

Receptors for IL4 play one of the key roles in the pathogenesis of asthma. Blockers of prescriptions for IL4 lead to a decrease in the exacerbation of bronchial asthma in patients and an improvement in the functions of external respiration¹⁵.

In asthma, signaling pathways associated with IL4Ra mediate airway inflammation, remodeling, and hyperreactivity, mucus hypersecretion, increased IgE, and subepithelial fibrosis in the airways. Treatment of mice with neutralizing antibodies to IL4Ra or soluble IL4Ra inhibited the development of the asthma phenotype¹⁶.

A relationship has been established between atopic asthma and polymorphism of genes for IL4 receptors, which transmit signals of these ligands to target cells. To clarify the role of the Gln551Arg and Ile50Val polymorphisms in the development of atopy, experiments were carried out to create cell lines containing four possible types of the IL4RA gene encoding Gln or Arg receptor variants at position 551 and Ile or Val at the position. Experiments with them showed that the sensitivity to IL-4 is significantly increased in cells with the Ile50 type of receptor and does not depend on the type of amino acid in position 551. Thus, convincing evidence of the functional significance of the Ile50Val polymorphism concerning AD was obtained and the role of the Gln551Arg missense mutation was rejected as a genetic factor of susceptibility to disease¹⁷.

Thus, IL4 and IL4R can be considered factors that play a significant role in the sensitization and pathogenesis of bronchial asthma. Taking into account the fact that most studies devoted to the cytokine IL4 make it possible to assess its role in the general inflammatory response in bronchial asthma, studies of the direct effect of IL4 on tracheal smooth muscle contraction under normal conditions and under sensitization conditions may be especially relevant. It is also interesting to carry out a comparative study that reveals the level of expression of the gene for IL4R and the level of expression of the molecular receptor IL4R in health and disease. Comparison of these processes in preparations of the trachea with and without ganglion is of particular importance. Such studies are being conducted for the first time and may be of great importance in eluci-

dating the mechanisms of asthma pathogenesis since the presence of the intramural metasympathetic ganglion can affect the level of receptor expression. Electrical stimulation of postganglionic nerves is also of great importance in experiments on isolated preparations, which brings the studied system as close as possible to natural physiological conditions. Therefore, this study aimed to study the level of expression of the IL4 gene (encoding the α -subunit), the level of expression of molecular IL4, and the effect of the cytokine IL4 on the contraction of the smooth muscle of the rat trachea using electrical stimulation of postganglionic nerves under conditions of the physiological norm and an experimental model of sensitization.

Equipment

In the experiments, a physiological complex was used that maintained the normal course of physiological processes in isolated preparations. The complex included special chambers for placing trachea and bronchial preparations in them, an ultrathermostat, an aerator, a peristaltic pump (ML0146/CV, MultiChamberOrganBaths, Panlab, Germany), electromechanical sensors (GrassFT-03 force-displacement transducer, AstroMed, WestWarwick, RI, USA), electro stimulator (direct-current stimulator, GrassS44, Quincy, MA, USA), personal computer, special software (Chartv4.2 software, PowerLab, ADInstruments, ColoradoSprings, CO, USA).

Animals manipulations

We studied 20 Wistar rats of both sexes with a body-weight of 190-270 g. Females were taken for experiments during the period of diestrus. The animals were kept in a vivarium, which met all the requirements for the conditions of keeping animals. To obtain samples of the respiratory tract, decapitation was performed with preliminary anesthesia. This approach ensured the rapid euthanasia of the animal (recommendations for the euthanasia of experimental animals, European Commission)¹⁸. Then the animal was fixed on the dissection table. After that, the chest was opened and then an operation was performed with the extraction of the animal's airways¹⁹. The airways were washed in Krebs-Henseleit solution, and then trachea preparations were prepared. Each specimen was a trachea sample 0.4-0.6 cm long and 0.5-0.7 cm wide. Samples of the trachea were taken from the bifurcation region (trachea preparations with ganglia) and in the area of straight sections (trachea preparations without ganglia). The tracheal incision line passed through the cartilaginous half rings. The smooth muscle remained intact. Tracheal preparations were placed in a chamber with Krebs-Henseleit solution, where one edge of the preparation was fixed with needles, and the other edge of the preparation was installed with hooks-holders attached to an electromechanical sensor recording the magnitude of the contractile response (measured in mn).

Experiment scheme

Two groups of animals were formed: a control group (received saline) and an experimental group with induced sensitization. Further, the assessment of tracheal muscle contraction, the level of expression of the IL4R gene, and the level of expression of receptors for IL4 in the tracheal preparations of the control and experimental groups were carried out.

This study was carried out under the principles of the Basel Declaration and the recommendations of the European Commission on the euthanasia of experimental animals.

Animal sensitization procedure

The rats were sensitized with the introduction of ovalbumin (Sigma-Aldrich, Germany). For a one-time sensitization procedure, 0.5 mg of ovalbumin was dissolved in 1 ml of saline. Then the ovalbumin solution was injected subcutaneously in 0.1 ml doses into the neck, back, both feet, groin, and 0.5 ml of ovalbumin solution was additionally injected intraperitoneally (the total volume of ovalbumin solution administered to one animal was 1 ml). Sensitization procedure with ovalbumin injections on the 1st, 14th, and 21st days. In parallel with the injections of the ovalbumin solution, inhalation with ovalbumin was carried out using a nebulizer on the 14th, 16th, 18th, 21st, and 24th days. For this, the rats were placed in an exposure chamber connected to a nebulizer (Omron, NEC29-E, Russia?). Ovalbumin in an amount of 1 g was dissolved in 100 ml of saline. The ovalbumin solution was sprayed for 30 minutes with a yield of 3 ml/mn and an average particle size of 3.2 μ m. The last inhalation with ovalbumin solution was carried out 72 hours before the euthanasia of the animals.

The nonsensitized group was injected with physiological saline intraperitoneally as a control²⁰.

Electrophysiological and mechanographic experiments

In all experiments, stimulation with an electric field was used. For this, two silver electrodes were placed in the chambers with the preparations. During the work, electrical stimulation of postganglionic nerve fibers was used (stimulus frequency - 30 Hz, duration - 0.5 ms, amplitude - 20 V, duration of stimulation - 10 C).

Electrical stimulation simulated the natural conduction of electrical impulses through the postganglionic link of the reflex circuit. In the experiments, the contractile reaction of the smooth muscles of the trachea of the rat was studied using electrical stimulation and pharmacological agents. First, electrostimulation of tracheal preparations was performed. Then the contractile reactions of the muscles were recorded. These answers were taken as the baseline (or 100%). After that, a solution of interleukin-4 was added and the contractile reactions of the muscles were recorded. Thus, the reactions of the tracheal muscles were recorded, taking into account electrical stimulation and the influence of interleukin-4. The magnitude of contractile reactions to the use of the drug largely depended

on the initial tone of smooth muscles, as well as on the control contractile reactions due to the use of electrical stimulation against the background of physiological solution. Even though all animals were of the same age, and the sample was homogeneous, the variability of the initial tone and control responses (measured in mn) of organs such as the trachea was quite high, and this fact determined the accounting for the reduction in percentage (calculated as a percentage of baseline activity level taken at 100%). Methods of electrical stimulation of postganglionic nerves are taken from research methods proposed by A. N. Fedin²¹.

Statistical analysis

Statistical analysis was performed using the SPSS statistical package, version 10.0 (SPSS Inc., Chicago, Illinois, USA). Comparison between groups of control and experimental results were performed using independent Student's t-tests (Student's t-test). A P value <0.05 was considered statistically significant. Data were expressed as mean, standard deviation.

Pharmacological procedure

Perfusion was performed with Krebs-Henseleit solution. In the chambers with the preparations, the required oxygen level, temperature (37°C), and pH (6.9 - 7.1) were maintained. The inflow of fresh Krebs-Henseleit solution was provided regularly, as was the outflow of the used one²¹.

In the course of the experiments, interleukin-4 (Sigma-Aldrich, USA) was injected exogenously into the chambers with preparations at a concentration of 150 ng/ml using perfusion for 120 min, after which the contractile activity was recorded.

Molecular genetic methods

To determine the level of gene transcripts, tissue samples of the trachea with the ganglion and the trachea without the ganglion were used.

Isolation of total RNA was performed using PureZOL™ RNA Isolation Reagent (Bio-Rad, USA). After adding 1/5 volume of chloroform, the tube was shaken and placed on ice for 5 min. Then the suspension was centrifuged at 14,000 g for 15 min at 4°C in an EPPENDORF 5417R centrifuge. The aqueous phase was transferred to a clean test tube, isopropanol was added, mixed, and left for 15 min at 4°C. Then the samples were centrifuged at 14,000 g for 15 min at 4°C. After centrifugation, the supernatant was removed, and the tRNA pellet was washed with 80% ethanol and centrifuged again at 12,000 g for 8 min at 4°C. After ethanol was removed, the total RNA pellet was dried for several minutes at room temperature, and 30 µL of RNase-free water was added. The amount and quality of total RNA were determined spectrophotometrically using a SmartSpecPlus device (Bio-Rad, USA). The purity criterion was the A_{260}/A_{280} ratio, which reflects the presence of protein impurities in the sample. Samples were considered clean if $A_{260}/A_{280} = 1.9$. To synthesize the first strand of complementary DNA (cDNA) on an RNA template pretreated with DNase (SibEnzyme, Russia), we

used the MMLVRT kit (Evrogen, Russia). The synthesized cDNA was stored at -200°C. The level of gene expression was assessed by real-time PCR on a LightCycler®96 device (Roche, Switzerland). 18S rRNA and GAPDH (Glyceraldehyde 3-phosphate dehydrogenase) were used as reference genes. For amplification, qPCRmix HS SYBR kits (Evrogen, Russia) were used. PCR protocol: cDNA denaturation for 5 min at 95°C; 35 cycles: denaturation at 95°C for 15 s; annealing at 60°C for 15 s; elongation at 72 °C 15 s. The specificity of the amplification products was checked by melting the PCR fragments. The PCR efficiency (98%) was assessed using a standard curve. The level of gene transcripts was calculated by the formula: level of transcripts = $2^{-\Delta Ct}$ (reference) - Ct (test sample), where Ct is the value of threshold cycles. PCR was repeated at least 3 times.

When designing primers for PCR, the computer program BeaconDesigner was used. Information on the structure of the studied genes was obtained from the international database of the National Center for Biotechnological Information (NCBI BLAST, GeneBank, USA). To exclude the possibility of obtaining a PCR product on a chromosomal DNA template, primers were designed according to the nucleotide sequence of different exons. Nucleotide sequences and size of PCR fragments for the IL4R gene were as follows: 5' AGCTGCATTCCAGTCTCTCC 3' (exon 2) and 5' CTCACACGTAGAAGTGCGGA 3' (exon 4), 185 ns item respectively²².

Immunohistochemical methods

For immunocytochemical studies with a fluorescent label, paraffin sections of the trachea prepared according to the standard technique were used. After 24 hours of fixation in buffered formalin, the tissue pieces were washed for several hours in running water. The process of tissue dehydration was carried out by carrying out an ascending concentration in alcohols. At the last stage, the tissue samples were kept in a mixture of absolute alcohol - xylene in a ratio of 1: 1 for an hour, then in xylene (30 min) and a mixture of xylene-paraffin (1: 1, 1 hour). Before embedding in paraffin, tissue samples were kept in two changes of paraffin (56°C, 1 hour). Sections with a thickness of 5-6 microns were made on a rotary microtome (Russia). To fix the sections, pretreated with Nikiforov's mixture, the slides were covered with a solution of gelatin with chrome alum. Dewaxing, rehydration, and antigen unmasking were performed using Trilogy™ solution (Cell-Marque, Merck, USA) according to the manufacturer's protocol. Then the sections were washed in distilled water and then in phosphate-saline solution (PBS) and permeabilized in 0.25% Triton X-100 solution for 30 minutes. To reduce the nonspecific binding of antibodies, the sections were incubated in a blocking solution of 2% bovine serum albumin (Sigma, USA). Solutions of Triton X-100 and BSA were prepared in 0.1 M PBS with the addition of 0.1% Tween-20. In our study, we used antibodies "IL-4R mouse monoclonal antibody" (ThermoFisherScientific, USA, dilution concentration 20 µg/mL. The working dilution of antibodies was determined by titration, taking into account the data specified in the passport. Incubation

with primary antibodies diluted in a blocking solution was carried out for 18 h at 4°C, after which it was washed with PBS 4 times for 5 minutes, and secondary antibodies were conjugated with the AlexaFluor 488 or 546 fluorochrome (ThermoFisher Scientific, USA) were applied. Hoechst 33342 (Invitrogen, ThermoFisher Scientific, USA) was used for staining cell nuclei. After incubation with the second antibodies, the sections were washed with PBS and embedded under coverslips in glycerol. The specificity of the antibodies was confirmed in control experiments.

To analyze the results obtained, we used an Olympus fluorescence microscope (Japan) equipped with a set of light filters and a camera. The results were processed using the ImageJ program.

Immunostaining

For immunohistochemical staining, the sections were processed by the method of indirect immunoperoxidase using heterologous antiserum. After inhibition of endogenous peroxidase activity using 1% H2O2 in PBS for 30 minutes, the sections were incubated in the presence of primary antibodies overnight at 41°C in a humid chamber. After incubation with primary antibodies, sections were treated with goat anti-mouse IgG (dilution, 1: 100; Chemicon, Temecula CA, USA) or goat-anti-rabbit IgG-peroxidase conjugate (dilution, 1: 100; Sigma, St. Louis M.O., USA).

Results

Results of immunohistochemical studies

In control preparations of the trachea with ganglion, 396.61 ± 102.86 cells expressed the molecular receptor for interleukin-4 (this indicator was 32.08 ± 8.32 IL4R-containing cells per 100 cells in a section). In experimental preparations of the trachea with ganglion, 909.05 ± 95.73 cells expressed the molecular receptor for interleukin-4 (66.94 ± 7.05 IL4R-containing cells per 100 cells per section) (Fig. 1, Fig. 2, Table 1). Differences in the levels of expression of molecular IL4R between cells in control and experimental preparations are significant (P = 0.03).

In control preparations of the trachea without ganglion, 419.71±97.36 cells expressed the molecular receptor for interleukin-4 (this figure was 31.64±7.34 IL4R-containing cells per 100 cells in a section). In experimental preparations of trachea without ganglion, 688.39±111.12 cells expressed the molecular receptor for interleukin-4 (50.06 ± 8.08 IL4R-containing cells per 100 cells per section) (Fig. 1, Fig. 2, Table 2). Differences in the levels of expression of molecular IL4R between cells in control and experimental preparations are significant (P = 0.04).

Fig. 1. Photos were taken with a light microscope. Cells containing the interleukin-4 receptor are marked with a red glow.

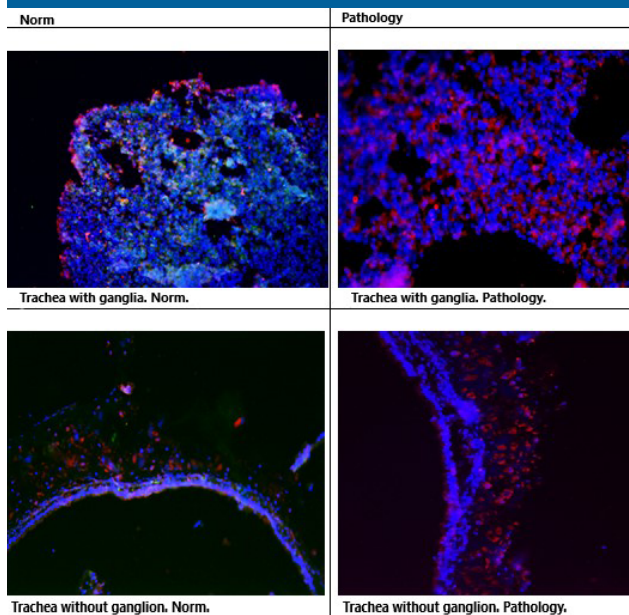
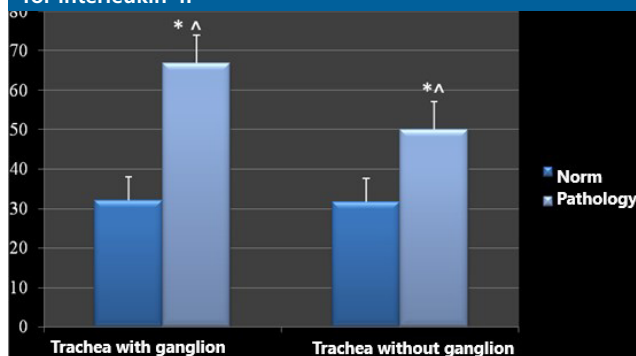


Fig. 2. Quantitative analysis of cells containing the receptor for interleukin-4.



* - Significant difference in the number of cells containing the receptor for interleukin-4 between control and experimental drugs.

^ - Significant difference in the number of cells containing a receptor for interleukin-4 between experimental preparations of mitrachea with ganglia and trachea without ganglia.

Tab. 1. The total number of cells stained in the section and quantitative analysis of cells containing the receptor for interleukin-4 (preparation of the trachea with ganglion).

Biological product	The total number of cells stained in the section	Total number of cells in a section with an interleukin-4 receptor	The number of cells in a section with an interleukin-4 receptor per 100 cells
Trachea with ganglion, norm	1236,33 ± 321,53	396,61 ± 102,86	32,08 ± 8,32
Trachea with ganglion, pathology	1358,05 ± 278,22	909,05 ± 95,73	66,94 ± 7,05

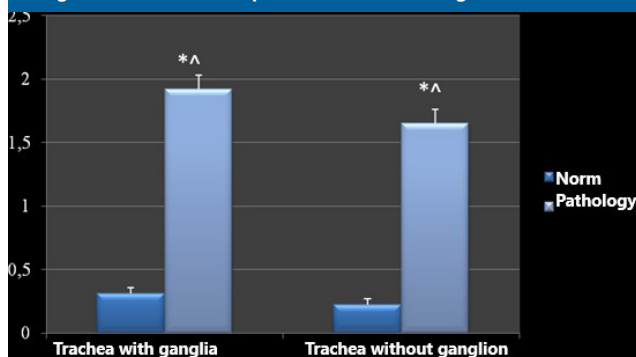
Tab. 2. The total number of cells stained in the section and quantitative analysis of cells containing the receptor for interleukin-4 (trachea preparation without ganglion).

Biological product	The total number of cells stained in the section	Total number of cells in a section with an interleukin-4 receptor	The number of cells in a section with an interleukin-4 receptor per 100 cells
Trachea without ganglion, norm	1326,51 ± 302,97	419,71 ± 97,36	31,64 ± 7,34
Trachea without ganglion, pathology	1375,13 ± 335,71	688,39 ± 111,12	50,06 ± 8,08

Results of molecular genetic studies

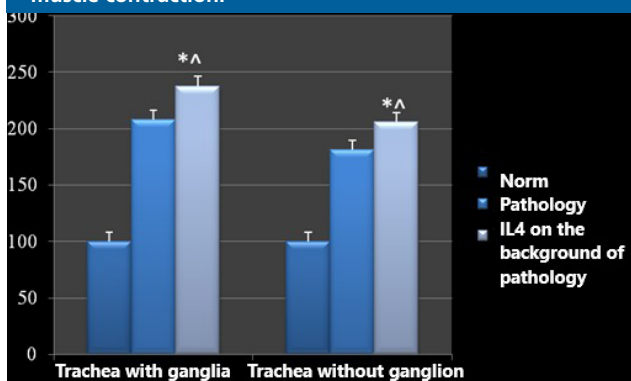
The expression level of the IL4R gene in the control preparations of the trachea with the ganglion was 0.31 ± 0.05 rel. Units. (taken as $100 \pm 16.12\%$). In experimental preparations of the trachea with ganglion, the level of IL4R gene expression increased to 1.92 ± 0.11 ($619.35 \pm 35.48\%$ of the norm) (Fig. 3, Table 3). Differences in the levels of IL4R gene expression between control and experimental drugs are significant ($P = 0.001$).

The expression level of the IL4R gene in control preparations of the trachea without ganglion was 0.22 ± 0.04 rel. Units. (taken as $100 \pm 17.12\%$). In experimental preparations of the trachea with ganglion, the level of IL4R gene expression increased to 1.65 ± 0.09 ($750.01 \pm 40.91\%$ of the norm) (Fig. 3, Table 4). Differences in the levels of IL4R gene expression between control and experimental drugs are significant ($P = 0.001$).

Fig. 3. The level of expression of the IL4R gene.

* - Significant difference in the level of IL4R gene expression between control and experimental drugs.

^ - Significant difference in the level of IL4R gene expression between experimental preparations of trachea with ganglia and trachea without ganglia.

Fig. 4. Influence of interleukin-4 on tracheal muscle contraction.

* - Significant difference in muscle contractions in response to IL4 administration between control and experimental drugs.

^ - Significant difference in muscle contractions in response to IL4 administration between experimental preparations of trachea with ganglia and trachea without ganglia.

Tab. 3. The content of mRNA in norm and pathology (preparation of the trachea with ganglion).

Biological product	mRNA content, rel.	mRNA content, % of the normal value
Trachea with ganglion, norm	0,31 ± 0,05	100 ± 16,12
Trachea with ganglion, pathology	1,92 ± 0,11	619,35 ± 35,48

Tab. 4. Content of mRNA in norm and pathology (preparation of trachea without ganglion).

Biological product	mRNA content, rel.	mRNA content, % of the normal value
Trachea without ganglion, norm	0,22 ± 0,04	100 ± 17,12
Trachea without ganglion, pathology	1,65 ± 0,09	750,01 ± 40,91

Results of mechanographic studies

Contractions of the smooth muscle of the trachea with the ganglion under the conditions of the physiological norm were 1.49 ± 0.07 mN, which was taken as $100.00 \pm 8.17\%$. The responses of the smooth muscle of the trachea with a ganglion in the experimental model increased to 3.06 ± 0.06 mN ($208.22 \pm 7.35\%$). Smooth muscle contractions in sensitized rats upon additional admission to chambers with isolated interleukin-4 preparations increased to 3.53 ± 0.07 mN ($237.12 \pm 9.05\%$) (Fig. 4, Table 5). responses of tracheal muscles in norm, pathology, and against the background of application of interleukin-4 are reliable ($P = 0.04$).

Contractions of the smooth muscle of the trachea without ganglion under physiological conditions were 1.32 ± 0.07 mN, which was taken as $100.00 \pm 7.92\%$. Tracheal smooth muscle responses without ganglion in the experimental model increased to 2.39 ± 0.07 ($181.53 \pm 7.89\%$). Smooth muscle contractions in sensitized rats upon additional admission to the chambers with isolated interleukin-4 preparations increased to 2.71 ± 0.08 mN ($205.46 \pm 9.55\%$) (Fig. 4, Table 5). The differences between the contractile responses of the tracheal muscle in normal, pathological conditions and against the background of interleukin-4 application are significant ($P = 0.04$).

Tab. 5. Contractions of the smooth muscle of the trachea in normal and pathological conditions (preparation of the trachea with ganglion).

Biological product	Muscle contraction in mN	Muscle contraction in %
Trachea with ganglion, norm	1,49 ± 0,07	100,00 ± 8,17
Trachea with ganglion, pathology	3,06 ± 0,06	208,22 ± 7,35
Trachea with ganglion, pathology + IL4	3,53 ± 0,07	237,12 ± 9,05

Tab. 6. Contractions of the smooth muscle of the trachea in normal and pathological conditions (preparation of the trachea without ganglion).

Biological product	Muscle contraction in mN	Muscle contraction in%
Trachea without ganglion, norm	1,32 ± 0,07	100,00 ± 7,92
Trachea without ganglion, pathology	2,39 ± 0,07	181,53 ± 7,89
Trachea without ganglion, pathology + IL4	2,71 ± 0,08	205,46 ± 9,55

Discussion

The total number of cells expressing the molecular receptor IL4R significantly increased in the tracheal tissues under pathological conditions as compared with the tracheal tissues of healthy animals. In preparations of the trachea with ganglion, the number of such cells increased from 32.08±8.32 in control animals to 66.94±7.05 in animals with sensitization. In trachea preparations without ganglion, the number of cells containing the receptor for interleukin-4 was also higher in the trachea samples from animals with sensitization (50.06±8.08 cells) than in control animals (31.64±7.34 cells) Thus, under sensitization conditions in rats, there is an increase in the expression of the receptor for interleukin-4, and the number of cells containing this receptor significantly increases.

If we compare preparations of the trachea with ganglion and preparations without ganglion, then in rats with sensitization there is a significant difference in the number of cells expressing IL4R. In samples of the trachea with ganglion, the number of such cells was higher than in samples without ganglion (66.94±7.05 cells in a section of the trachea with a ganglion and 50.06±8.08 cells in a section of a trachea without a ganglion). In control animals, there was no difference in the number of IL4R-containing cells. It can be assumed that neurons of the intramural ganglion enhance the expression of the molecular receptor under pathological conditions.

The level of expression of the IL4R gene was also significantly higher in tracheal preparations of animals with sensitization. In trachea samples with ganglia, the amount of mRNA was 0.31±0.05 rel. Units. in control animals and 1.92±0.11 relative units. in experimental animals. In trachea preparations without ganglia, the amount of mRNA was 0.22±0.04 relative units. in control animals and 1.65±0.09 relative units. in experimental animals. It can be concluded that under pathological conditions in the tracheal tissues there is a strong increase in the ex-

pression of genes encoding molecular IL4R. It should also be noted that the level of IL4R gene expression in trachea specimens with ganglia is higher than in trachea specimens without ganglion. Perhaps this is because drugs with ganglia increase neurogenic inflammation, which is accompanied by an attack of the trachea by proinflammatory agents, including the cytokine interleukin-4, which itself is known to induce IL4R gene expression through the STAT-6 signaling molecule. These results find indirect confirmation in the works of MikitaT., CampbellD¹¹, and SchindlerU., WuP.¹². Mediators, metabolic products of nerve cells, or other signaling molecules secreted by them, directly or indirectly (through the processes of neurogenic inflammation) may serve as a signal for the start of expression of the IL4R gene.

Studies on the effect of interleukin-4 on tracheal smooth muscle contractions have shown that this cytokine leads to a significant increase in muscle contractile responses, increasing obstructive events. Against the background of pronounced contractile responses in sensitized rats, interleukin-4 caused an additional increase in contractile responses from 3.06±0.06 mN to 3.53±0.07 mN in the tracheal muscle with ganglion and from 2.39±0.07 mN to 2.71±0.08 mN in tracheal muscle without ganglion.

The large values of the contractile responses of the tracheal muscle with the ganglion can be associated with the greater expression of IL4R receptors in these regions of the respiratory tract as compared to the parts of the trachea without ganglia. Also, the sections of the trachea with the ganglion may differ in increased contractile activity due to the presence of local intramural metasympathetic reflex arcs in these sections. Our data on the effect of interleukin-4 on the contractile responses of rat smooth muscle find indirect confirmation in the studies of Che, Xiao-wen, Y.¹, and Chuchalin A. G.⁴

Conclusions

Thus, it can be concluded that under sensitization conditions in the rat trachea tissues there is a strong expression of the IL4R genes, significant expression of the molecular receptor for interleukin-4, and an increase in the constrictor effect on smooth muscle due to the influence of the cytokine interleukin-4 on it. In trachea regions containing intramural ganglia (bifurcation region), the level of expression of IL4R genes, the level of expression of the molecular receptor IL4R, and the magnitude of muscle contractile responses in response to interleukin-4 administration were significantly higher than in trachea samples without ganglia. It is assumed that these differences are due to the presence in these parts of the trachea of local intramural metasympathetic reflex arcs passing through the ganglia and the possible influence of

neuronal biologically active molecules (mediators, signaling molecules, metabolic products, or other compounds) on the expression intensity of the IL4R gene.

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