Caspian Journal of Environmental Sciences
Online ISSN: 1735-3866
Print ISSN: 1735-3033

# The role of mast cells, C-fibers, and cholinergic transmission in the reduction of tracheal muscle and mronchi of sensitized animal

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## ABSTRACT

The article considers the role of stabilization of mast cell membranes, blockade of neuromuscular transmission and inactivation of C-fibers in the contractile activity of smooth muscles of the trachea and bronchi of sensitized rats. The studies were conducted on isolated preparations using electrical stimulation of postganglionic nerves (frequency - 30 stim/s, duration - 0.5 ms, amplitude - 20 V, duration of stimulation - 10 s). The study used cromolyn sodium, atropine, and capsaicin. As a result of the experiments, it was found that the leading role in the contraction of the muscles of the trachea and bronchi of a sensitized rat belongs to mast cells. However, the maximum effect of normalization of smooth muscle contraction can be achieved only in the case of a complex effect - blockade of neuromuscular transmission, stabilization of mast cell membranes, and inactivation of C-fibers.

**Keywords:** Romolyn sodium, Atropine, Capsaicin, Acetylcholine receptor blockade, Electrical stimulation, Postganglionic nerves.

## INTRODUCTION

Studies of smooth muscle contractions of the trachea and bronchi in sensitized animals are relevant, since they allow us to establish pathological mechanisms of increased muscle activity, many details of which are still poorly understood. The aim of this study was to establish the role of mast cells, C-fibers and cholinergic transmission in the contraction of the muscles of the trachea and bronchi of sensitized animals. Mast cells are known to play an important role in the pathological reaction of the smooth muscle of the respiratory tract. The number of these cells increases in the area of the pathological focus, the intensity of their degranulation increases (Begueret *et al.* 2007). Mediators and cytokines secreted by mast cells act on adjacent structures: smooth muscle, C-fibers, epithelium, neurons of the intramural ganglion (Fedin *et al.* 2014). To eliminate the effects of mast cells, sodium cromoglycate and its analogues are used. The drug stabilizes mast cell membranes, preventing their degranulation with the subsequent release of mediators and cytokines. As a result, an improvement in the function of external respiration is noted (Anderson *et al*. 2010; Kippelen *et al*. 2010), reduction of contractile responses of the trachea and bronchi (Lin *et al.* 2011). With regard to C-fibers, their complex role in the conditions of the normal functioning of the body has been established. C-fibers are excited by small doses of capsaicin and secrete tachykinins, which cause constrictor (due to neurokinin A, SP) (Undem & Kollarik 2005; Kirilina *et al.* 2018) or dilatational (as a result of VIP; NO) effects (Matsuzaki & Hamasaki 2014). There is evidence of an increased excretion of tachykinins with C-fibers under conditions of pathologies such as bronchial asthma, chronic obstructive pulmonary disease (Elekes *et al*. 2007). Regarding cholinergic transmission, there is information about its leading role in the contraction of the muscles of the trachea and bronchi under normal conditions. It is reported that with blockade of cholinergic transmission of atropine, the size of muscle contractions drops sharply (Masakazu *et al.* 2006; Kiver *et al.* 2016). However, most studies with respect to all of the above structures were performed on isolated preparations without the use of electrical stimulation. Such studies make it possible to assess muscle tone in response to pharmacological

Caspian J. Environ. Sci. Vol. 18 No. 5 (Special Issue: Environmental Aspects of Economic and Social Sustainable Development)
pp. 495~502 Received: April 05. 2020 Accepted: Sep. 23. 2020
DOI: Article type: Research
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effects and do not provide an opportunity to evaluate muscle response in a natural organism experiencing electrical effects associated with processes occurring in the nervous system. The present study was carried out using electric field stimulation of postganglionic nerve fibers, which brings the studied system closer to natural conditions and distinguishes this work from others. In accordance with the goal, in this study, was made an attempt to determine the contribution of mast cells, C-fibers and cholinergic transmission to the pathological contraction of the smooth muscle of the rat trachea and bronchi. As a result of the study, it will be possible to determine which of the following structures plays a leading role in muscle contraction. Also, the aim of the study was to reduce smooth muscle contractions to normal values by blocking the structures most significant in contracting. The indicated positions distinguish this study from similar ones and determine its novelty.

## MATERIALS AND METHODS

**Pet procedure**: 32 Wistar rats of both sexes with a body weight of 190-270 were examined. There were no differences in the contractile reactions of the muscles of males and females, since the females were taken for experiments during the diestrus period. The animals were kept in a vivarium, which met all the requirements for animal welfare. Animals were anesthetized by placing the animal in a chamber with chloroform. The effect of chloroform was manifested in a fairly rapid loss of consciousness in animals, after which decapitation was performed. This approach ensured quick and painless euthanasia of the animal (recommendations for euthanasia of experimental animals, European Commission) (Close *et al.* 1997). Thereafter, the animal was fixed on the opening table; the chest was opened and then an operation was performed to extract the respiratory tract of the animal. Parenchymal lung tissue was removed mechanically with a wooden spatula (Hatziefhimiou *et al*. 2005). The airways were rinsed in a Krebs-Henselight solution, and then trachea and bronchial preparations were prepared. Each preparation was a sample of the trachea or bronchi 0.4-0.6 cm long and 0.5-0.7 cm wide. Samples of the trachea and bronchi were taken from the bifurcation region, since intramural ganglia are present in these areas. The incision line of the trachea and bronchi passed through the cartilaginous half rings. The smooth muscle remained intact. Tracheal and bronchial preparations were placed in a chamber with Krebs-Henseleit solution, where one edge of the preparation was fixed with needles, and the second edge of the preparation was set with hook-holders attached to an electromechanical sensor that records the contractile response (measured in mn).
**Equipment:** In the experiments, a physiological complex was used that supports the normal course of physiological processes in isolated preparations. The complex included special chambers for placing trachea and bronchial preparations in them, an ultra-thermostat, an aerator, a peristaltic pump (ML0146 / CV, Multi Chamber Organ Baths, Panlab, Germany), electromechanical sensors (Grass FT-03 force displacement transducer, Astro Med, West Warwick, RI, USA), electrical stimulator (direct-current stimulator, Grass S44, Quincy, MA, USA), personal computer, special software (Chart v4.2 software, Power Lab, AD Instruments, Colorado Springs, CO, USA ).
**Electrophysiological experiments:** In all experiments, electric field stimulation was used. For this, two silver electrodes were placed in the drug chambers. During operation, electrical stimulation of postganglionic nerve fibers was used (stimulus frequency: 30 Hz, duration: 0.5 ms, amplitude: 20 V, stimulation duration: 10 s) Electrical stimulation simulated the natural conductivity of electrical impulses through the postganglionic link of the reflex chain. The experiments were studied contractile reaction of smooth muscles of the trachea and bronchi of rats using electrostimulation and pharmacological agents. First, electrostimulation of preparations of the trachea and bronchi was carried out. Muscle contractile reactions were recorded. These responses were taken as the base level (or 100%). After this, pharmacological substances were added and muscle contractile reactions were recorded. Thus, tracheal and bronchial musculature reactions were recorded taking into account electrical stimulation and pharmacological preparations. Value of contractile reactions to use the drug was largely dependent on the initial smooth muscle tone, as well as on the control contractile reactions in the case of the use of electric stimulation in connection with saline. Despite the fact that all animals were of the same age and the sample was homogeneous, the variability of the initial tone and control reactions (measured in many) of organs such as the trachea and bronchi was quite high, and this fact determined the reduction in percentage (calculated as %) from the base level of activity taken at 100%). Methods of electrical stimulation of postganglionic nerves are taken from research methods proposed by Fedin (1997).

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**Pharmacological procedure:** Perfusion was carried out with a Krebs-Henseleit solution of the following composition: sodium chloride (118 mmol $L^{-1}$ as perfusion, Sigma-Aldrich, Germany); potassium chloride (4.8 mmol $L^{-1}$ as perfusion, Sigma-Aldrich, Germany); magnesium sulfate (1.18 mmol $L^{-1}$ as perfusion, Sigma-Aldrich, Germany); potassium dihydrogen phosphate (1.2 mmol $L^{-1}$ as perfusion, Sigma-Aldrich, Germany); 2.5 mmol $L^{-1}$ calcium chloride as perfusion, Sigma-Aldrich, Germany); sodium bicarbonate (25.0 mmol $L^{-1}$ perfusion, Sigma-Aldrich, Germany); glucose (5.5 mmol $L^{-1}$ as perfusion, Sigma-Aldrich, Germany). The necessary oxygen level, temperature (37 °C) and pH (6.9 - 7.1) were maintained in the dishes with the preparations. The inflow of a fresh Krebs – Henseleit solution was provided regularly, as well as the outflow of the used (Fedin *et al.* 1997).

During the experiments, the following substances were exogenously introduced into the drug chambers: capsaicin (3 nmol $L^{-1}$ as perfusion for 30 min, Sigma-Aldrich, Germany) for inactivation of c-fibers (Kirilina, Smirnova, Blazhevich *et al*. 2018), atropine (0.2 nmol $L^{-1}$ in perfusion for 30 min, Sigma-Aldrich, Germany) to interrupt neuromuscular transmission (Fedin, Kiver, Smirnova *et al.* 2014), cromolyn sodium (240 μmol $L^{-1}$ as perfusion for 30 min, Sigma-Aldrich, Germany) to stabilize mast cell membranes (Lin, Chou, Chu & Wang 2011). In the study, it was necessary to exclude the effect of the epithelium; its physiological effects were inhibited by indomethacin (3 nmol, as perfusion for 30 min, Sigma-Aldrich, Germany) in all experiments. Indomethacin had no effect on the contraction of the smooth muscles of the trachea and bronchi of rats. This was verified earlier before proceeding to the main series of experiments (Vanhoutte 2013).

**Animal Sensitization Procedure:** Rats were sensitized by a single intraperitoneal administration of 0.23 nmol of ovalbumin (Sigma-Aldrich, Germany) mixed with 120 μmol of aluminum hydroxide (Sigma-Aldrich, Germany) as an adjuvant. After 14 days, ovalbumin (1.17 nmol $mL^{-1}$) was applied to the chamber with experimental preparations. The primary management of ovalbumin contributes to the development of sensitization of the animal. Repeated application of ovalbumin leads to mast cell degranulation in experimental preparations. A non-sensitized group was injected with saline intraperitoneally as (Masakazu *et al.* 2006).

**Experiment design:** Two groups of animals were formed: the control group (received saline) and the experimental group (received an injection of ovalbumin with its reintroduction after 14 days). In the experimental group, tracheal and bronchial muscle contractions were evaluated after repeated administration of ovalbumin. Atropine, capsaicin and cromolyn sodium were introduced into the chambers with preparations of the trachea and bronchi on the 14th day before the repeated administration of ovalbumin in order to block the corresponding structures until the moment of repeated contact with a foreign protein. Next, an assessment of smooth muscle contractions after repeated administration of ovalbumin under conditions of pre-blocked cholinergic receptors, inactivated C-fibers, and stabilized mast cells was performed.

### 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 was carried out using independent t-tests. A value of $p < 0.05$ was considered statistically significant. Data were expressed as mean value, standard deviation.

## RESULTS AND DISCUSSION

The contractile responses of the smooth muscle of the trachea and bronchi under conditions of electrical stimulation of postganglionic nerves against the background of physiological Krebs-Henselight physiological solution in the control group of animals were within the physiological norm and were taken as 100%. In sensitized animals, after repeated administration into the chambers with ovalbumin preparations, muscle responses under conditions of electric stimulation of postganglionic nerves increased to 198.81 ± 1.91% in trachea preparations (n = 8, significant difference compared with the responses with physiological saline, p = 0.02), and up to 187.22 ± 2.11% in the bronchi (n = 8, a significant difference in comparison with the answers against the background of physiological saline, p = 0.03).

Against the background of the stabilization of mast cell membranes by sodium cromoglycate, the trachea responses decreased to 135.17 ± 2.21% (n = 8, a significant difference compared with the responses to physiological saline, P = 0.04), bronchi to 132.45 ± 2.15% (n = 8, a significant difference in comparison with the responses against the background of physiological saline, P = 0.04) (Fig. 1, Table 1).

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**Table 1.** The magnitude of the contractions of the smooth muscle of the trachea and bronchi in mNewton and percent.

| Pharmacological drug | Trachea | | Bronchi | |
|---|---|---|---|---|
| | Amplitude of contraction (mN) | Amplitude of contraction (%) | Amplitude of contraction (mN) | Amplitude of contraction (%) |
| Krebs-Henseleit solution | 1.52 mN ± 0.02 | 100.00 % ± 1.32 | 1.48 mN ± 0,05 | 100.00 % ± 3.39 |
| Ovalbumin | 3.03 mN ± 0.03 | 198.81% ± 1.91 | 2.77 mN ± 0.03 | 187.22 % ± 2.11 |
| Ovalbumin + Cromoglycate Sodium | 2.05 mN ± 0.03 | 135.17 % ± 2.21 | 1.96 mN ± 0.03 | 132.45 % ± 2.15 |

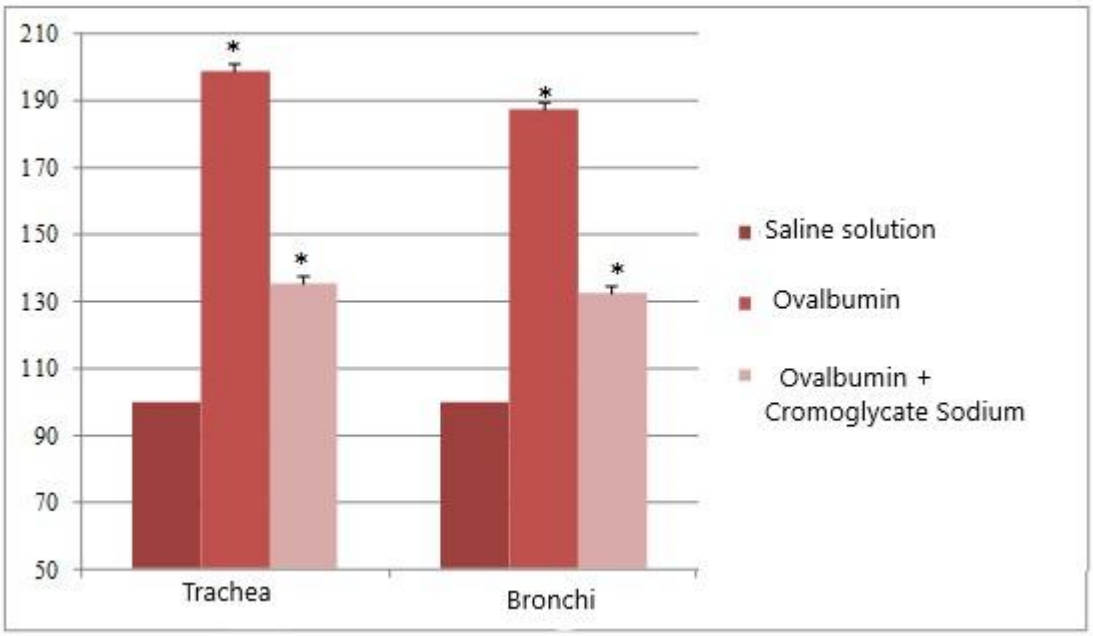


**Fig. 1.** The contractile responses of smooth muscles of the trachea and bronchi in the Krebs-Henseleit solution, after repeated administration of ovalbumin, after stabilization of mast cell membranes. The abscissa indicates the preparations used. The ordinate shows changes in smooth muscle reactions in %. *. significant differences.

In experiments where repeated administration of ovalbumin was preceded by atropine perfusion, contractile responses decreased to 149.57 ± 3.21% in trachea preparations (n = 8, a significant difference compared with the responses on the background of ovalbumin without cholinergic receptor blockade, p = 0.02) , and up to 141, 12 ± 2.95% in the bronchi (n = 8, a significant difference compared with the responses against the background of ovalbumin without blocking cholinergic receptors, p = 0.02).

In a complex experiment, where repeated administration of ovalbumin is preceded by perfusion of atropine and sodium cromoglycate, tracheal responses decreased to 121.11 ± 2.37% (n = 8, significant difference compared with the responses of ovalbumin with blockade of cholinergic receptors, p = 0.04) and bronchi - up to 117.32 ± 2.52% (n = 8, a significant difference compared with the responses against the background of ovalbumin with blockade of cholinergic receptors, p = 0.04) (Fig. 2, Table 2).

**Table 2.** The size of the smooth muscle contractions of the trachea and bronchi in mNewton and percent.

| Pharmacological drug | Trachea | | Bronchi | |
|---|---|---|---|---|
| | Amplitude of contraction (mN) | Amplitude of contraction (%) | Amplitude of contraction (mN) | Amplitude of contraction (%) |
| Krebs-Henseleit solution | 1.52 mN ± 0.02 | 100.00 % ± 1.32 | 1.48 mN ± 0.05 | 100.00 % ± 3.39 |
| Ovalbumin | 3.03 mN ± 0.03 | 198.81% ± 1.91 | 2.77 mN ± 0.03 | 187.22 % ± 2.11 |
| Ovalbumin + Atropine | 2.26 mN ± 0.05 | 149.57 % ± 3.21 | 2.08 mN ± 0.04 | 141.12 % ± 2.95 |
| Ovalbumin + Atropine + Sodium Cromoglycate | 1.84 mN ± 0.04 | 121.11% ± 2.37 | 1.38 mN ± 0.04 | 117.32 % ± 2.52 |

Caspian J. Environ. Sci. Vol. 18 No. 5 (Special Issue: Environmental Aspects of Economic and Social Sustainable Development)
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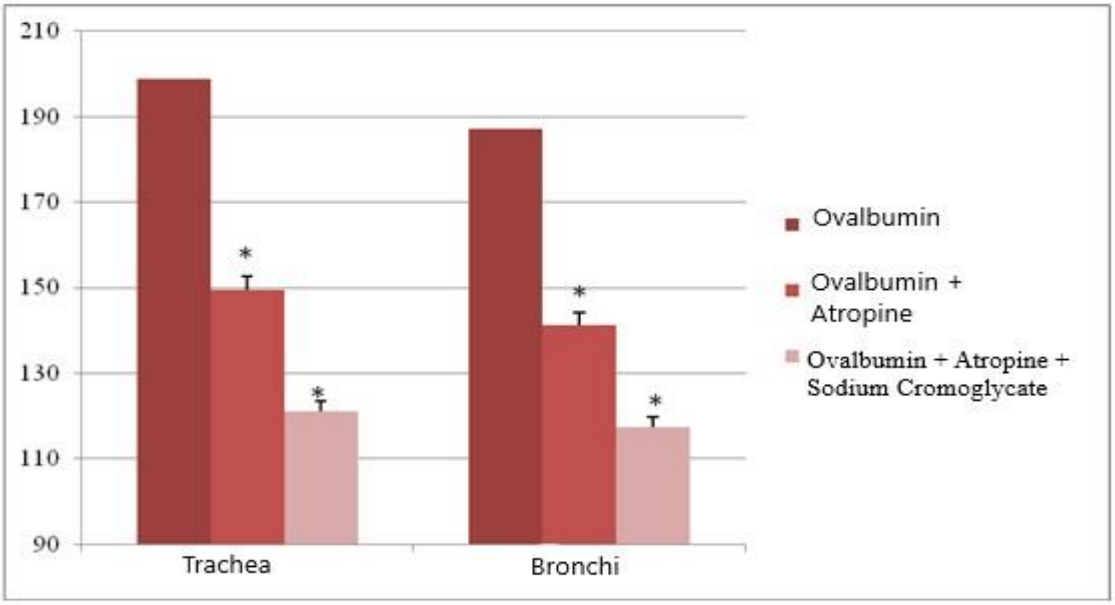


**Fig. 2.** The contractile responses of smooth muscles of the trachea and bronchi after repeated administration of ovalbumin, after interruption of neuromuscular transmission and after simultaneous stabilization of mast cell membranes and interruption of neuromuscular transmission. The abscissa indicates the preparations used. The ordinate shows changes in smooth muscle reactions in%. *. significant differences.

In an experiment where blockade of C-fibers with capsaicin was performed against the background of repeated administration of ovalbumin, the trachea responses decreased to 188.45 ± 2.43% (n = 8, a significant difference compared with the responses after repeated administration of ovalbumin, p = 0.04), bronchi - up to 176.19 ± 2.32% (n = 8, significant difference in comparison with the answers on the background of repeated administration of ovalbumin, p = 0.04). In a complex experiment, where repeated administration of ovalbumin was preceded by perfusion of atropine, cromolyn sodium and capsaicin, the contractile responses of the trachea and bronchi were characterized by the most pronounced decrease in values. The tracheal contractions were 118.18 ± 2.18% (n = 8, a significant difference compared with the responses with physiological saline, p = 0.04), bronchial muscle contractions were 115.32 ± 2.22% (n = 8, a significant difference in comparison with the responses against the background of physiological saline, p = 0.04) (Fig. 3, Table. 3).

**Table 3.** The magnitude of the contractions of the smooth muscle of the trachea and bronchi in mNewtons and percent.

| Pharmacological drug | Trachea | | Bronchi | |
|---|---|---|---|---|
| | Amplitude of contraction (mN) | Amplitude of contraction (%) | Amplitude of contraction (mN)) | Amplitude of contraction (%) |
| Krebs-Henseleit solution | 1.52 mN ± 0.02 | 100.00 % ± 1.32 | 1.48 mN ± 0.05 | 100.00 % ± 3.39 |
| Ovalbumin | 3.03 mN ± 0.03 | 198.81% ± 1.91 | 2.77 mN ± 0.03 | 187.22 % ± 2.11 |
| Ovalbumin + Capsaicin | 2.86 mN ± 0.04 | 188.45 % ± 2.43 | 2.61 mN ± 0.03 | 176.19 % ± 2.32 |
| Ovalbumin + Atropine + Sodium Cromoglycate + Capsaicin | 1.79 mN ± 0.03 | 118,18 % ± 2.18 | 1.71 mN ± 0.03 | 11532 % ± 2.22 |

Repeated administration of ovalbumin into chambers with preparations of the trachea and bronchi of sensitized animals led to a marked increase in contractile responses to pathological values (up to 198.81 ± 1.91% in trachea preparations and up to 187.22 ± 2.11% in bronchial preparations). The introduction of each of the pharmacological preparations — cromoglycate sodium, atropine and capsaicin — led to a decrease in responses. The strongest decrease in responses was observed due to the administration of sodium cromoglycate (up to 135.17 ± 2.21% in trachea preparations and up to 132.45 ± 2.15% in bronchial preparations). Such response is associated with the stabilization effect of mast cell membranes, as a result of which the release of mast cell mediators was blocked, contributing to the development of an allergic reaction and increased smooth muscle contraction due to the effects

Caspian J. Environ. Sci. Vol. 18 No. 5 (Special Issue: Environmental Aspects of Economic and Social Sustainable Development)
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of allergy mediators on adjacent structures (epithelium, intramural ganglion neurons, adrenergic and cholinergic nerve endings, smooth muscle cells, C-fibers) (Kryukova *et al.* 2001).

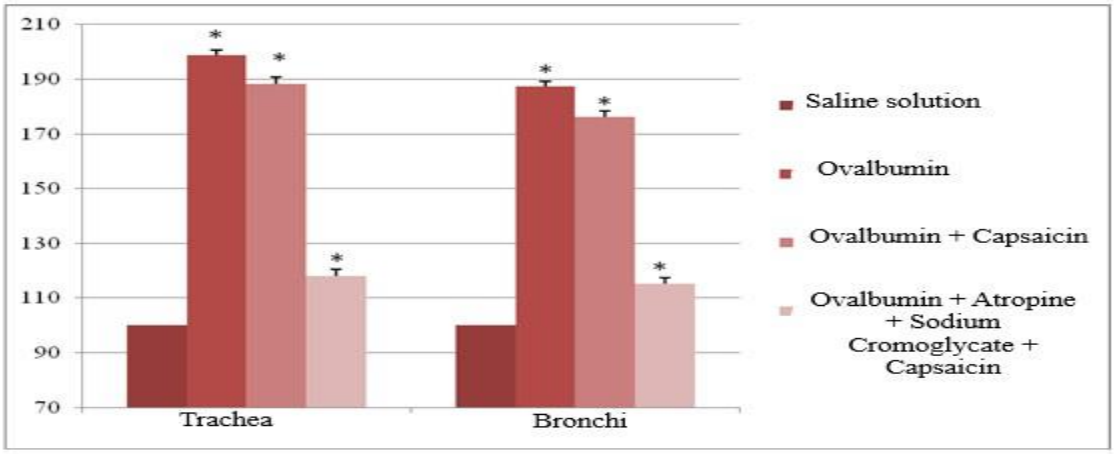


**Fig. 3.** The contractile responses of smooth muscles of the trachea and bronchi after repeated administration of ovalbumin, after inactivation of C-fibers and after simultaneous stabilization of mast cell membranes, interruption of neuromuscular transmission and inactivation of C-fibers. The abscissa indicates the preparations used. The ordinate shows changes in smooth muscle reactions in %. *. significant differences.

The introduction of atropine into the chambers with trachea and bronchial preparations led to a less pronounced decrease in contractile responses (to 149.57 ± 3.21% in trachea preparations and to 141, 12 ± 2.95% in bronchi). Nevertheless, sufficiently pronounced values indicate the significant role of cholinergic transmission in the implementation of smooth muscle contraction in a sensitized animal.

When capsaicin preparations were admitted to the chambers, the lowest values for reducing contractile responses were observed. The contractions of the trachea decreased to 188.45 ± 2.43%, the contractile responses of the bronchi to 176.19 ± 2.32%. Apparently, inactivation of C-fibers plays the most insignificant role in the contraction of the smooth muscle of the trachea and bronchi of a sensitized rat (Kirilina *et al.* 2018).

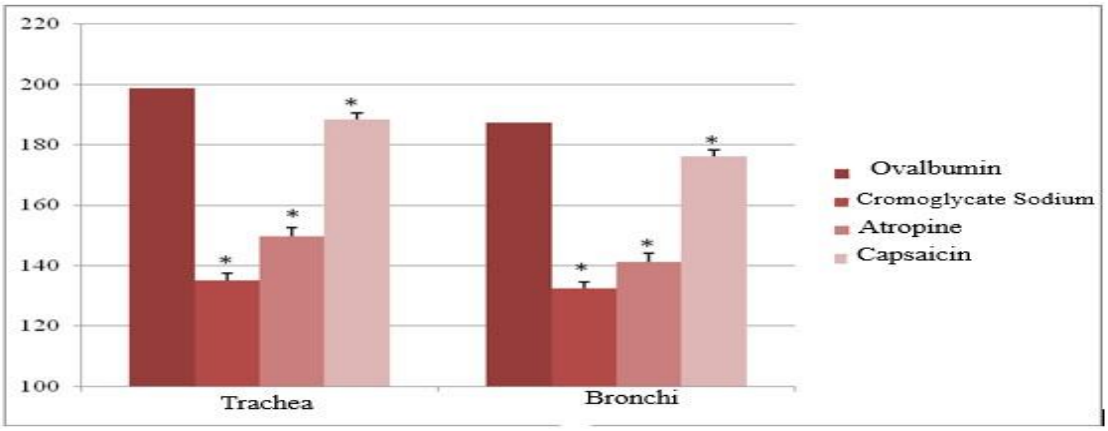


**Fig. 4.** Contractile responses of smooth muscles of the trachea and bronchi after repeated administration of ovalbumin, after stabilization of mast cell membranes, after interruption of neuromuscular transmission, after inactivation of C-fibers. The abscissa indicates the preparations used. The ordinate shows changes in smooth muscle reactions in %. *. significant differences.

## CONCLUSION

Thus, the leading role in the contraction of the muscles of the trachea and bronchi of a sensitized rat belongs to mast cells. However, the maximum effect of normalization of smooth muscle contraction can be achieved only in the case of a complex effect - blockade of neuromuscular transmission, stabilization of mast cell membranes, and inactivation of C-fibers.

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Caspian J. Environ. Sci. Vol. 18 No. 5 (Special Issue: Environmental Aspects of Economic and Social Sustainable Development)
pp. 495~502 Received: April 05. 2020 Accepted: Sep. 23. 2020
DOI: Article type: Research
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# نقش ماست‌سل‌ها ماست ، فیبرهای C و انتقال کولی‌نرژیک در کاهش عضله نای و نایچه حیوانات حساس

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(تاریخ دریافت: ۹۹/۰۱/۱۷ تاریخ پذیرش: ۹۹/۰۶/۰۲)

**چکیده**

نقش تثبیت غشای ماست سل، محاصره انتقال عصبی عضلانی و غیرفعال شدن فیبرهای C در فعالیت انقباضی عضلات صاف نای و نایچه موش های حساس در این مطالعه در نظر گرفته شده است. این مطالعات بر روی آماده‌سازی‌های جدا شده با استفاده از تحریک الکتریکی اعصاب پس از گلژیون (فرکانس - ۳۰ تحریک در ثانیه ، مدت زمان - ۵/۰ میلی ثانیه ، دامنه - ۲۰ ولت ، مدت تحریک - ۱۰ ثانیه) انجام شد. در این مطالعه از کرومولین سدیم، آتروپین و کپسایسین استفاده شده است. در نتیجه این آزمایش‌ها، مشخص شد که نقش اصلی در انقباض عضلات نای و نایچه‌های موش حساس به ماست سل‌ها تعلق دارد. با وجود این، بیشینه اثر نرمال سازی انقباض عضله صاف فقط در صورت یک اثر پیچیده - محاصره انتقال عصبی عضلانی، تثبیت غشای ماست سل و غیرفعال شدن فیبرهای C - قابل دستیابی است.

*مولف مسئول

***Bibliographic information of this paper for citing:***

Kirilina, VM, Blazhevich, LE, Smirnova, OE 2020, The role of mast cells, C-fibers, and cholinergic transmission in the reduction of tracheal muscle and mronchi of sensitized animal, Caspian Journal of Environmental Sciences, 18: 495-502

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Caspian J. Environ. Sci. Vol. 18 No. 5 (Special Issue: Environmental Aspects of Economic and Social Sustainable Development)
pp. 495~502 Received: April 05. 2020 Accepted: Sep. 23. 2020
DOI: Article type: Research
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