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# The role of stabilization of mast cell membranes by sodium cromoglycate in smooth muscle contraction of trachea and bronchi of the rat in the conditions of the physiological norm

El papel de la estabilización de las membranas de los mastocitos por cromoglicato de sodio en la contracción del músculo liso de la tráquea y los bronquios de la rata en las condiciones de la norma fisiológica

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## ABSTRACT:

The article discusses the role of stabilization of mast cell membranes with sodium cromoglycate in the contractile activity of smooth muscles of the trachea and bronchi. The studies were carried out 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). As a result of the study, it was found that the stabilization of mast cell membranes with sodium cromoglycate promotes an increase in the contractile activity of smooth muscle under physiological conditions. An increase in muscle contraction in response to sodium cromoglycate developed even after the atropine blockade of cholinergic receptors. When C-fibers were inactivated, the muscle contractile responses obtained after the administration of sodium cromoglycate decreased. Probably, the bronchoconstrictor effect of sodium cromoglycate is caused by the elimination of the dilating function of mast cells under normal physiological conditions.

**KEYWORDS:** sodium cromoglycate, mast cells, acetylcholine receptor blockade, 105281, zenodo5651304.

## RESUMEN:

El artículo analiza el papel de la estabilización de las membranas de los mastocitos con cromoglicato de sodio en la actividad contráctil de los músculos lisos de la tráquea y los bronquios. Los estudios se llevaron a cabo en preparaciones aisladas utilizando estimulación eléctrica de los nervios posganglionares (frecuencia - 30 estimulación / s, duración - 0,5 ms, amplitud - 20 V, duración de la estimulación - 10 s). Como resultado del estudio, se encontró que la estabilización de las membranas de los mastocitos con cromoglicato de sodio promueve un aumento de la actividad contráctil del músculo liso en condiciones fisiológicas. Se desarrolló un aumento de la contracción muscular en respuesta al cromoglicato de sodio incluso después del bloqueo de los receptores colinérgicos por atropina. Cuando se inactivaron las fibras C, disminuyeron las respuestas contráctiles musculares obtenidas

después de la administración de cromoglicato de sodio. Probablemente, el efecto broncoconstrictor del cromoglicato de sodio se debe a la eliminación de la función dilatadora de los mastocitos en condiciones fisiológicas normales.

**PALABRAS CLAVE:** cromoglicato de sodio, mastocitos, bloqueo del receptor de acetilcolina.

## INTRODUCTION

The qualitative and quantitative composition of pollutants in the surrounding air is constantly increasing, and therefore the epidemiological situation concerning the incidence of allergic bronchial asthma is deteriorating. For this reason, there is a growing interest in drugs that stabilize mast cell membranes. Several membrane stabilizers are fairly well understood. For example, the physiological effect of a drug such as nedocromil sodium on the human respiratory tract is described in the works of Joos G., Connor B.<sup>1</sup>. Recently, sodium cromolyn is often used for therapeutic purposes. However, the mechanism of action of this drug and its physiological effects remain unclear. Established membrane-stabilizing and anti-inflammatory function of sodium cromoglycate. A significant decrease in the number of mast cells, eosinophils, CD4-lymphocytes, and macrophages as a result of the use of sodium cromoglycate was found<sup>2</sup>. Stabilization of cell membranes is associated with an indirect blockade of the penetration of calcium ions through the membrane. This leads to the blocking of the formation of the connection of the membranes of the mast cell granules with the outer membrane.

As a result, the release from granules of histamine, bradykinin, leukotrienes, prostaglandins, and other biologically active substances mediating inflammation and bronchospasm is inhibited<sup>3</sup>. Most of the current research on the use of this drug is aimed at assessing the overall therapeutic effects. A significant decrease in the number of attacks in patients with allergic bronchial asthma after prolonged use of pharmacological preparations based on sodium cromoglycate has been proven<sup>1</sup>. It was found that sodium cromoglycate prevents the development of allergic and inflammatory reactions, prevents bronchospasm during sensitization of the body, and severe allergic asthma<sup>4</sup>. In models of ovalbumin-sensitized guinea pigs with asthma, it was found that sodium cromoglycate significantly inhibits the late stage of the allergic reaction and, to a lesser extent, the early stage. In such animals, sodium cromoglycate suppresses the inflammatory effects of interleukin-5, prevents tissue damage by reactive oxygen species, and prevents further development of obstructive pathology<sup>5</sup>. It has been shown that a decrease or complete cessation of smooth muscle contraction in guinea pigs after the introduction of sodium cromoglycate, preceding the introduction of antigen and sensitization<sup>6</sup>. Hasan S. S. in his studies indicates the dose-dependent effect of sodium cromoglycate on the muscle of the bronchi. When treating guinea pig bronchial preparations previously sensitized with ovalbumin, sodium cromolynate reduced or completely stopped bronchoconstriction in direct proportion to the applied concentration: at a concentration of 10-8 g/ml, the drug practically did not eliminate bronchoconstriction, and at a concentration of 10-6 g/ml completely stopped pathological contraction muscles<sup>2</sup>. The effect of sodium cromoglycate was studied in a model of chronic obstructive pulmonary disease induced in rats by exposure to nitrogen dioxide. It has been shown that the suppression of mast cell degranulation and the release of endogenous histamine by stabilizing the cell membrane with sodium cromoglycate prevents the development of hyperreactivity of bronchial smooth muscles.<sup>7</sup>

This study aimed to study the physiological role of stabilization of mast cell membranes with sodium cromoglycate in contractions of the muscles of the trachea and bronchi of rats under physiological conditions using electrical stimulation of postganglionic nerves against the background of blockade of C-fibers and cholinergic receptors. This article presents the results of experiments on the effect of sodium cromoglycate on the contraction of the muscles of the trachea and bronchi both against the background of saline and against the background of eliminating the effects of the intramural metasympathetic ganglion and C-fibers. In the

modern scientific literature, there is rarely information about the role of mast cell membrane stabilizers in smooth muscle contractions, taking into account the effects of the metasympathetic nervous system. At the same time, such studies are of particular importance, since the effects of activation and stabilization of mast cells should be considered taking into account the anatomical proximity of the ganglion and its physiological effects. Such studies may help to uncover new data on the mechanisms of action of sodium cromoglycate. It is also important to note that most studies on the effects of this membrane stabilizer have been conducted without the use of electrical nerve stimulation. In this study, stimulation of nerve structures with an electric field was used, autonomic nervous influences were taken into account, which brings isolated drugs as close as possible to the natural conditions of the body.

## MATERIALS AND METHODS

The object of the study was 20 Wistar rats of both sexes with a bodyweight of 180-250 g. Prepared preparations of the trachea and bronchi were placed in chambers with Krebs-Henseleit saline solution, where the required oxygen level and temperature regime were maintained. For research, we took the trachea and bronchi areas in the bifurcation area, since these zones of the respiratory tract are characterized by a large accumulation of intramural metasympathetic ganglia. We studied the change in the responses of the smooth muscle of the trachea and bronchi, caused by electrical stimulation of postganglionic nerves to pharmacological drugs. The work analyzed the maximum and minimum amount of contraction. The minimum amount of contraction can be considered as a relaxation effect, and the maximum - as a constrictor one (taken into account as a percentage of the background level of activity, taken as 100%).

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, Multi Chamber Organ Baths, Panlab, Germany), electromechanical sensors (Grass FT-03 force-displacement transducer, Astro Med, West Warwick, RI, USA), electro 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).

Statistical analysis was performed using the SPSS statistical package, version 10.0 (SPSS, USA). Comparison between groups of control and experimental results were performed using independent t-tests. A p-value <0.05 was considered statistically significant. Data were expressed as mean, standard deviation.

The electrical stimulator made it possible to irritate the drugs with an electric field. Silver electrodes were placed on both sides of the specimen. When the postganglionic nerves were stimulated, the frequency was 30 stim/s, the duration was 0.5 ms, the amplitude was 20 V, and the stimulation duration was 10 s. The registration of contractile activity was carried out by an electromechanical sensor. The contraction (tension) of the smooth muscle was converted into an electrical signal, which was fed to a personal computer for further processing<sup>8</sup>.

During the experiments, the following substances were injected exogenously: adenosine (10 µg/ml to activate mast cells as a 0.2 ml application, Sigma-Aldrich, Germany), sodium cromoglycate (100 µg/ml as perfusion to stabilize mast cell membranes Sigma -Aldrich, Germany), atropine (5 µg/ml as perfusion to block neuromuscular transmission, Sigma-Aldrich, Germany), capsaicin (1 µg/ml as perfusion for 30 minutes to inactivate C-fibers, Sigma- Aldrich, Germany).

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<sup>9</sup>.

## RESULTS

Before the effect of stabilization of mast cell membranes on smooth muscle contraction was evaluated, experiments were carried out to determine the significance of membrane destabilization with mast cell degranulation in muscle contraction. Against the background of saline and upon stimulation of postganglionic nerves, the mast cell activator, adenosine, caused an increase in the contractile responses of tracheal smooth muscle to  $114.81 \pm 2.61\%$ . On the bronchi, the responses were slightly lower than  $108.22 \pm 1.91\%$  (Fig. 1).

When cromoglycate sodium entered the baths with preparations of the respiratory tract, the contractile responses increased both relative to the Krebs-Henseleit saline solution and relative to the action of adenosine. The responses of the trachea reached  $148.75 \pm 2.41\%$ , bronchi -  $140.11 \pm 2.14\%$  ( $p < 0.05$ ).

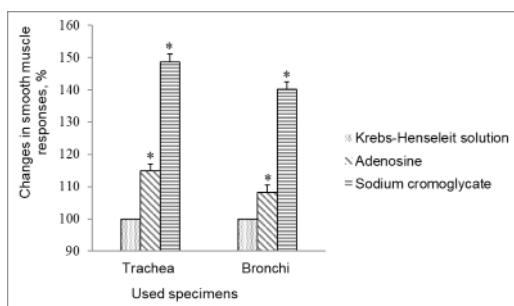


FIG. 1.

The contractile responses of the smooth muscle of the trachea and bronchi against the background of the action of adenosine and sodium cromoglycate.

\* - significant ( $p < 0.05$ ) difference from control values

Adenosine leads to partial degranulation of mast cells and increases the contractile responses of the tracheal and bronchi muscles within normal physiological values. Stabilization of mast cell membranes with sodium cromoglycate leads to a strong increase in contractile responses in the conditions of the physiological norm.

Blockade of cholinergic receptors with atropine (Fig. 2) led to inhibition of smooth muscle contraction in response to electrical stimulation. The tracheal muscle responded to stimulation of postganglionic nerves equal to  $18.32 \pm 2.91\%$ , bronchi -  $15.25 \pm 1.82\%$ . When cromoglycate sodium was introduced into the bath with preparations, while maintaining the blocked state of cholinergic receptors, the contractile responses increased again. The responses of the trachea, in that case, increased to  $82.11 \pm 3.11\%$ , bronchi - to  $75.43 \pm 2.03\%$  ( $p < 0.05$ ).

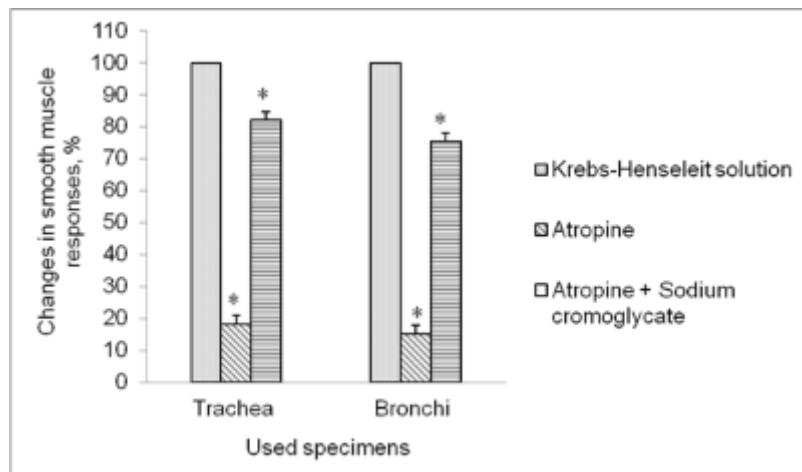


FIG. 2.

Contractile responses of the smooth muscle of the trachea and bronchi against the background of the action of atropine and sodium cromoglycate.

\* - significant ( $p < 0.05$ ) difference from control values

Atropine leads to a strong decrease in contractile responses of the tracheal and bronchi muscles. Stabilization of mast cell membranes with sodium cromoglycate against the background of atropine leads to an increase in contractile responses.

With the depletion of sensitive C-fibers with high doses of capsaicin (Fig. 3), contractile responses decreased to  $94.31 \pm 1.81\%$  in tracheal preparations. The muscle of the bronchi showed less muscle contraction, which was  $85.23 \pm 1.54\%$ . When cromoglycate sodium was introduced into baths with preparations, an increase in contractile responses occurred. Tracheal muscle responses increased up to  $135.77 \pm 3.22\%$ . The bronchial responses were slightly lower and amounted to  $130.21 \pm 3.56\%$  ( $p < 0.05$ ).

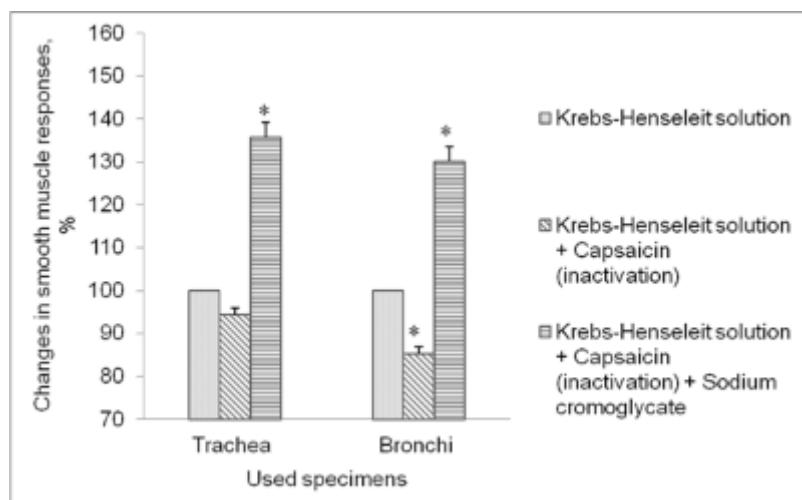


FIG. 3.

The contractile responses of the smooth muscle of the trachea and bronchi against the background of the action of high doses of capsaicin and cromolyn sodium.

\* - significant ( $p < 0.05$ ) difference from control values

Inactivation of C-fibers with capsaicin leads to a decrease in the contractile responses of the trachea and bronchi. Sodium cromoglycate on the background of inactivated C-fibers leads to an increase in contractile responses.

## DISCUSSION

The injection of adenosine, a substance that causes IgE-independent activation of mast cells, into the baths with preparations of the respiratory tract, a small increase in the contractile responses of the smooth muscle of the trachea, and bronchi was recorded in the case when stimulation of the postganglionic nerves was used. This increase can be regarded as a variant of normal physiological contraction. After the preparation was completely washed out with saline, sodium cromoglycate was injected into the trachea and bronchial tract trays. Further, electrical stimulation was performed. The smooth muscle responses, in this case, were significantly increased. Stabilization of mast cell membranes, both in tracheal preparations and in bronchial preparations, leads to an increase in contractile responses. That is, in conditions of smooth muscle contractions fluctuating within the physiological norm, sodium cromoglycate causes a constrictor effect. Most likely, this is due to the elimination of partial degranulation of mast cells, which is observed under physiological conditions. With partial degranulation, small doses of histamine are released, which leads to a relaxation effect due to the activation of H<sub>2</sub> and H<sub>3</sub> receptors. When comparing the contractile responses of the muscles of the trachea and bronchi, obtained as a result of the administration of adenosine, with the responses of the muscles, obtained as a result of the administration of sodium cromoglycate (Fig. 1), it can be seen that the responses to sodium cromoglycate exceed those to adenosine. These results may indicate the presence of any additional receptors to sodium cromoglycate, which mediate the constrictor effect under normal physiological conditions or indicate a normal range of contractions during partial degranulation of mast cells in response to their moderate destabilization by adenosine.

To identify the physiological effect of sodium cromoglycate and the role of mast cells, experiments were carried out with the use of an anticholinergic drug - atropine. Atropine made it possible to block cholinergic receptors and thereby eliminate the influence of local intramural ganglia. After the application of atropine, the responses of the trachea and bronchi were greatly reduced, since the leading reflex pathways in the contraction of smooth muscles pass through the ganglion. However, the addition of sodium cromoglycate to atropine resulted in increased responses. The responses of the trachea and bronchi, in this case, were lower than the control values obtained against the background of saline, but significantly higher than the responses obtained against the background of atropine alone. Thus, sodium cromoglycate, even against the background of a blockade of neuromuscular transmission under conditions of a physiological norm, leads to an increase in the contractile activity of smooth muscle. This can be explained by the elimination of the dilating function of mast cells under normal physiological conditions. If we compare the magnitude of muscle contraction as a result of sodium cromoglycate administration against the background of saline (Fig. 1) and the background of atropine (Fig. 2), we can see the predominance of contractile responses against the background of saline. This may indicate that, under physiological conditions, the metasympathetic ganglion has a synergistic effect on the effects of mast cells mediated by sodium cromoglycate. In the effects associated with sodium cromoglycate, muscle contraction is sufficiently associated with mast cell blockage. Perhaps this effect is associated with the great significance of the dilating effect of mast cells under normal conditions or with the presence of any other structures that have receptors for sodium cromoglycate and lead to an increase in contraction.

The results obtained in our studies concerning the physiological effect of sodium cromoglycate contradict the data of Hasan S.S., Salat M. Y. obtained in experiments on guinea pigs<sup>2</sup> and with the data of Lin Y.Y., Chou Y.L. obtained in experiments on rats<sup>10</sup>. Most likely, the discrepancy in the results is because these authors worked under conditions of a pathological organism (sensitized animals with allergic asthma). Under such conditions, the use of sodium cromoglycate led to a decrease in the contractile responses of the smooth muscle of the respiratory tract, due to the stabilization of mast cell membranes and the cessation of volumetric degranulation. Since mast cells under conditions of a physiological norm have a relaxation effect, and under

conditions of allergy - a constrictor (due to the release of different concentrations of histamine), sodium cromoglycate leads to different physiological effects in different situations.

C-fibers play an important role in the contraction of the muscles of the trachea and bronchi. Taking into account the mutual physiological influence of C-fibers and mast cells, and their joint significance in the contraction of smooth muscles, it is important to establish the role of blockade of mast cells with sodium cromoglycate under conditions of blocked C-fibers. In the modern scientific literature, there is no information on the role of sodium cromoglycate in muscle contraction against the background of the effects of C-fibers. In our studies, with blockade of C-fibers, contractile responses decreased, which is associated with the elimination of the excitatory effect of these structures. But when cromoglycate sodium was introduced into baths with preparations, there was a strong increase in muscle responses up to  $135.77 \pm 3.22\%$  in tracheal preparations and up to  $130.21 \pm 3.56\%$  in bronchi. However, if we compare the magnitude of muscle responses to sodium cromoglycate against the background of saline (Fig. 1) and the background of C-fiber blockade (Fig. 3), then the prevalence of contraction against the background of saline is obvious. Such results may indicate the role of C-fibers in the physiological effect of sodium cromoglycate. While maintaining the activity of C-fibers (muscle responses against the background of a saline solution), stabilization of membranes with sodium cromoglycate leads to greater contractile responses, in comparison with the responses obtained under conditions of inactivation of C-fibers. The obtained result may indicate a synergistic role of C-fibers in the contractile activity of smooth muscle together with mast cells and intramural ganglion. However, the role of C-fibers should be characterized as less significant than the effect of the ganglion in muscle contraction mediated by the stabilization of mast cell membranes. It is also possible that C-fibers have receptors for sodium cromoglycate, which has an exciting effect on them. In the literature, there was information about the presence of receptors for sodium cromoglycate on the membranes of cells involved in the immune response<sup>2</sup>, but we could not find data on the presence of receptors for this compound on afferent C-fibers.

## CONCLUSION

Thus, under physiological conditions in rats, stabilization of mast cell membranes with sodium cromoglycate promotes an increase in the contractile activity of smooth muscle of the trachea and bronchi, which is probably associated with the cessation of partial degranulation of mast cells and, to a sufficient extent, with the effect of the intramural ganglion. To a lesser extent, smooth muscle contractions mediated by the stabilization of mast cell membranes with sodium cromoglycate are associated with the effects of C-fibers.

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