RELAXING ACTION OF OLIGOPEPTIDES ISOLATED FROM THE TRACHEAL MUCOSA AND LUNG PARENCHYMA ON SMOOTH MUSCULATURE OF THE ISOLATED RAT TRACHEA

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The existence of peptidergic regulation of contractility of the smooth muscle formations has been convincingly demonstrated for the gastrointestinal tract and cardiovascular system. However, no regulatory peptides possessing a specific action on the respiratory organs, like endothelin and the natriuretic factor for the cardiovascular system or intestinal hormones for the digestive organs, have yet been found. Meanwhile there are grounds for considering that the mucosa of the trachea and bronchi possesses an effective specific system of regulation of smooth muscle tone in the respiratory passages, evidence for which has been obtained in numerous investigations conducted on de-epithelized preparations of the trachea and bronchi [8, 9]. The writers found previously that low-molecular-weight peptides isolated from the tracheal mucosa and lung parenchyma of calves possess broncholytic activity on a model of experimental allergic bronchospasm and an antiinflammatory action on models of acute and chronic inflammation in lung tissue [1, 2, 7].

The aim of this investigation was to study the effect of peptides isolated from the respiratory organs of calves on cholinergic constriction of an isolated smooth-muscle preparation of the rat trachea.

EXPERIMENTAL METHOD

Peptide preparations obtained from the tracheal mucosa (TMP) and lung parenchyma (LPP) of calves were used. As preparations for comparison, we used peptides obtained from the vascular wall (calf aorta, VWP) and also thymalin, a peptide preparation obtained from the thymus by a similar technology (Leningrad Meat Combine Medical Preparations Factory). TMP, LPP, and VWP were obtained by the method in [3, 4]. Peptides were assayed by Lowry's method. The peptide preparations were standardized for molecular weight by gradient disk PAG electrophoresis and gel-filtration on Sephadex G-25 ("Pharmacia," Sweden), and also by peptide mapping, using methods of thin-layer chromatography, two-dimensional electrophoresis, and isoelectric focusing. As standard cholinergic agents we used acetylcholine and atropine ("Serva," Germany). The investigations were conducted on noninbred male albino rats weighing 150-200 g. The animals were killed by cervical dislocation. The trachea was exposed by a longitudinal skin incision from the ventral aspect and thoracotomy, separated from the esophagus and surrounding tissues, mobilized, and divided along the midline from the ventral aspect. Taking account of differences in the innervation of different parts of the trachea in rats [5, 11], strips consisting of six segments from the cervical (2-3 segments below the larynx) and thoracic (2-3 segments above the bifurcation) parts of the trachea were used in the experiments. The strips of trachea were placed in a constant-temperature continuous-flow chamber, which was perfused with aerated Krebs–Henseleit solution (KHS) by means of a peristaltic pump at a speed of 1 ml/min. The temperature of the solution in the chamber was kept between 37.0 and 37.5°C by means of a U-10 ultrathermostat (East Germany), at

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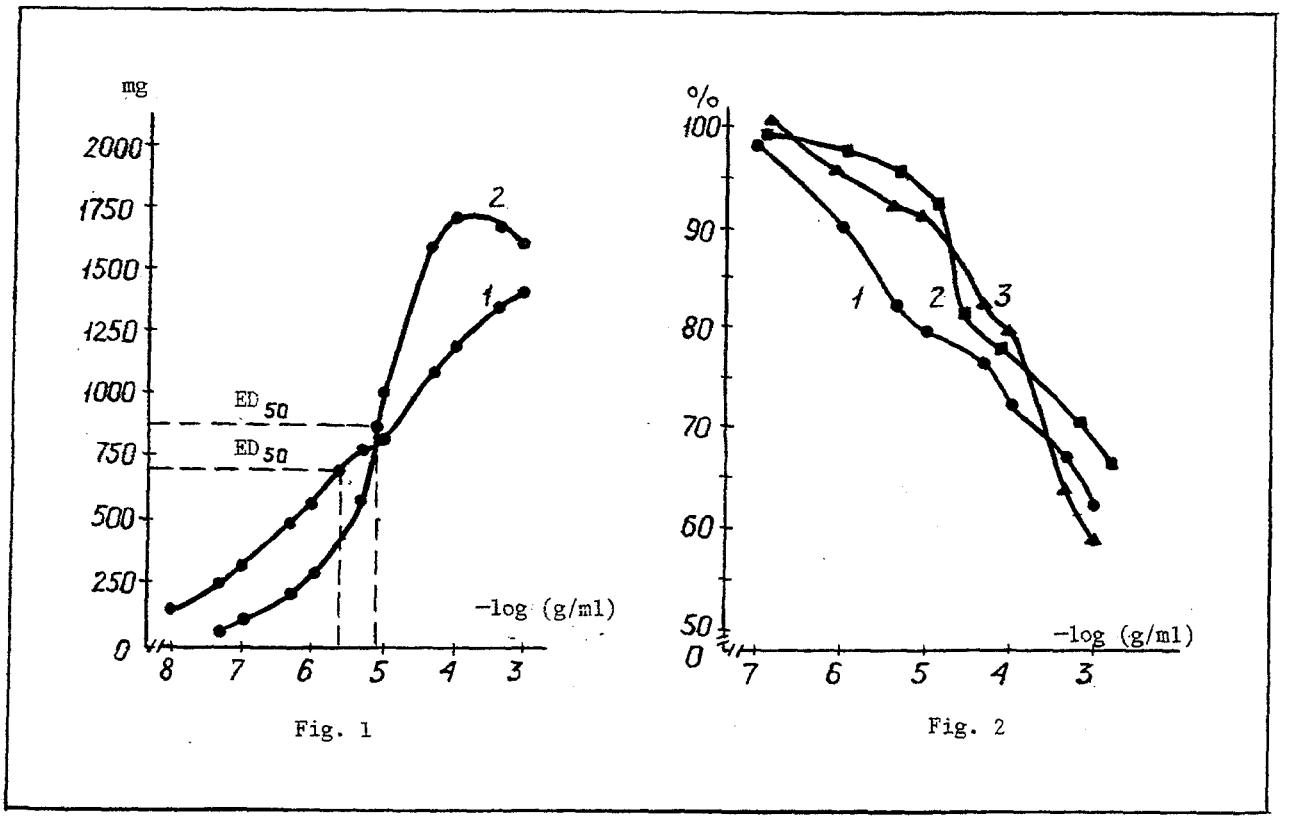

Fig. 1

Fig. 2

Fig. 1. Effect of acetylcholine on smooth muscle of rat trachea. Abscissa, negative logarithm of acetylcholine concentration (in g/ml); ordinate, tonic contraction of tracheal smooth muscle (in mg). 1) Cervical part, 2) thoracic part of trachea.

Fig. 2. Effect of TMP and LPP on time taken to achieve 50% relaxation of tracheal smooth muscle, in which spasm was induced by acetylcholine. Abscissa, negative logarithm of concentration (in g/ml), ordinate, time taken to achieve 50% relaxation of tracheal smooth muscle, in which spasm was induced by acetylcholine (in % of control, Krebs–Henseleit physiological saline), 1) TMP (thoracic part of trachea), 2) LPP (thoracic part of trachea), 3) TMP (cervical part of trachea).

pH 7.2-7.4. The smooth-muscle preparation was securely fixed on the side of the cartilaginous half-rings with tungsten needles to the base of the chamber. The opposite side was connected by a thread to the rod of a 6MKhlS mechanotron (USSR), the output signal from which was amplified and recorded on a type N-390 automatic writer (USSR). The initial tension on the preparation was 500-700 mg, and preincubation under continuous-flow conditions continued for not less than 30 min. The effect of the test substances was evaluated by changes in the time taken to reach 50% relaxation of the smooth-muscle preparation, in which spasm was induced by acetylcholine beforehand, the chamber being subsequently perfused either with KHS only or with the same solution containing the test substance in the necessary concentration. The time required to reach 50% relaxation of the preparation on rinsing with KHS was taken as the control level (100%), relative to which the effect of the test substances was calculated as a percentage. The results were subjected to statistical analysis by parametric and nonparametric tests.

EXPERIMENTAL RESULTS

Perfusion of the chamber with the smooth-muscle preparation with acetylcholine solution induced tonic contraction of the smooth muscle of the rat trachea, the intensity of which was clearly dose-dependent in character (Fig. 1). Under these circumstances, with acetylcholine in a concentration of between 10^{-8} and 10^{-5} g/ml, responses of the cervical and thoracic parts of the trachea did not differ significantly from each other. Meanwhile, with higher

TABLE 1. Effect of Test Substances on Time Taken to Achieve 50% Relaxation of Smooth Muscle in Thoracic Part of Trachea, in which Spasm Was Induced by Acetylcholine

Substance	n	Concentration of substance, g/ml	50% relaxation time, %
KHS	12	—	100
TMP	9	5×10^{-4}	63,8±3,5*
LPP	8	5×10^{-4}	70,7±4,1*
VMP	5	5×10^{-4}	99,7±4,4
Thymalin	5	5×10^{-4}	97,2±3,6
Atropine	8	10^{-6}	54,0±6,5*

Legend. n) Number of animals, asterisk indicates statistically significant differences ($p < 0.05$) compared with control (KHS).

concentrations of acetylcholine (10^{-4} g/ml) the response of the thoracic part of the trachea was significantly higher compared with the cervical part (1720 ± 81 and 1210 ± 119 mg respectively, $p < 0.01$). However, a further increase in the acetylcholine concentration led to weakening of the tonic response of the thoracic part of the trachea, whereas the tension developed by the cervical part reached 1430 ± 107 mg (with acetylcholine in a concentration of 10^{-3} g/ml). ED_{50} for the thoracic and cervical parts of the trachea was $8.3 \cdot 10^{-6}$ and $2.2 \cdot 10^{-6}$ g/ml respectively.

Other workers also have observed a similar difference in the responses of the upper and lower parts of the respiratory tract [10, 11], possibly associated with differences in the density of acetylcholine receptors [10] or unequal cholinesterase activity [11]. Weakening of the contractile response of the thoracic part of the trachea observed with high acetylcholine concentrations may be associated with activation of inhibitory neurons of the intramural ganglia of the trachea [6].

In further investigations acetylcholine was used in a concentration of 10^{-5} g/ml, evoking a response of both parts of the trachea of not less than 50% of maximal: 865 ± 84 and 885 ± 65 mg for the thoracic and cervical parts of the trachea respectively.

Rinsing the tracheal smooth muscle, when in spasm induced by acetylcholine, with Krebs–Henseleit solution led to a 50% reduction of tension of the preparation after 5.7 ± 0.3 min in the cervical and 5.5 ± 0.4 min in the thoracic part of the trachea (control). The results of the use of different concentrations of peptide preparations from the tracheal mucosa (TMP) and the lung parenchyma (LPP) for rinsing are given in Fig. 2. The action of TMP was investigated on the cervical and thoracic parts of the trachea, that of LPP on the thoracic part only. It can be seen that perfusion of the preparation with TMP and LPP solutions led to more rapid 50% relaxation of the tracheal smooth muscle; the effect, moreover, was dose-dependent in character. A significant increase in the rate of relaxation under the influence of TMP was observed on the thoracic part of the trachea, starting with a concentration of 10^{-6} g/ml and of LPP with a concentration of 10^{-5} g/ml, and of TMP on the cervical part also with a concentration of 10^{-5} g/ml. With higher concentrations, the relaxing effect of TMP and LPP was intensified, to reach a maximum (acceleration of relaxation by 30-40% compared with the control) with a concentration of 10^{-3} g/ml.

Within the range of concentrations from 10^{-5} to 10^{-3} g/ml the action of the preparations did not differ significantly, regardless of which part of the trachea was used. This may mean that both TMP and LPP equally contain biologically active peptides which have a relaxing action on tracheal smooth muscle. Under these circumstances, the action of these peptides probably is independent of the presence of intramural ganglia, which are found mainly in the thoracic part of the trachea. The existence of peptides closely similar in activity in preparations of the tracheal mucosa and lung parenchyma may be connected with the relatively high specific gravity of the middle and small bronchi (and, consequently, the bronchial mucosa also) in the lung parenchyma.

As comparison preparations we used vascular wall peptides obtained by a similar method (calf aorta, VWP), the thymus peptide preparation thymalin, and atropine. Table 1 gives results (percentages of the control) illustrating the effect of the test substances on the time taken to achieve 50% relaxation of the thoracic part of the smooth-muscle preparation of the trachea in spasm under the influence of acetylcholine. Neither VWP nor thymalin

had any relaxing activity in the system used. Meanwhile atropine, a specific acetylcholine receptor blocker, in a concentration of 10^{-6} g/ml, virtually doubled the rate of relaxation of the tracheal smooth muscle.

Thus the relaxing effect of TMP and LPP is specific to peptides obtained only from the tracheal mucosa and the lung parenchyma. The higher activity of atropine than of TMP and LPP can be explained not only by its selective blocking effect on acetylcholine receptors, but also by the fact that peptides with relaxing action probably constitute only a small part of the total peptide preparations. Nevertheless, the fact that the tracheal mucosa and lung parenchyma contain chemically stable oligopeptides with relaxing activity toward the smooth-muscle apparatus of the trachea is sufficiently important on its own account, not only from the standpoint of the study of the mechanisms of regulation of smooth muscle tone in the respiratory passages, but also from the point of view of prospects for the development of fundamentally new broncholytics based on natural low-molecular-weight peptides with specific biological activity.

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