Ultralow Doses of Antibodies to Inflammatory Mediators: Antitussive Properties of Antibodies to Bradykinin, Histamine, and Serotonin

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We studied antitussive activity of antibodies to inflammatory mediators (bradykinin, histamine, and serotonin) in ultralow doses. Experiments were performed on guinea pigs with cough induced by citric acid and capsaicin. Test preparations suppressed cough produced by citric acid. Antibodies to bradykinin in ultralow doses were most potent in relieving capsaicin-induced cough (up to 85%); antibodies to serotonin and, particularly, to histamine produced a smaller effect. Potentiated histamine and serotonin possessed polymodal properties.

Key Words: *antitussive activity; cough models; citric acid; capsaicin; ultralow doses; antibodies; bradykinin; histamine; serotonin*

Recent studies indicate that cough is realized not only via the central mechanisms. Other pathogenetic mechanisms of the cough reflex, including activation of receptors for physiological mediators bradykinin, serotonin, and histamine, received little attention.

Receptors for bradykinin, which acts as a neuropeptide modulator of vascular tone, permeability, and pain sensations, play an important role in the induction of cough. Bradykinin possesses vasoactive properties and induces cough by affecting the mucosal layer of the upper and lower respiratory tract [8]. The mechanism of bradykinin-induced cough in patients with arterial hypertension treated with inhibitors of angiotensin-converting enzyme (*e.g.*, enalapril and captopril) is poorly understood. It was hypothesized that the increase in blood bradykinin level induced by these inhibitors stimulates arachidonic acid metabolism [7]. The ratio between blood concentrations of thromboxane B2 and prostaglandin F1 undergoes changes. This imbalance contributes to stimulation of irritant receptors in the mucosal layer and induction of cough, which is resistant to standard antitussive preparations and requires special therapeutic treatment. Clinical observations indicate that the direct action of bradykinin on the lower respiratory tract in patients with bronchial asthma causes bronchoconstriction and cough [8]. *In vitro* experiments on guinea pigs showed that the immunoreactive analogue of bradykinin acting as the antagonist of its receptors rapidly binds to B2 receptors on epitheliocytes of the tracheal mucosa [9].

Serotonin-reactive structures in the carotid and aortic zone and mucosal layer of the respiratory tract play a role in the pathogenesis of cough [4]. The role of receptors for 5-hydroxytryptamine (serotonin) in the cough reflex was studied on guinea pigs with selective agonists and antagonists. It was shown that not only 5-HT1 and 5-HT2, but also 5-HT1A receptors in the central nervous system are involved in the pathogenesis of cough. Induction or suppression of the cough reflex associated with activation of these receptors depends on the dose of agonists [10].

The involvement of histamine receptors in the cough reflex received little attention [5]. Experimental data suggest that histamine produces the indirect effect and stimulates cascade reactions of arachidonic acid metabolism. The intensity of this process increases during inflammation of the respiratory tract [1].

Here we studied the effects of potentiated antibodies against bradykinin (PAB-B), histamine (PAB-H), and serotonin (PAB-S) synthesized at the "Materia Medica Holding" Research-and-Production Company and administered in ultralow concentrations on experimental cough.

MATERIALS AND METHODS

Experiments were performed on 97 male and female guinea pigs weighing 500-700 g and kept in a vivarium of the All-Russia Research Center for Safety of Biologically Active Substances under standard condi-

All-Russia Research Center for Safety of Biologically Active Substances, Staraya Kupavna; *"Materia Medica Holding" Research-and-Production Company, Moscow

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TABLE 1. Antitussive Activity of PAB-B, PAB-H, and PAB-S and Suppression of the Cough Reflex (%) after Administration of PAB-B and PAB-H to Guinea Pigs Treated with Citric Acid and Capsaicin (Number of Cough Attacks, *M*±*m*)

Series		Cough-producing agent	
		citric acid	capsaicin
Control		20.86±2.69	8.28±0.54
PAB-B (*n*=28), ml	40	14.40±1.07* (30.96)	1.14±0.26* (31.7)
	80	8.86±1.47* (55.38)	1.33±0.20* (14.5)
Control		16.25±0.94	9.40±0.56
PAB-H (*n*=40), ml	40	11.1±1.6** (86.23)	7.10±1.14 (24.5)
	80	13.9±1.3 (83.94)	8.3±0.4 (11.7)
Control		—	12.60±1.89
PAB-S (*n*=5), 80 ml		—	11.2±1.98

Note. *$p<0.01$ and **$p<0.05$ compared to the control (before PAB administration). Suppression of cough in shown in brackets. *n*, number of animals.

tions. The animals were deprived of food 24 h before the experiment.

Antibodies against bradykinin, histamine, and serotonin were administered in ultralow doses (equivalent concentration 10^{-24} wt %). Antitussive activity of preparations was studied on classic models of cough according to methodical recommendations of the Russian Ministry of Health [2].

The effect of preparations was evaluated on 2 models of cough induced by citric acid and capsaicin. The animals were divided into 11 groups of 5-12 specimens each. They received PAB-B (40 or 80 μl, citric acid and capsaicin groups), PAB-H (40 or 80 μl, citric acid and capsaicin groups), and PAB-S (40 or 80 μl, citric acid group; and 80 μl, capsaicin group). During the experiment guinea pigs were kept in individual cages.

In series I cough was produced by citric acid [11]. The aerosol of 17% citric acid (2 ml, Sigma) was inhaled via a Pari nebulizer for 5 min. The reaction of animals to citric acid was tested 1 day before the experiment. Further studies were performed on highly reactive guinea pigs with serious cough attacks. On the next day test compounds in various doses (1 drop, 20 μl; 2 drops, 40 μl; and 4 drops, 80 μl) were applied to the mucosal layer of the mouth cavity in highly reactive animals via a micropipette. Citric acid was aerosolized after 30 min. We estimated the number of cough attacks over 30 min. The results were expressed in percents of the control (untreated animals, 100%).

Antitussive activity of PAB-S was studied on guinea pigs with citric acid-induced cough. They were divided into 2 subgroups of highly reactive and low reactive animals.

In series II guinea pigs were treated with the selective cough-inducing agent capsaicin.

The method for preparation and testing of animals was similar. To induce cough the animals inhaled 60 μg/ml capsaicin (15 μM, Sigma) for 5 min using a nebulizer. Capsaicin (1.2 mg) was dissolved in 20 ml mixture of 10% ethanol and 10% Tween 80 to prepare the matrix solution.

In this series the method for administration of test compounds and criteria of their antitussive activity were similar to those in experiments with citric acid-produced cough.

The effect of test compounds on the number of cough attacks was analyzed by Student's *t* test for dependent variables.

RESULTS

PAB-B produced the antitussive effect and markedly decreased the number of cough attacks induced by

TABLE 2. Antitussive Activity of PAB-S in Guinea Pigs with Citric Acid-Produced Cough (Number of Cough Attacks, *M*±*m*)

Dose, ml	Subgroup 1		Subgroup 2	
	control	PAB-S	control	PAB-S
40	17.14±0.83	20.00±2.35	19.40±1.57*	8.80±2.73
80	32.75±5.00	19.0±2.1**	18.6±3.2	22.00±2.95

Note. *$p<0.01$ and **$p<0.05$ compared to the control (before PAB-S administration). *n*, number of animals.

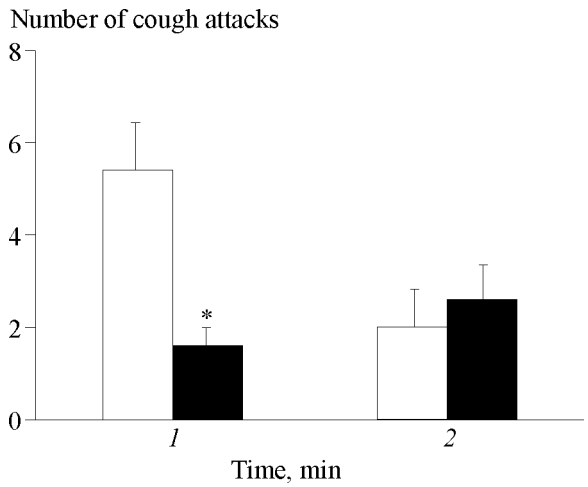

Fig. 1. Effect of PAB-S in a dose of 80 ml on the incidence of cough in guinea pigs treated with capsaicin. Light bars: number of cough attacks before PAB-S administration. Dark bars: number of cough attacks after PAB-S administration. Period of registration: first (*1*) and next 10 min (*2*) after inhalation of capsaicin. *$p<0.05$ compared to the parameter before PAB administration.

citric acid and capsaicin (Table 1). PAB-B in doses of 40 and 80 µl suppressed cough produced by citric acid by 30 and 55%, respectively. Suppression of capsaicin-induced cough was more pronounced (80%). It should be emphasized that in these experiments antitussive activity of PAB-B did not depend on the dose (Table 2). PAB-H in a dose of 40 µl significantly decreased the incidence of cough attacks produced by citric acid. PAB-H in a dose of 80 µl also decreased the severity of cough (statistically insignificant, Table 1). PAB-H in doses of 40 and 80 µl relieved cough produced by citric acid by 31.7 and 14.5%, respectively (Table 1). However, PAB-H only tended to suppress cough produced by capsaicin. In doses of 40 and 80 µl PAB-H decreased the incidence of cough attacks (Table 1).

The effects of PAB-S were studied in animals with cough produced by citric acid or capsaicin. PAB-S in a dose of 40 µl markedly decreased the incidence of cough attacks in some animals treated with citric acid (Table 2). In other animals the reaction to citric acid remained unchanged. Moreover, in 3 guinea pigs the severity of cough attacks slightly increased after administration of PAB-S. Similar results were obtained in experiments with PAB-S in a dose of 80 µl. In some animals PAB-S did not produce the antitussive effect (Table 2), while in others cough was suppressed by 42%. In highly reactive animals of group 1 the average number of cough attacks after inhalation of citric acid was greater than in group 2 guinea pigs (Table 2). PAB-S significantly decreased the number of cough attacks in animals highly reactive to citric acid (Table 2), but had no effect on low reactive guinea pigs.

PAB-S in a dose of 80 µl did not produce the antitussive effect in animals with capsaicin-induced cough. We analyzed the results obtained over the first 10 min of observations. After administration of PAB-S the incidence of cough attacks differed from the baseline level. The average number of cough attacks over the first 10 min after treatment with PAB-S was much lower than the baseline level (statistically significant). However, the severity of cough did not differ between control and PAB-S-receiving animals over the next 10 min observations.

Guinea pigs had different sensitivity to PAB-S, which was probably associated with individual variations in the density and heterogeneity of serotonin receptors (5HT1 and 5HT2).

Our results show that PAB-B were potent in suppressing cough (particularly that produced by capsaicin). The effect of PAB-B did not depend on the dose. It should be emphasized that no dose-dependent effects were revealed in experiments with antibodies to other mediators.

The action of PAB-S on cough demonstrates their polymodality. PAB-S in doses of 40 and 80 µl produced the opposite effects on animals with citric acid-induced cough. Besides this, PAB-S caused various changes in animals with capsaicin-induced cough.

Polymodal effects produced by antibodies to inflammatory mediators in ultralow doses probably reflect modulation of the substrate. If so, the directionality of action depends on the initial state of mediators in the body. It cannot be excluded that the variable effect of antibodies in ultralow concentrations reflects the Arndt-Shultz law, which describes differences in the influence of substances in various doses.

Our results indicate that affinely purified PAB-B, PAB-H, and PAB-S possess antitussive activity. The effect of preparations depends on their dose and type of cough. PAB-B are most potent in suppressing cough attacks. It may be suggested that these antibodies have high therapeutic activity.

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