Antitussive Activity of Anar

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Affinity-purified antibodies to morphine (mixture of homeopathic dilutions C30+C200) produced an antitussive effect on male and female guinea pigs with cough induced by citric acid and capsaicin.

Key Words: *antitussive preparations; morphine; antibodies to morphine; ultralow doses*

Antitussive drugs are used for the therapy of patients with various diseases of the upper and lower respiratory tract, pleurisy, neoplasms, and tracheobronchial dyskinesia accompanied by nonproductive cough, in the postoperative period, and during preparation for bronchoscopy. Congestion in the pulmonary circulation accompanied by persistent cough requires antitussive therapy. These preparations improve the state of patients, decrease the risk for severe complications of cough, and constitute a necessary component of pathogenetic therapy.

Despite a variety of antitussive agents, only few preparations are highly efficient and produce the selective central effect. These antitussive preparations are required in clinical practice, especially in oncology and pediatrics. Physiological parameters and peculiarities of pathological processes in these categories of patients limit the use of traditional antitussive drugs with the central and peripheral mechanism of action, since they often produce side effects and can cause death. Some patients have the syndrome of persistent cough resistant to the commonly used drugs. The search for highly efficient antitussive preparations producing the central effect and effective in ultralow doses is of considerable importance. Here we studied the original homeopathic preparation Anar synthesized at the "Materia Medica Holding" Research-and-Production Company.

MATERIALS AND METHODS

Experiments were performed on 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 conditions. The animals were deprived of food and water 24 h before the experiment.

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We studied the original homeopathic preparation Anar containing affinity-purified antibodies to morphine (mixture of homeopathic dilutions C30 and C200, equivalent concentration 10^{-60} wt %). The preparation was approved by the Russian Ministry of Health as a medicine for the therapy of patients with opium abstinence. Morphine hydrochloride was used as the reference preparation. The antitussive effect of Anar was studied on classic models of cough according to methodical recommendations on experimental studies of antitussive drugs (Russian Pharmacological Committee, 2001) [4].

Guinea pigs were kept in individual cages. Each group consisted of 8-10 animals. Cough was induced by citric acid [12]. To this end, citric acid was sprayed using a Pary nebulizer for 5 min (17% aerosol, 2 ml). The reaction of animals to citric acid was tested 1 day before the experiment. Guinea pigs were divided into highly reactive (18-43 cough attacks), low reactive (11-13 cough attacks), and very-low reactive animals (1-6 cough attacks). On the next day the test compounds in various doses were applied to the oral mucosa in highly reactive animals with micropipette (20 μl) and citric acid was sprayed again. The number of cough attacks over 30 min was determined.

Morphine hydrochloride in the same volume was used as the reference preparation.

The method for preparation and testing of animals with capsaicin-produced cough was similar. To induce cough the animals inhaled 15-30 μM capsaicin (Sigma) within 5 min through a nebulizer. Capsaicin (1.2 mg) was dissolved in 20 ml mixture of 10% ethanol and 10% Tween 80 and this stock solution was used for preparing preparations contained capsaicin in concentrations of 15×10^{-6}, 30×10^{-6}, 10^{-9}, and 10^{-10} M. The optimum concentration of capsaicin was chosen in special experimental series on 15 guinea pigs. Capsaicin in very low doses (1 and 0.1 nM) produced only insignificant cough. Cough attacks induced by capsaicin in doses of 15 and 30 μM were practically simi-

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TABLE 1. Effect of Anar on Cough Attacks Produced by Citric Acid and Capsaicin in Highly Reactive Guinea Pigs (*M*±*m*)

Exposure	Initial level	Morphine	Anar, µl		
			20	40	80
Citric acid	28.99±2.28	10.10±2.86*	8.90±2.34*	6.70±0.99*	—
% of inhibition	—	53.6±10.6	67.6±11.2	74.9±3.7	—
Capsaicin	11.30±0.94	11.50±0.91	—	6.30±0.64**	6.10±0.83**
% of inhibition	—	—	—	42.97±4.76	41.7±4.2

Note. *p<0.001 and **p<0.01 compared to the initial level; +compared to untreated animals.

lar. However, treatment with capsaicin in a dose of 30 µM was followed by dyspnea and tachycardia in animals. In further experiment we used capsaicin in a dose of 15 µM. Anar was administered perorally 30 min before inhalation of 15 µM capsaicin.

The capsaicin model of cough is important for detailed studies of antitussive drugs. Capsaicin-produced changes are mediated by various mechanisms. On the one hand, the effect of capsaicin is blocked by morphine and, therefore, is realized via opioid receptors [10]. On the other hand, the influence of capsaicin is mediated by receptors for neuropeptides [6] (*e.g.*, substance P).

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

RESULTS

Anar produced an antitussive effect on guinea pigs with citric acid-induced cough. Antitussive activity of Anar surpassed that of the reference preparation (considering the dose, Table 1).

Therefore, a new homeopathic preparation Anar was therapeutically effective in guinea pigs with citric acid-produced cough.

As differentiated from the response to citric acid, the reactions to capsaicin was similar in all animals. However, capsaicin was less potent than citric acid in producing cough attacks (Table 1). Our results agree with clinical data on the absence of correlation between the severity of cough produced by citric acid and capsaicin in volunteers [7].

Anar markedly decreased the incidence of cough attacks produced by capsaicin. Increasing the dose of Anar by 2 times did not potentiate the antitussive effect (Table 1). It should be emphasized that the reference preparation did not affect cough produced by capsaicin (Table 1), which was probably associated with low dose of morphine. Previous studies showed that morphine-like substances (*e.g.*, codeine) suppress cough only in doses of 10 mg/kg and higher [8]. Since the test compound contains potentiated antibodies to morphine, it can be suggested that ANR produces a morphine-like effect and prevents cough via the central mechanism.

The specific morphine-like antitussive effect of Anar (multiply diluted antibodies to morphine) is probably associated with *in vitro* formation of structures spatially and electronically similar to morphine molecules.

In the organism morphine undergoes transcoding into its functional analogue. Immunization of humans and animals with morphine-protein conjugates leads to generation of antibodies to morphine. Antibodies interacting with the same receptors simulate the effects of morphine. The system of antibodies against low-molecular-weight compounds+anti-idiotypic antibodies is an important component of the immunochemical system of homeostasis, the system recognizing a variety of chemical substances and encoding, storing, and using this information during adaptation of the organism to environmental conditions [2,3]. The homeopathic preparation Anar (multiply diluted solution of antibodies to morphine) probably undergoes transcoding. By the specificity of binding, the formed structure acts as anti-idiotypic antibodies. However, this structure complementary to morphine receptors is not the peptide structure, but a structure of fluid used for potentiation (multiple homeopathic dilution) of antibodies to morphine [1]. This nonprotein structure interacts with morphine receptors and produces an antitussive effect (similarly to anti-idiotypic antibodies). These data suggest that the antitussive effect of Anar is associated with signal transcoding and storage of information in the form of a nonprotein substrate. Molecules of water (structures of water clusters) are good candidates for the storage of information about chemical compound (structure of the active center in antibodies to morphine).

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