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EFFECT OF ANTITUBULIN ANTIBODIES ON ACTIVITY OF THE TASTE RECEPTOR APPARATUS

A. I. Esakov and O. D. Meshcheryakova

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The attention of research workers has recently been increasingly drawn to the functional role of the cytoplasmic tubulin—microtubules complex in mechanisms of sensory reception [7]. Depression of responses to stimulation has been demonstrated on olfactory [8] and mechanically sensitive receptors [11, 15] of certain invertebrates to stimulation after treatment with colchicine or vinblastine, pharmacological agents connected with tubulin and, consequently, destroying microtubules [16].

The present writers showed previously on chemoreceptors of the frog tongue that colchicine causes a sharp decrease in their sensitivity, so that afferent impulse generation may cease completely [1]. This effect was shown to be reversible under the influence of substances leading to an increase in the intracellular cAMP level: cAMP, dibutyryl-cAMP, the phosphodiesterase inhibitor theophylline, adrenalin, and GTP [2], and also during activation of the sympathetic nervous system [3]. The specificity of the restorative action of cAMP was demonstrated because inactive forms of nucleotides 2',3'-cAMP and 5'-AMP were ineffective and did not restore responses of the tongue chemoreceptors blocked by colchicine. Administration of cGMP or acetylcholine as a rule potentiated the effect of colchicine. The results were evidence of the importance of the colchicine-sensitive and cAMP-dependent process in maintenance of the chemosensitive function of the receptors. However, the nature of this process remained unexplained because the spectrum of action of colchicine is rather wide. It could not only interact specifically with tubulins, leading to destruction of the microtubular apparatus, but could also interfere with the course of various processes: inhibit nucleic acid and protein synthesis [9], inhibit energy metabolism [10], and block intracellular transport of materials [13] and activity of individual enzymes [6, 14].

To test the hypothesis that the tubulin—microtubules system plays an essential role in the mechanisms of function of the tongue chemoreceptors, in the investigation described below the effect of selective disturbances of this system, caused by means of purified monospecific antitubulin antibodies, was studied.

EXPERIMENTAL METHOD

Antitubulin antibodies were isolated from total fractions of immunoglobulins from a rabbit injected with purified tubulin. The method of immunosorption of tubulin, immobilized on Biogel P-300 by the method in [17], on a column was used. The nonimmune fraction of γ-globulins, not containing antitubulin antibodies, and also antibodies against specific nerve tissue proteins (S-100, GP-25) were used as the control. Antitubulin antibodies were injected subepithelially into the tongue in a dose of 0.2 mg/ml and in a volume of 0.2 ml.

The indicator of activity of the taste receptors was the spike discharge recorded from fibers of the glossopharyngeal nerve of the frog *Rana temporaria* by means of silver electrodes on a C-9 oscilloscope (from Nihon Kohden, Japan). A 0.5 M solution of NaCl, a 1.0 M solution of glucose, and tap water, which was applied to the dorsal surface of the tongue in a volume of 6.0 ml, were used as taste stimuli. Recording was carried out for 10 sec, after which the tongue was rinsed with Ringer's solution.

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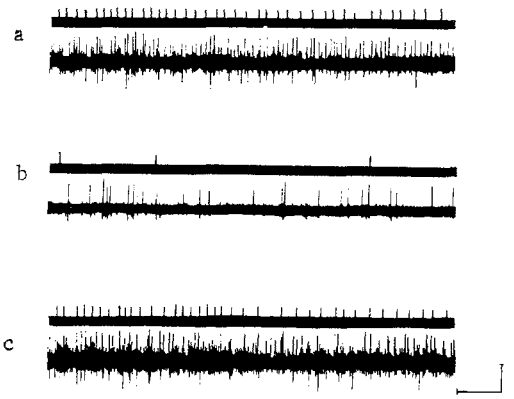

Fig. 1. Effect of antitubulin antibodies and of γ-globulin fraction not containing antitubulin antibodies on responses of taste receptor apparatus. a) Response of receptors to stimulation by NaCl in control: bottom trace obtained 5-8 sec after the beginning of stimulation, top trace consists of shaped pulses from counting device with coefficient 1:10, b) the same, 48 h after injection of antitubulin antibodies, c) the same 48 h after injection of γ-globulin fraction not containing antitubulin antibodies. Calibration, 50 μV, 1 sec.

EXPERIMENTAL RESULTS

Considerable changes in responses of the taste receptors to stimulation were observed (Fig. 1) 48 h after subepithelial injection of monospecific antitubulin antibodies, as shown by a decrease ($P < 0.05$) in the response to salt (71.6%) and water (73%). The change in responses to stimulation by glucose solution was less clear: Whereas in some experiments these responses were reduced by 54.7% ($P < 0.05$), in other experiments responses of the taste receptors to stimulation were in general unchanged. Depression of responses of the taste receptors by antitubulin antibodies still persisted 6 days after injection.

The effect of inhibition of responses of the receptors by antitubulin antibodies was characterized by definite specificity. Evidence and support of this specificity of action is given by data obtained in experiments in which the γ-globulin fraction not containing antitubulin antibodies and the γ-globulin fraction enriched with these antibodies were injected. Whereas nonimmune γ-globulins (Fig.1c) did not cause any change in response of taste receptors to adequate stimulation at these times, the γ-globulin fraction enriched with antitubulin antibodies reduced responses of the taste receptors to stimulation by salt solution by 34%, and by stimulation with water by 56%. No change was observed in the responses of the taste receptors to stimulation by glucose solution. Control injection of physiological saline likewise was ineffective.

It must be pointed out that injection of antibodies against specific nerve tissue proteins (S-100, GP-25) caused a marked reduction in responses of the taste receptors to stimulation. For instance, responses to salt were reduced by 66.6%, to water by 59.5%, and to glucose by 47.7%. Antibodies against protein GP-25 had an even stronger effect: Responses of the receptors to stimulation by salt solution were reduced by 84.2%, to stimulation by water by 78.9%, and by glucose by 56%.

The inhibitory effect of antitubulin antibodies may be abolished by activation of the sympathetic nervous system due to stimulation of sympathetic ganglion I. Only 15 min after stimulation of this kind, the responses of the taste receptors on the ipsilateral side were increased six-eight fold. An increase of this kind continued for 1 h of observation or even longer (Table 1).

Depression of responses of the taste receptors to stimulation, caused by administration of monospecific antitubulin antibodies, was thus connected with disturbance of the functioning of the tubulin–microtubules system and is evidence of the important role of that system and of processes coupled with it in the maintenance of receptor reactivity to stimulation.

TABLE 1. Effect of Sympathetic System on Responses of Tongue Chemoreceptors Blocked by Antitubulin Antibodies (M ± m)

Intensity of response after injection of antibodies, spikes/sec	Intensity of response of chemoreceptors after stimulation of sympathetic ganglion I, spikes/sec			
	15 min	30 min	45 min	60 min
	Glucose			
53,00±16,00	94,50±13,50 (78,3)	127,00±13,00† (139,6)	120,50±15,50 (127,4)	151,00±14,00† (184,9)
	NaCl			
22,00±3,00	50,50±13,50* (129,5)	127,50±4,50* (479,5)	130,00±6,00* (490,9)	103,50±11,50† (370,5)
	Tap water			
14,50±3,50	123,50±8,50* (751,7)	140,50±4,50* (868,9)	110,00±6,00* (658,6)	147,50±5,00* (817,2)

Legend. Increase in intensity of responses (in % of initial) shown in parentheses. *P < 0.01, †P < 0.05 compared with response before stimulation of ganglion.

Since tubulin molecules may be localized not only in the microtubular apparatus of the receptor cell, but also in chemosensitive and synaptic membranes, the question of the localization of the source of the effects of specific antitubulin antibodies which we observed remains unexplained. Blocking of responses of the taste receptors under the influence of antibodies against monospecific proteins of the S-100 group may be connected with the recently found specific interaction of this antigen with tubulin [4]. The effect of anti-GP-25 antibodies, however, was probably localized at the level of the nerve terminal membrane, for GP-25 itself is probably the nerve cell membrane glycoprotein [5]. Evidence that the mechanism of inhibition of receptor responses by antitubulin antibodies differs from that of antibodies against GP-25 may be provided by data showing that sympathetic stimulation is ineffective in the case of blocking of responses by anti-GP-25 antibodies.

Restoration of responses of receptors, caused by stimulation of the sympathetic nervous system and blocked by **antitubulin antibodies, is** evidence, first, of the integrity and effectiveness of sympathetic influences under these conditions and, second, of a connection between these influences and the functioning of the tubulin—microtubules system. This connection is evidently mediated by cyclic nucleotides, but the discovery of adenylate cyclase on the chemoreceptor cell membrane [12] provides a rational explanation of this mediation.

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