RESTORATION OF COLCHICINE-BLOCKED RESPONSES OF FROG TONGUE CHEMORECEPTORS BY ELECTRICAL STIMULATION OF THE SYMPATHETIC NERVOUS SYSTEM

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In recent years considerable attention has been paid to the physiological role of the cytoplasmic proteins known as tubulins and of the microtubular apparatus, formed from them, in the mechanisms of sensory reception. The property of tubulins of specifically interacting with plant alkaloids of the colchicine or vinblastine type has enabled the state of the microtubular appratus to be deliberately controlled and the changes observed in receptor function under these circumstances to be studied [6].

It was shown for the first time on the mechanically sensitive cell of the cockroach that exposure to colchicine leads to destruction of the microtubular apparatus of the cell and to cessation of electrophysiological responses to adequate stimulation [9]. Loss of sensitivity to adequate stimulation after a single treatment with colchicine also was found on chemoreceptors of insects in electrophysiological [4] and behavioral experiments [10].

In investigations on chemoreceptors of the frog tongue the present writers showed previously that 3-4 days after a single subepithelial injection of colchicine into the tongue a considerable decrease is observed in their responses to adequate stimulation, possibly amounting even to complete cessation of afferent impulse generation [2]. Responses blocked by cholchine were shown to be restored by injection of cyclic 3',5'-AMP (cAMP) and of inhibitors of cyclic nucleotide phosphodiesterase. The observed reversibility of the colchicine effect and restoration of reactivity of the receptor apparatus can be explained by participation of the cyclic nucleotide system in the polymerization and assembly of the microtubular apparatus of the cell, as the results of biochemical studies showed [13]. It was also found that responses of the receptor apparatus may be restored not only after injection of cAMP, but also after injectin of adrenaline, but not of acetylcholine or of cyclic 3',5'-GMP, which modify receptor activity under normal conditions.

Considering the important role of adrenaline in the activity of the chemoreceptor apparatus of the tongue, since it is the mediate of sympathetic fibers, which innervate this apparatus [7], in amphibians it was suggested that sympathetic influences may behave as the natural mechanism whose activation can restore colchicine-blocked receptor responses. In addition, the efficacy of sympathetic stimulation would be evidence of the absence of any poisoning effect of colchicine on sympathetic fibers under the conditions chosen (20 μM colchicine, testing after 3-5 days), for colchicine is known to affect the transport and liberation of mediators.

The aim of the present investigation was to study the effect of electrical stimulation of sympathetic ganglion I on responses of receptors of the frog tongue under normal conditions and after treatment with colchicine.

EXPERIMENTAL METHODS

Experiments were carried out on frogs (*Rana temporaria*) immobilized by destruction of the brain and spinal cord. Afferent spike discharges recorded from fibers of the lingual

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TABLE 1. Effect of Electrical Stimulation of Sympathetic Ganglion I on Responses of Chemoreceptors of Ipsilateral Side of Tongue to Stimulation by 0.5 M NaCl Solution and 1.0 M Glucose Solution under Normal Conditions and after Injection of Colchicine (M ± m)

Experimental conditions	Intensity of response, spikes/sec				
	control	15 min	30 min	60 min	90 min
Normal NaCl	175,5±3,1	233,1±35,6*	260,8±72,3	342,0±28,6†	275,8±54,7*
Colchicine NaCl	64,6±27,2		148,0±13,1*	121,0±15,1*	210,0±8,4†
Colchicine NaCl	18,6±4,7		53,3±23,8	51,3±20,7	67,3±21,9*
Normal Glucose	87,5±24,7	208,0±21,2*	252,0±82,0	249,0±43,8*	191,0±29,7
Colchicine Glucose	38,5±11,9	169,0±59,0	128,0±28,2*	184,5±10,6*	101,5±33,2
Colchicine Glucose	15,0±7,1	31,5±16,2	40,5±0,7*	78,0±2,0*	80,0±0,0*

*P < 0.05 compared with control.
†P < 0.01 compared with control.

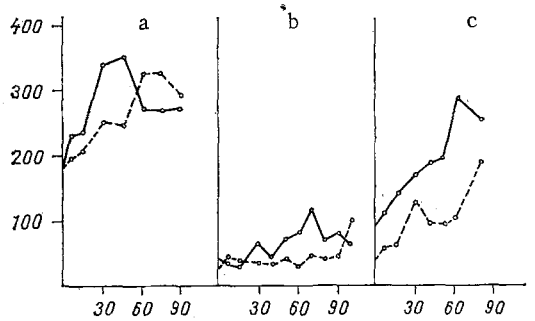

Fig. 1. Changes in responses of chemoreceptors on ipsilateral and contralteral sides of tongue under normal conditions and after treatment with colchicine to stimulation with 0.5 M NaCl solution under the influence of electrical stimulation of sympathetic ganglion I. a) Normal conditions, b, c) different levels of decrease in receptor activity as a result of injection of colchicine. Continuous line, ipsilateral side; broken line, contralateral side. Abscissa, time (in min); ordinate, intensity of response (in spikes/sec).

nerve in response to adequate stimulation of the tongue by tasty solutions (0.5 M NaCl, 1 M glucose) and tapwater were used as the indicator of responses of the tongue chemoreceptors. Responses of the receptors were recorded simultaneously from both lingual nerves, so that the effect of sympathetic stimulation could be compared on the side of the stimulated ganglion and also on the opposite side. Experiments were carried out in winter and spring (February-May) on animals kept under standard laboratory conditions at 10°C. Two pairs of silver electrodes with an interelectrode distance of 2 mm were used for recording with the VC-9 oscilloscope (Nihon Kohden, Japan). Bipolar silver electrodes were used for electrical stimulation of sympathetic ganglion I. The parameters of stimulation were: 2 msec, 60 Hz, 5 V.

EXPERIMENTAL RESULTS

Electrical stimulation of sympathetic ganglion I for 45 sec led to a marked increase in responses of the chemoreceptors to stimulation by NaCl and glucose solutions. This effect was characterized by a long latent period: Although on the ipsilateral side strengthening of the responses could be observed as easly as after 5 min, this strengthening did not reach statistically significant values until after 15 min, and it remained at that level for 1.5-2 h after stimulation (Table 1). Sympathetic facilitation of receptor responses to stimulation

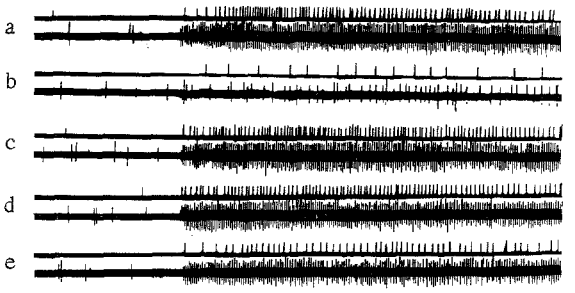

Fig. 2. Changes in responses of chemoreceptors on ipsilateral side of tongue to stimulation by 0.5 M NaCl solution under the influence of colchicine and electrical stimulation of sympathetic ganglion I. a-e) Afferent spike activity; trace of spikes shaped by means of a counting device (with coefficient 1:10) shown above. a) Initial response before treatment with colchicine; b) after treatment with colchicine; c-e) 30, 60, and 90 min, respectively, after stimulation of sympathetic ganglion.

by glucose solution was found to be stronger and reached its maximum earlier than in the case of salt stimulation (Table 1).

During simultaneous recording of responses of receptors from the two lingual nerves supplying the tongue, a sympathetic facilitatory effect was observed both on the side of the stimulated ganglion and on the contralateral side. Under these circumstances the effect observed on the contralateral side could reach the same value as on the side of stimulation, but it was delayed in time (25-40 min; Fig. 1a).

A sharp decrease in responses of the tongue chemoreceptors to the stimuli used took place 3-5 days after a single injection of 20 μM colchicine. Electrical stimulation of sympathetic ganglion I under these conditions led to considerable recovery of the intensity of responses of the tongue chemoreceptors to stimulation by the above-mentioned solutions (Fig. 2). Responses of the chemoreceptors could be increased by 2.5-3 times 30 min after stimulation, to reach and, in some cases, to exceed the intensity of the control responses under normal conditions (Fig. 1; Table 1). Characteristically the rate of recovery of the responses and their magnitude depended on the degree of inhibition of the responses of the receptors by colchicine: For instance, whereas responses to salt were reduced by 60%, their significant recovery ($P < 0.05$) was observed 30-45 min after stimulation of the sympathetic ganglion; with deeper inhibition of the responses (by 90% or more) significant recovery could be observed only after 60-70 min (Fig. 1b, c). No significant difference was observed in the time of recovery of responses of the tongue chemoreceptors on the side of the stimulated ganglion and on the contralateral side.

The facilitating effect of sympathetic stimulation on responses of the tongue receptors thus confirms previous data [5]. In the present investigation, it will be noted, the facilitatory action of the sympathetic system was demonstrated on receptors located not only on the side of the electrically stimulated ganglion, but also on the contralateral side. The considerable delay of the effect observed in that case compared with the effect on the ipsilateral side was probably due to the later arrival of catecholamines, liberated in response to sympathetic stimulation, at the contralateral receptors on account of diffusion processes or transport with the blood flow. The sympathetic facilitatory action on the afferent flow from chemoreceptors indicates a role of sympathetic fibers as one channel of centrifugal regulation of receptors. As previous intracellular investigations showed, regulation of this sort is postsynaptic in character and adapted to processes on the membrane of the chemoreceptor cell [1]. It can be tentatively suggested that regulatory influences on the chemoreceptor cell are not confined to the postsynaptic membrane, but spread also to intracellular cytoplasmic processes in the receptor cell. The discovery of adnylate cyclase and phosphodiesterase activity in the chemoreceptor cell by biochemical [12] and electron-histochemical methods [8] indicates the existence of a system linking processes on the postsynaptic membrane

with cytoplasmic processes inside the cell. Sympathetic stimulation probably leads to elevation of the cAMP level in cells of the taste papilla in the same way as in cells of the salivary glands, where parallel activation of adenylate cyclase [11] and phosphodiesterase [15] takes place in response to such stimulation.

An important place in changes in chemoreceptors sensitivity in the tongue to adequate stimulation after stimulation of the sympathetic ganglion is evidently occupied by connection (interaction) of the cyclic nucleotide system with the system regulating structural transformation of tubulins, which are subunits of the microtubular apparatus found in receptor formations. Although direct evidence of the role of tubulins in the chemosensitive function of receptors is insufficient, the experiments with colchicine indicate a significant role of changes in the structural state of the microtubular apparatus in receptor function. If blocking the sensitivity of the chemoreceptor apparatus by colchicine is the results of specific interaction between it and tubulin and destruction of the microtubular apparatus of the receptor cell, blocking the colchicine effect during sympathetic stimulation suggests that sympathetic fibers may participate in the intracellular control of certain cytoplasmic processes connected with the state of the tubulins in the cell. The effect described above is one concrete expression of the adaptive and trophic influences of the sympathetic system on metabolic processes in the cells. The possibility of restoration of receptor responses when blocked by colchicine, demonstrated as a result of stimulation of the sympathetic system, indicates that neurotransmitter processes realizing the sympathetic facilitatory effect on receptor cells are preserved [3, 14].

The results thus showed that sympathetic influences on the chemoreceptor apparatus may behave as a factor in the restoration of the pharmacologically blocked activity of that apparatus. Meanwhile the sympathetic nervous system is probably the natural mechanism responsible for maintaining a high level of sensitivity of receptors, effected at the intracellular level through changes in the state of the tubulins of the receptor cell.

LITERATURE CITED

1. A. I. Esakov, in: "Sensory Systems" [in Russian], Leningrad (1977), p. 60.
2. A. I. Esakov and O. D. Meshcheryakova, Dokl. Akad. Nauk SSSR, 250, No. 5, 1274 (1980).
3. A. Kotyk and K. Janacek, Membrane Transport [Russian translation], Moscow (1980), p. 113.
4. E. F. Block and W. J. Bell, J. Insect. Physiol., 20, 993 (1974).
5. K. E. Chernetsky, J. Neurophysiol., 27, 493 (1964).
6. P. Dustin, Microtubules, Berlin (1978), p. 329.
7. G. Gabella, Neurol. Sci., 9, 237 (1969).
8. K. Kurihara and N. Koyama, Biochem. Biophys. Res. Commun., 40, 30 (1972).
9. D. T. Moran and F. G. Varela, Proc. Natl. Acad. Sci. USA, 68, 957 (1980).
10. D. E. Matsumoto and R. D. Farley, Comp. Biochem. Physiol., 66, 93 (1980).
11. T. C. Muir and D. Templeton, J. Physiol. (London), 259, 47 (1976).
12. H. Nomura, Chem. Senses Flavour, 3, 319 (1978).
13. G. B. Olmsted and G. G. Borisy, Biochemistry (Washington), 14, 2996 (1975).
14. A. M. Poisner and J. Bernstein, J. Pharmacol. Exp. Ther., 177, 102 (1971).
15. B. Weiss, in: Frontiers of Catecholamines Research (E. Usclin and S. H. Snyder, eds.), New York (1973), p. 373.