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Study of Frequency Potentiation Mechanisms of cAMP-Dependent Responses of Snail Neurons

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Cyclic adenosine monophosphate (cAMP) is the intracellular mediator of various physiological responses of the nerve cell. The cAMP influence on the neuron electrical activity is studied by cAMP injection into the cytoplasm through an intracellular microelectrode. It has been found on mollusks that intracellular injection of cAMP by microionophoresis or under pressure causes a short-term membrane depolarization, the mechanism of which in different neurons are connected with conductivity changes for ion channels of various types [6].

The effect of frequency potentiation of cAMP-dependent responses developing in some snail neurons has been shown during frequent multiple cAMP injections [2]. It was taken into account that this effect depends on the microionophoresis technique peculiarities, namely that the quantity of substance released from the micropipette depends not only on the injection current strength and duration, but also on the "blocking" current strength and duration commonly used between injections [1, 4]. Our experiments with switching off the "blocking" current have not yielded, however, a clear answer to the question of the interrelations of the latter with the studied effect. This led us to assume that the effect of the frequency potentiation of the cAMP responses might depend on physiological factors, notably on the stimulation of the Ca-mediated cAMP responses [3, 7], in addition to the microelectrode factor.

The present paper demonstrates the analysis of the participation of the physiological and microelectrode factors in the development of the effect of the cAMP responses frequency potentiation.

MATERIAL AND METHODS

Experiments were carried out on the B_4 and F neurons [5] of snail isolated nerve ganglia in a circulating solution: NaCl 120 mM, KCl 5 mM, $CaCl_2$ 6 mM, $MgCl_2$ 3.5 mM; pH 7.5-7.9. Recording of intracellular potentials, transmission of the polarizing current, and cAMP intracellular injection were carried out with a multibarreled microelectrode. The microelectrodes were assembled from semi-finished seven-barreled products of the WPI firm (USA). The recording barrel was filled with a 2 M sodium citrate solution. The resistance was 5-15 mOhm. The same solution was in the barrel for the polarizing current and the barrel serving as an indifferent electrode during the intracellular microionophoresis. The barrels were filled with a 0.1 M

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solution of cAMP sodium salt (Serva, Germany). cAMP intracellular injection was carried out by a 5-40 nA current passing for 1-10 sec between the cAMP barrel and the indifferent barrel. The "blocking" current strength was 2-4 nA.

RESULTS

The intracellular injection of cAMP into the B_4 and F neuron (n=8) caused a rapid and reversible membrane depolarization. In 5 out of 8 cases the repetitive cAMP injections led to the generation of cAMP responses with an increased amplitude, that is the effect of frequency potentiation of the cAMP responses (reported earlier in detail [2]) was observed. When the intervals between the injections exceeded 5 min, the amplitude of the cAMP responses did not change noticeably during several hours of the experiment.

Our experiments did not reveal a dependence of the cAMP response amplitude on the preliminary generation of action potentials (AP). Thus, our results differ from those obtained by N. I. Kononenko *et al.* [3, 7], who reported an increase of cAMP responses after the Ca^{2+} level rises in the neuron cytoplasm due to different methods, including AP generation.

In our experiments the cAMP response frequency potentiation effect was preserved during membrane hyperpolarization, when the cAMP-dependent depolarization did not reach the AP generation threshold. Switching off the hyperpolarizing current followed by an AP series did not alter the amplitude of the subsequent cAMP responses.

The above-mentioned results and the fact of the cAMP response frequency potentiation effect abolition for switching off the "blocking" current, which had been observed (though not in all the neurons) in previously reported experiments [2], made it possible to assume that the factor associated with the enhanced leakage of cAMP from the microelectrode during the following injections is instrumental in the cAMP responses frequency potentiation [1, 4].

To check this hypothesis we conducted experiments on a comparative study of the cell responses to frequency injection of cAMP from two different barrels of the same multibarreled microelectrode. If the investigated frequency potentiation effect of the cAMP responses is indeed of microelectrode origin and is associated with the enhanced cAMP leakage from the microelectrode barrel, the exchange of the barrels during the frequency injection should lead to the abolition of the potentiation effect. And, *vice versa*, the cAMP response dynamics will not depend on which of the microelectrode barrels is applied for cAMP injection when the studied effect is of physiological origin.

The experiments with cAMP injection from two barrels of the multibarreled microelectrode did not yield evidence for assuming a physiological nature of the cAMP response frequency potentiation phenomenon. The exchange of microelectrode barrels during the frequency injection led to the abolition of the potentiation effect in all the cases studied (n=4).

The results of one of the experiments are presented in Fig. 1. The upper part of the figure shows a single response to cAMP injected from the second microelectrode barrel. It can be seen that after the cAMP response potentiation development caused by frequency injection of the substance from the first

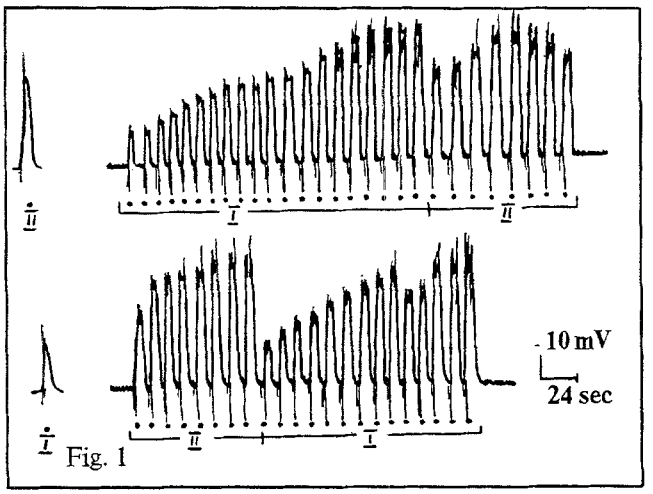

Fig. 1. Effect of cAMP response potentiation during cAMP injection from two barrels of a multibarreled microelectrode.
I) first barrel, II) second barrel of microelectrode. Injection current strength 20 nA, duration 1 sec. the cAMP injection are noted by points. MP=-90 mV; cell of the F group.

barrel, cAMP injection from the second barrel does not cause a potentiatied response. To develop potentiation of the responses to cAMP from the second barrel, frequency injection of the substance from this very barrel is required. The lower part of the Fig. 1 shows the same result, but with a different order of involvement of microelectrode barrels.

The results give evidence that the microelectrode factor, associated with the quantity of substance leaking from the microelectrode during the repetitive injections makes a significant contribution to the development of the cAMP response frequency potentiation effect caused by microionophoretic injections of cAMP to the cell.

Our study shows for the first time the pronounced influence of the microelectrode-dependent factor on the electrophysiological responses to cAMP dynamics. There have been reports of similar effects of a number of physiologically active substances. Thus, for example, prolonged ""heating-up" of the application micropopette tip is characteristic for the case when it is filled with norepinephrine, but is weakly expressed when it is filled with GABA [1].

This conclusion is proved by our observations confirming that frequency potentiation is uncharacteristic for the exciting responses to acetylcholine, applied with the aid of microionophoresis on the external surface of snail neurons (unpublished data). The question as to what features of the molecules of the substance excreted from the micropipette are responsible for this microelectrode factor remains open.

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PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY

Thermo-Induced Structural Rearrangements of Platelet Membranes by the Action of an Inductor and Inhibitors of Aggregation.

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An universal property of biological membranes is their capacity for structural rearrangements [2]. The structural rearrangements occurring in the plasma membrane reflect the intracellular metabolic processes. In studies of the effect of inhibitors and inductors of aggregation on the spectrum parameter S it was found by several authors that ADP and aspirin decrease, while α-tocopherol (vitamin E) increases the value of this parameter [6,8]. The variations under constant temperature reveal no distinctions between inductor and inhibitor actions on the membrane.

In this study an attempt was undertaken to demonstrate the generalities as well as the features inherent in the action of these compounds.

MATERIAL AND METHODS

In the present study acetylsalicylic acid (aspirin), α-tocopherol (vitamin E), and ADP (Sigma) were used.

As a hydrophobic spin probe for platelet membrane testing the stearic acid derivative 5-doxyl stearate was used at the final concentration 10^{-4}M. EPR spectra were recorded with a Varian E-104 radiospectrometer equipped with a variable temperature accessory at 10 mW microwave power, 1,6 G modulation amplitude and 100 G/8 min scan time. The temperature of the sample was controlled by a thermocouple within 0,5 °C.

Figure 1 shows a typical spectrum from this probe in the platelet membrane. This spectrum is characteristic of the anisotropic motion of the spin probe incorporated into the lipid bilayer of the platelet membrane. To

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