ROLE OF cAMP IN REGULATION OF HIPPOCAMPAL UNIT ACTIVITY *IN VITRO*

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An important role in the regulation of reactivity of nerve cells is nowadays ascribed to cyclic nucleotides and, in particular, to cAMP [13]. According to one hypothesis [10, 11], the electrical response of neurons to certain mediators is formed with the participation of cAMP as secondary messenger. The intracellular cAMP level is known to be controlled by the activity of two enzyme systems: adenylate cyclase and phosphodiesterase (PDE). Inhibition of PDE, an enzyme inactivating cAMP, leads to accumulation of the latter in neurons.

This hypothesis was based on the results of biochemical investigations and also on data obtained on peripheral synapses. The question of the role of the adenylate cyclase system in the work of synapses in central structures of the mammalian brain has not yet been adequately studied. It is thus interesting to discover whether the adenylate cyclase system participates in regulation of activity of hippocampal synaptic systems and, in particular, how cAMP accumulation influences reactivity of hippocampal neurons.

EXPERIMENTAL METHOD

Experiments were carried out on surviving hippocampal slices of C57BL/6 mice by the method described previously [1, 14]. After decapitation of the animal and removal of the upper cranial bones, transverse slices were cut through the hippocampus. The slices were placed in an experimental chamber through which balanced Simms' salt solution saturated with a gas mixture containing 95% O_2 and 5% CO_2, and heated to 33-34°C, was passed.

Bipolar glass stimulating electrodes, filled with Simms' solution, were introduced into the radial layer, in which lie Schaffer's collaterals running from neurons of area CA_3 to neurons of area CA_1, where the recording glass microelectrode filled with 2 M potassium citrate solution was located (Fig. 1a).

The evoked response began to be recorded 1 h after cutting of the slice, when its electrophysiological parameters had stabilized. Rhythmic pulses of current of between 10 and 50 V, 0.2 msec in duration, with a frequency of 0.2 Hz were used for stimulation. The strength of stimulation was chosen so as to evoke a population spike (PS), which is a synchronized discharge of pyramidal neurons in area CA_1 (Fig. 1b), whose amplitude reflects reactivity of this particular hippocampal synaptic system [7].

To raise the cAMP level in the slices dibutyryl-cAMP (DB-cAMP), an analog of cAMP distinguished by its ability to pass freely through cell membranes, and 8-(C_1-acetylaminoethylthio)-cAMP, a synthetic structural analog of cAMP [2], which is an irreversible PDE inhibitor which passes sparingly through cell membranes, were used. The substances were added to the incubation solution in a concentration of 10^{-4} M. The data were recorded and processed on a PDP-8a minicomputer by programs compiled by A. G. Gusev [3].

EXPERIMENTAL RESULTS

It will be clear from Fig. 2 that addition of the PDE inhibitor to the solution surrounding the slice caused a gradual increase in amplitude of PS. When the slice was rinsed with standard Simms' solution, the amplitude of the response decreased a little, although it still remained higher than initially. Repeated addition of the inhibitor again increased the re-

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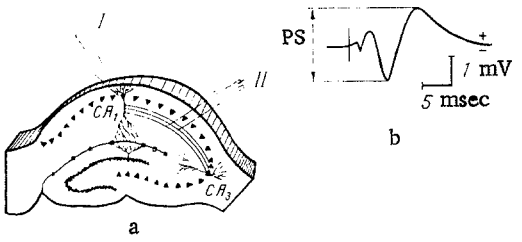

Fig. 1. Diagram of experimental arrangements: a) diagram of hippocampal slice; I) recording electrode located in cellular layer of area CA_1, II) stimulating bipolar electrode located in region of Schaffer's collaterals running from neurons of area CA_3; b) form of response of area CA_1 cells evoked by stimulation of Schaffer's collaterals: PS) amplitude of PS.

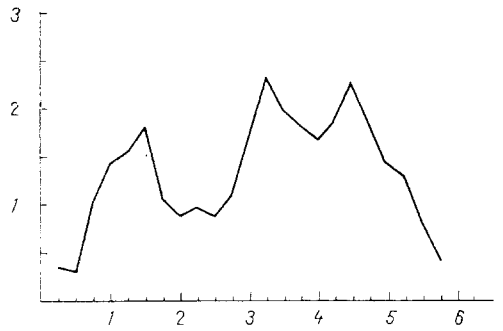

Fig. 2. Changes in amplitude of PS under the influence of PDE inhibitor (10^{-4} M). Peaks correspond to addition of substance to incubation solution. Abscissa, time of observation (in h); ordinate, amplitude of PS (in mV).

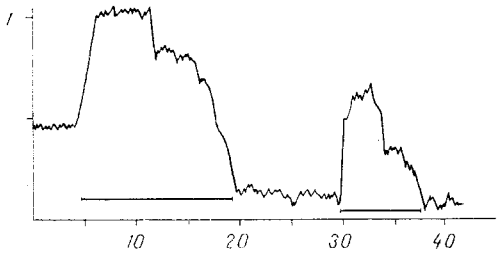

Fig. 3. Changes in amplitude of PS under the influence of DB-cAMP (10^{-4} M). Horizontal lines denote addition of substance. Abscissa, time of observation (in min); ordinate, amplitude of PS (in mV).

sponse, and this effect continued during subsequent rinsing of the slice; this may be evidence of irreversible binding of the inhibitor with the enzyme.

Under the influence of DB-cAMP, the response increased rapidly and then decreased and disappeared completely (Fig. 3). In some cases, only the phase of depression of PS was observed.

According to data in the literature, obtained both on molluscan [4, 6] and mammalian [9] neurons, intracellular injection of cAMP itself usually leads to increased conductance of the membrane and depolarization of the neuron. Meanwhile, experiments in which the cAMP level in the cells was raised with the aid of PDE inhibitor (theophylline, for example) showed that after addition of the inhibitor in a low concentration, activation of the neuron was observed,

whereas an increase in the concentration of the substance inhibited unit activity [5]. These changes were reversible in character. A distinguishing feature of the PDE inhibitor which we used is that it binds irreversibly with the enzyme, and this, combined with the poor penetration through the cell membrane, should give rise to a slow but permanent rise in the intracellular cAMP level. Such a gradual increase in the cyclic nucleotide concentration within the neuron probably leads to moderate activation of phosphorylation of membrane proteins and increased excitability of the membrane, whereas DB-cAMP, added in the same concentration, but passing readily through the membrane, evidently causes a sharp increase in the intensity of phosphorylation of proteins, including membrane proteins, with the probable result of rapid depolarization of the neuron, accompanied by inactivation of the spike generating mechanism.

It can be postulated on the basis of these results that the secondary messenger system, which is the role played by cAMP, participates in the regulation of hippocampal unit reactivity. Changes in PS under the influence of PDE inhibitor are similar in their parameters to changes observed during prolonged post-tetanic potentiation [12, 14], characteristic of hippocampal synaptic systems. This suggests that the adenylate cyclase system participates in the realization of this phenomenon, and experimental confirmation of this hypothesis is required.

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