DYNAMIC CHANGES IN RESPONSES OF MOLLUSCAN NEURONS TO REPEATED INJECTIONS OF CYCLIC AMP

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Cyclic AMP (cAMP) is regarded as an important link in synaptic transmission, for it may be involved as intracellular intermediary in the generation of electrophysiological responses of neurons to some mediators [12, 14]. This view is confirmed by results indicating the ability of cAMP, when injected into a neuron, to evoke an electrophysiological response. These experiments can be undertaken particularly effectively on large molluscan neurons, in which a number of different workers have demonstrated the appearance of both rapid and reversible depolarization, and hyperpolarization, after intravenous injection of cAMP, and under voltage clamp conditions they have demonstrated inward and outward currents respectively [2, 3, 7-11, 13].

The investigation described below shows that after injection of cAMP into *Helix pomatia* neurons aftereffects develop, which persist for several minutes, and are manifested as a change in amplitude of responses of the neurons to the next injections of cAMP.

EXPERIMENTAL METHOD

Experiments were carried out on large neurons of active snails in December to April. The neurons were identified in accordance with Sakharov's classification [5]. Isolated nerve ganglia of *H. pomatia* were placed in a chamber with a volume of 3 ml containing continuously flowing Ringer's solution of the following composition (in mM): NaCl 120, KCl 5, $CaCl_2$ 6, $MgCL_2$ 3.5; the pH was adjusted to 7.5-7.9 with Tris-HCl. Four- and five-barreled glass microelectrodes with a resistance of the recording barrel of 5-15 MΩ were used. Three barrels of the microelectrode, which served to record potentials, to pass the polarizing current, and as reference electrode for intracellular microiontophoresis, were filled with 2 M potassium citrate solution. The two remaining barrels were filled with 0.1 M solution of the sodium salt of cAMP (from "Reanal," Hungary) and a 0.1 M solution of the disodium salt of 5'-AMP (also from "Reanal"). Microelectrodes with low resistance of connection between the barrels were used in the experiments. The intracellular injection of the various drugs was given by passing a current of 5-40 nA and duration of 1-10 sec between the barrel filled with cAMP or 5'-AMP and the reference barrel. The strength of the blocking current was 5 nA.

EXPERIMENTAL RESULTS

On virtually all identified nerve cells and also large unidentified neurons of *H. pomatia* microiontophoretic injection of cAMP caused membrane depolarization with a latent period of 0.1-2 sec; the time to reach a maximum was 2-30 sec and a total duration 10-60 sec.

The amplitude of depolarization to cAMP was increased on hyperpolarization of the membrane. Under these circumstances the reversal potentials of the cAMP responses differed considerably in different neurons and varied from +10 to −30 mV. The amplitude of the cAMP responses was estimated within the range of values of membrane potential (MP) that corresponded to the linear portion of the current-voltage characteristic curve of the membrane (from −30 to −80 mV). Reversal potentials were determined by extrapolation.

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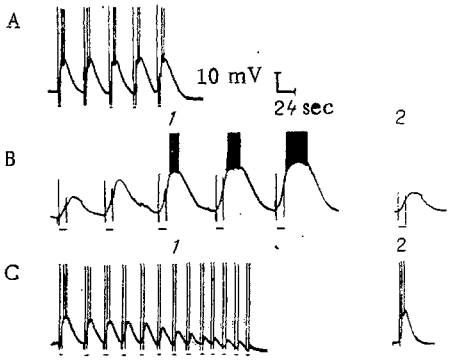

Fig. 1

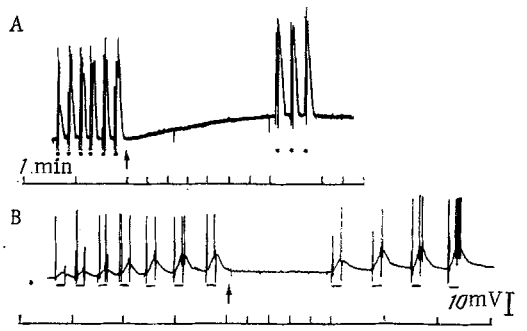

Fig. 2

Fig. 1. Responses of neurons to repeated injections of cAMP. A) Neuron V_4: MP = −52 mV, amplitude of responses to repeated injections unchanged; B) neuron RPa_2: MP = −50 mV, amplitude of responses to repeated injections of cAMP rises. Interval of 6 min between traces 1 and 2; C) neuron from F group: MP = −55 mV, amplitude of responses to repeated injections of cAMP decreases. Interval between traces 1 and 2 is 5 min. Duration of injection of cAMP indicated by marks below traces. Strength of injection current: A) 25 nA, B) 15 nA, C) 20 nA.

Fig. 2. Responses of neurons to cAMP after switching off retaining current. A) Neuron V_4: MP = −80 mV, amplitude of responses to repeated injections of cAMP rises, i.e., potentiation of cAMP responses is observed. Strength of injection current 20 nA, duration 2 sec. Arrow indicates time of switching off retaining current. Marked membrane depolarization and an increase in amplitude of cAMP responses are observed next, with no potentiation of the latter; B) neuron RPa_2: MP = −50 mV, amplitude of responses to repeated injections of cAMP rises. Strength of injection current, no membrane depolarization is observed and potentiation of cAMP responses is preserved. Points on A and straight lines on B denote time of cAMP injection. Time calibration signal shown below traces, varying in the course of the trace.

If 5'-AMP was injected into the *H. pomatia* neurons under the same conditions as cAMP, it caused no change in MP of the neurons.

Repeated injections of cAMP into the same cell at intervals of not less than 5 min showed that responses to cAMP are completely reproducible and remain unchanged in amplitude throughout the experiment (for several hours). Only in a few experiments under the above conditions was a gradual increase in the duration of the trailing edge of the response observed. With shortening of the intervals between injections to 30 sec, no marked changes in amplitude of cAMP-depolarization due to repeated injections could be observed in most experiments (n = 40; Fig. 1A).

Meanwhile in 15 experiments (cells RPa_1, RPa_2, LPa_3, V_4, V_2, F) it was found that if cAMP was injected at intervals shorter than 5 min the amplitude of responses to the repeated injections was not equal to the amplitude of responses to previous injections of cAMP. Each subsequent injection of cAMP evoked a response which exceeded its predecessor in amplitude and duration. The response to cAMP reached a definite maximum after 5-10 injections, and remained unchanged during subsequent injections (Fig. 1B). In some cases a 10-15-fold increase in amplitude of membrane depolarization could be observed in response to repeated injections of cAMP.

The effect of the preceding injection of cAMP on the succeeding cAMP response gradually weakened with an increase in the interval between injections and disappeared completely after 3-6 min.

Injection of cAMP into identified neurons of different preparations did not always cause potentiation of cAMP responses. In neuron V_4, for instance, a potentiation effect was observed in two of three preparations, in neuron LPa_3 in two of four preparations, and so on.

In four experiments (cells V_2, F) an effect opposite to that described was found, namely, a gradual decrease in amplitude of the cAMP responses with intervals of less than 5 min between injections (Fig. 1C).

The question arises whether the effect of a change in cAMP responses during repeated injections observed in these experiments is a physiological phenomenon or is connected with expulsion of a different dose of cAMP from the microelectrode. This latter possibility is indicated by data showing the effect of a retaining current on the quantity of substance expelled from the microelectrode [1, 4].

To answer this question experiments were carried out in which the retaining current was switched off. Under these circumstances (depending on the thickness of the microelectrode tip) the experimental results were distributed into two groups. In the first group of experiments (three cases) switching off the retaining current led to marked membrane depolarization, evidently due to leakage of cAMP from the microelectrode and its accumulation in the cell. In these cases the amplitude of responses to cAMP was much higher than initially, and effects of potentiation of cAMP responses were not observed (Fig. 2A). In the second group of experiments (two cells) switching off the retaining current did not lead to membrane depolarization, evidence that significant diffusion of cAMP from the microelectrode into the cell did not occur. In these experiments effects of potentiation of cAMP respones were preserved (Fig. 2B).

The last observations indicate that effects of potentiation of cAMP responses can develop even in the absence of a retaining current. Abolition of these effects when the retaining current is switched off in cells where the intracellular cAMP level was considerably increased as a result of leakage of the latter from the microelectrode can be explained, in our view, by an increase in the total dose of cAMP or by a change in activity of cAMP-sensitive enzymes in the cell. The control thus provides evidence in support of the physiological nature of the phenomenon observed. Other observations also support this view, such as recording of both the development of potentiation of cAMP responses during repeated injections by the same microelectrode, and its absence in different neurons.

However, the fact that during successive injections different quantities of the substance may be expelled from the microelectrode, resulting in responses of different amplitude, seems to us to be not completely ruled out. Quite possibly both factors (physiological and microelectrode) participate in the genesis of this phenomenon.

The most natural physiological explanation of the increase in amplitude of cAMP responses observed in most experiments with short intervals between injections is accumulation of cAMP in the cell because of its incomplete decomposition by phosphodiesterase. The residual amount of cAMP is below the threshold for an electrophysiological response and is preserved for several minutes. If during this time interval cAMP is again injected, its total quantity will be more than that at the previous injection, and it will evoke an electrophysiological response with higher amplitude.

The existence of other possible mechanisms of the phenomenon we are discussing can be postulated. For instance, it is shown in [13] that the cAMP response of *H. pomatia* neurons contains a Ca-dependent component. Meanwhile, according to data obtained by the same workers [2], an increase in the intracellular Ca^{++} concentration has a potentiating effect on cAMP responses. In our view these data can be used to explain the effect of autopotentiation of cAMP responses observed in our own experiments.

Reductions in amplitude of cAMP responses observed in four of our experiments during repeated injections may conjecturally be explained by the ability of cAMP-dependent structures in these cells to undergo desensitization.

Despite the abundance of the literature on intracellular injection of cAMP into molluscan neurons, we could find no description of such effects of potentiation or depression of cAMP responses during repeated injections. It can be tentatively suggested that the authors cited used longer intervals between injections of cAMP or worked with cells in which this effect is manifested only weakly.

The phenomenon of after-activation or deactivation of the cAMP system, which has recently been found, may in our opinion be of great physiological importance. These effects may be involved in processes of a transient increase in the efficiency of synaptic transmission during intensive functions of the synapse. This hypothesis is based on steadily ac-

cumulating data on mediation of several neurotransmitter responses by cAMP [12, 14]. Two such neurotransmitters for *H. pomatia* are serotonin and dopamine [10], Further confirmation of this hypothesis is given by data on the ability of certain *H. pomatia* neurons to increase or reduce the amplitude of their responses to serotonin if it is applied repeatedly [6].

LITERATURE CITED

1. A. A. Aleksandrov, The Technique of Microelectrophoresis in Physiology [in Russian], Leningrad (1983).
2. N. I. Kononenko and A. D. Shcherbatko, in: Neurochemical Mechanisms of Regulation of Memory [in Russian], Pushchino (1984), p. 5.
3. E. A. Liberman, S. V. Minina, and N. E. Shklovskii-Kordi, Biofizika, 27, 542 (1982).
4. R. Pervis, Microelectrode Methods of Intracellular Recording and Iontophoresis [Russian translation], Moscow (1983).
5. D. A. Sakharov, The Genealogy of Neurons [in Russian], Moscow (1974).
6. E. I. Solntseva and L. V. Bezrukova, Dokl. Akad. Nauk SSSR, 256, 1499 (1981).
7. J. B. Aldenhoff, G. Hofmeier, H. D. Lux, et al., Brain Res., 276, 289 (1983).
8. P. Deterre, D. Paupardin-Tritsch, J. Bockaert, et al., Nature, 290, 783 (1981).
9. A. H. Drummond, J. A. Benson, and I. B. Levitan, Proc. Nat. Acad. Sci. USA, 77, 5013 (1980).
10. A. H. Drummond, Adv. Cycl. Nucl. Res., 15, 373 (1983).
11. R. Gilette and D. J. Green, Brain Res., 273, 384 (1983).
12. P. Greengard, Nature, 260, 101 (1976).
13. N. I. Kononenko, P. G. Kostyuk, and A. D. Shcherbatko (A. D. Scherbatko), Brain Res., 268, 321 (1983).
14. J. L. Nathanson, Physiol. Rev., 57, 157 (1977).

CAUSES OF DISTURBANCE OF FATTY ACID OXIDATION IN ISOLATED MITOCHONDRIA OF THE ISCHEMIC HEART

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Activation of respiration of cardiac mitochondria (Mc) in state 3 with succinate by cytochrome c is considerably enhanced during ischemia [1]. Cytochrome activated oxidation of glutamate + malate is enhanced more than twofold during ischemia, but no activating effect of cytochrome c on fatty acid (FA) oxidation could be observed [7]. Activation of oxidation of pyruvate + malate (P + M) by cytochrome c was enhanced during ischemia by a greater degree than oxidation of 3-hydroxybutyrate [2]. These data show that cytochrome c did not activate respiration during ischemia, or activated it less, only in medium without malate or succinate. However, these dicarboxylates had no significant effect on FA oxidation under control conditions [6]. Inhibition of oxidation of palmitate was actually observed in another study [5] both in the control and during ischemia.

To examine this problem it was decided to study the effect of cytochrome c on FA oxidation under control and ischemic conditions in medium with and without malate. Depression of carnitine acyltransferase activity, which is observed even in the early stages of ischemia [8], may play an essential role in the disturbance of FA oxidation in ischemia. It has been suggested that injury to the outer membrane of Mc in ischemia can lower not only the cytochrome c content in Mc [1], but also external carnitine acyltransferase activity, and this fall must lead to a decrease in the ratio between velocities of oxidation of palmitoyl-CoA

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