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Unusual biochemistry of changes in neuron membrane permeability evoked by cAMP

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Influence of different metabolic poisons on cAMP-evoked neuron membrane permeability is investigated. Drugs preventing cAMP binding with R subunits of protein kinase decrease the cAMP-evoked current, but the inhibitor of the C subunit, H8, has no effect. The cAMP-dependent current is increased by uncouplers and decreased by inhibitors of glycolysis and oxidative phosphorylation. The mechanism of cAMP action on neuron permeability is discussed.

cyclic AMP; Neuron permeability; Protein kinase; Glycolysis; Oxidative phosphorylation; (*Helix lucorum*)

1. INTRODUCTION

It was shown that intracellular injection of cyclic AMP evoked a generator potential [1] increasing Na^+ permeability and decreasing K^+ permeability [2]. It was generally believed that cAMP changes neuron activity by way of protein kinase activation and neuron membrane protein phosphorylation. However, the C subunit of protein kinase did not imitate the cAMP-dependent increase in Na^+ permeability [3,4] described by us. The delay of the cAMP response is so short that cAMP cannot be transported during this time from the electrode tip placed at the neuron center to the neuron membrane with the usual diffusional process [5]. Experiments described in this paper show that the biochemistry of the cAMP-dependent system controlling generator potential is not usual.

2. MATERIALS AND METHODS

Experiments were performed on neurons of suboesophageal ganglia of the land snail, *Helix lucorum*. The ganglia were isolated and placed in bath solution containing 80 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM Tris-HCl buffer (pH 7.5). Neurons were impailed by 3 microelectrodes filled with testing drugs such as 0.1 M adenosine 3',5'-cyclic monophosphate (cAMP), 0.5 M ATP, 0.1 M N^6,O^2-dibutyryl adenosine 3'-5'-cyclic monophosphate (dibutyryl cAMP), 0.3 M tolbutamide, 10 mM *N*-[2-(methylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H8), 0.25 mM TTFB, 0.3 M iodoacetate, 0.3 M 6-deoxy-D-glucose.

Some drugs were added to the bathing solution in the following final concentrations: 1 mM sodium metavanadate, 1-5 mM potassium arsenate, 0.06-0.12 M dinitrophenol (DNP), 20 μg/ml oligomycin, 10 μg/ml antimycin A, 10^{-8} M *p*-trifluoromethoxycarbonyl cyanide phenylhydrazone (FCCP), 4×10^{-9} M 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile (SF 6847), 6×10^{-6} M rotenone, 10 mM sodium azide.

Measurement of the neuron electric activity and intracellular injection were made with a Nova 3D computer as described previously [6]. Computer simulation of cAMP-evoked current was achieved as shown in [7].

3. RESULTS AND DISCUSSION

The dibutyryl analog of cAMP penetrating through a cell membrane is often used to study the cAMP effect. However intraneuronal injection of this drug did not cause the quick ionic current as in the cAMP case (fig.1A). When there are two electrodes in the neuron, one with cAMP and the other with dibutyryl cAMP, the neuron response to

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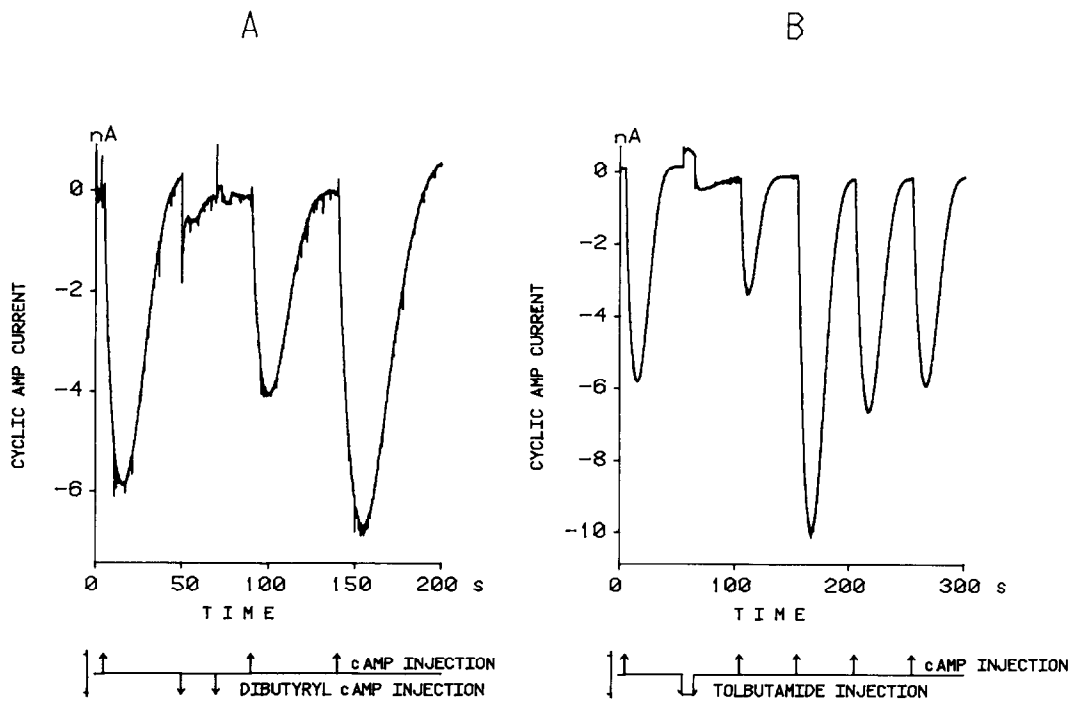

Fig.1. Change of cAMP-evoked input current after injection of drugs influencing cAMP binding to protein kinase. Duration of intracellular injection with pressure pulses is represented with arrows: up one for cAMP and down one for dibutyryl cAMP (A) and tolbutamide (B).

cAMP decreased if dibutyryl cAMP injection was made 50 s before. After 100 s the value of cAMP current became normal. The dibutyryl derivative of cAMP binds with the R subunit of the cAMP-dependent protein kinase without activation of the enzyme and in this way it could prevent cAMP action.

A decrease of the cAMP effect is also observed after injection of tolbutamide that decreases protein kinase activation by cAMP preventing cAMP interaction with the R subunit [8]. Fig.1B shows that tolbutamide evoked a temporary decrease of the cAMP current when cAMP was tested some seconds after the inhibitor injection. Unexpectedly, at the second injection, the value of the cAMP effect not only became normal but rose above the initial value.

A strong inhibitor of protein kinases, H8, prevents interaction of the C subunit of protein kinase with ATP [9]. However, we could not observe any effect of H8 on the cAMP current. Nevertheless we suggested that the interaction of cAMP with the R subunit of protein kinase is a necessary step in cAMP-dependent current generation.

cAMP-dependent protein kinase (R_2C_2) has 4 binding sites for cyclic nucleotide molecules (two sites on each R subunit) [10]. Computer simulation of a cAMP-evoked neuron membrane current [7] allows one to determine the number of cAMP molecules needed to bind with the holoenzyme in order to evoke the neuron electric response (fig.2). When cAMP is injected by pressure the initial cAMP concentration is large and the cAMP current increase does not depend on binding site number (fig.2a,b).

During iontophoretic injection the cAMP concentration grows slowly and there is a big difference between curves obtained for cases with four binding sites and those with one (fig.2). In the first case the current increases slowly but decreases quickly. The kinetics of the cAMP response are the same as those shown in experiments with iontophoretic injection of this nucleotide [6].

This mathematic model takes into account the diffusion of cAMP from the microelectrode tip

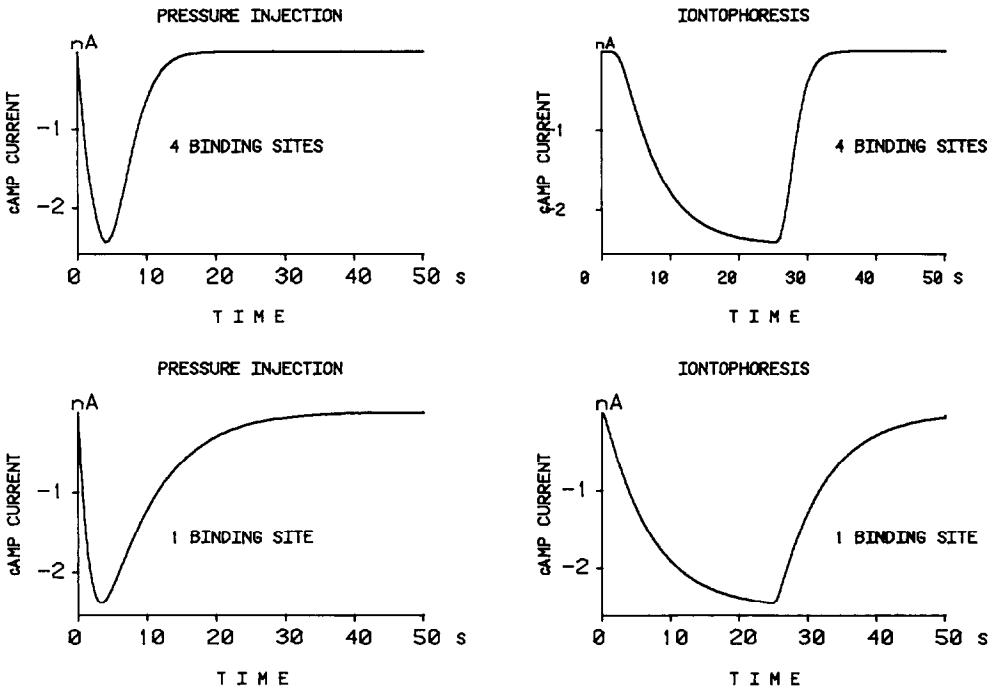

Fig.2. Dependence of the shape of a computer simulated cAMP current on the kinetics of the cAMP interaction with protein kinase bound to MAP2. cAMP injected by pressure or current and enzyme has four or one binding sites.

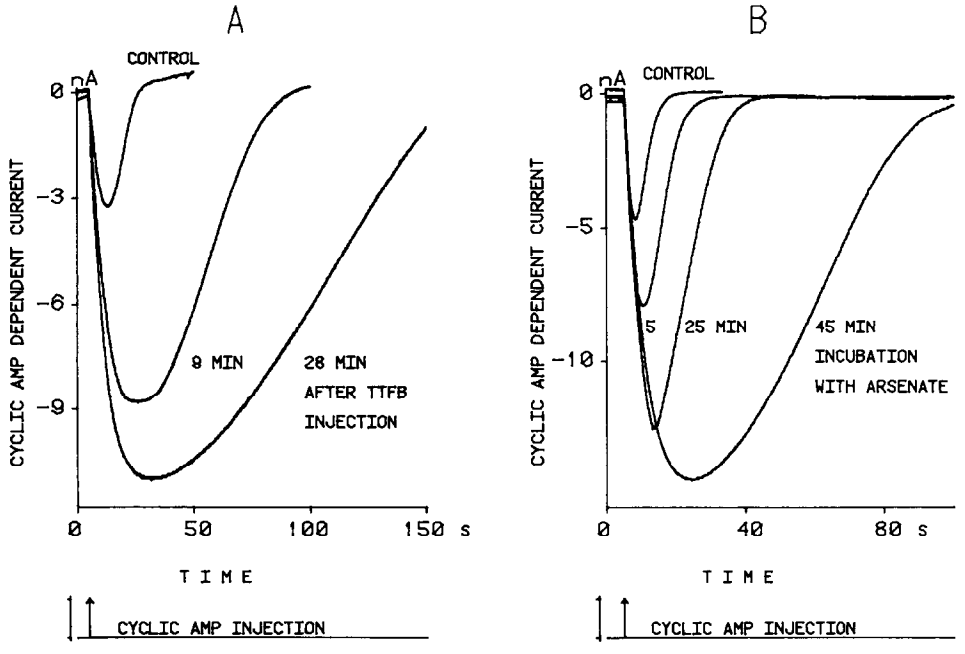

Fig.3. Influence of uncouplers on the cAMP effect. (A) Increase in the cAMP current 9 and 28 min after TTFB intracellular injection. (B) Increase in the cAMP current 5, 25 and 45 min after addition of 5 mM arsenate to the bathing medium.

over the entire cell volume in which R subunits of protein kinases are uniformly distributed. In the neuron, a considerable proportion of protein kinase is bound to microtubule-associated protein MAP2 [11]. We supposed [2] that the cAMP-dependent generator potential was evoked by the interaction of cAMP with R subunits of this enzyme. This suggestion was confirmed by data concerning a very short delay in the cAMP response [5,6] and a decrease of the response in the presence of microtubular-destroying agents [2]. But the experiments with H8 and the following data show that protein phosphorylation is not necessarily an essential step in the cAMP-evoked neuron response.

Uncouplers of oxidative phosphorylation stop ATP synthesis and accelerate its hydrolysis. Physical uncouplers, proton carriers [12] and calcium ions, decrease the membrane potential of mitochondria. An addition of these drugs (DNP, TTFB, SF 6847, FCCP) to extraneuronal medium or their intracellular injection increased the cAMP response (fig.3A and [13]).

Physical uncouplers do not affect glycolysis. However, as shown in fig.3B, arsenate, the chemical uncoupler of glycolysis and oxidative phosphorylation, increased the cAMP response most effectively. By the method of penetrating ions [12] we observed that arsenate diminished the membrane potential of mitochondria up to a level produced with ADP. Thus, in the presence of arsenate mitochondria maintain the membrane potential and, consequently, the intracellular calcium concentration increases less than in the presence of the physical uncouplers. The increase of the amplitude of the cAMP current in the presence of arsenate was also observed when an EGTA-Ca^{2+} buffer supporting an intracellular concentration of free calcium ions was injected into the neuron [2].

Inhibitors of glycolysis and oxidative phosphorylation also decrease the level of ATP in a cell.

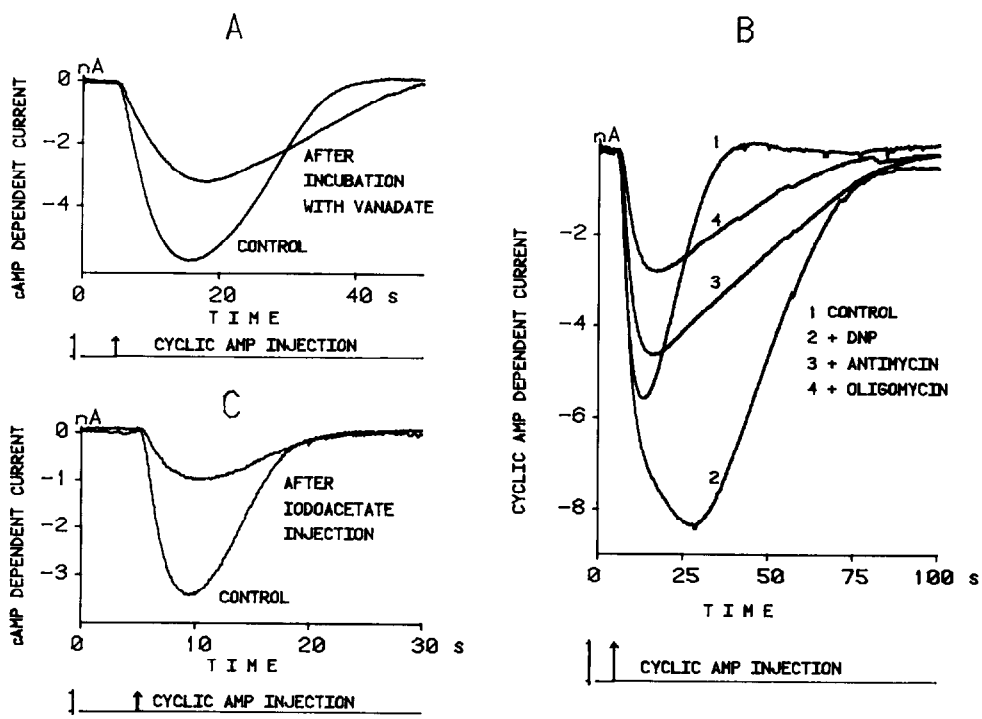

Fig.4. Effect of different inhibitors of oxidative phosphorylation and glycolysis on the cAMP current. (A) Decrease of this current after 20 min incubation of ganglia with 1 mM vanadate. (B) The cAMP current was initially increased by 0.06 mM DNP, then antimycin A (10 μg/ml) and oligomycin (20 μg/ml) were consequently added to the experimental bath. (C) The cAMP current was diminished by intracellular iodoacetate injection.

Iodoacetate, 6-deoxy-D-glucose (inhibitors of glycolysis) and rotenone, antimycin A, azide, vanadate (inhibitors of oxidative phosphorylation) decreased the cAMP-evoked current (fig.4). It was reduced most effectively when inhibitors of glycolysis and oxidative phosphorylation were added simultaneously. The effect of oxidative phosphorylation inhibitors was well defined after uncoupler action (fig.4B). Not only inhibitors of the respiratory chain but also ATPase inhibitor oligomycin decreased the cAMP current (fig.4B). Oligomycin worked even after inhibition of the respiratory chain, when mitochondrial ATPase acts as ATP-hydrolase.

The data described above show that the cAMP effect may not be connected with protein phosphorylation but requires a proceeding of the oxidative and glycolytic reactions which are accelerated by uncouplers and are retarded by the inhibitors of these processes. It is possible that cAMP interacts with R subunits bound to microtubular-associated proteins. As a result of this interaction a mechanical signal could arise [5,14]. This signal spreads through the cytoskeleton to the neuron membrane and causes opening of the Na^+ channels and closing of the K^+ channels. We think that the frequency of this signal is about 10^{10} Hz [5]. Thus energy is needed to prevent quick fading of the signal. If we presuppose that the energy released during metabolic reactions is used for this purpose, the increase in the cAMP response caused by uncoupler and the decrease of the cAMP response caused by inhibitors become clear [14].

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