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Mechanical influence and cAMP injection evoke the same reaction of neuron ionic channels

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Intracellular cAMP injection and negative pressure in the patch-electrode increase the interburst closed time of the same potassium ionic channels in the snail neuron membrane. Sodium channels which were registered as change of background noise are activated both by cAMP injection and by negative pressure. These results are considered in connection with data about the unusual biochemistry of the neuron reaction to cAMP.

Cyclic AMP; Cytoskeleton; Neuron ionic channel; Patch-clamp technique; *Helix lucorum*

1. INTRODUCTION

Cyclic adenosine 3′,5′-monophosphate (cAMP) intracellularly injected into snail neurons evokes a generator potential [1–4], increases Na^+ permeability and decreases K^+ permeability of the external membrane [3–7]. Some paradoxal features were shown to be inherent to the cAMP-dependent system controlling this generator potential [8–11]. Drugs which prevent cAMP binding with regulatory (R) subunits of the cAMP-dependent protein kinase decrease cAMP-evoked current, in spite of the fact that the inhibitor of catalytic (C) subunit, H8, has no effect. The cAMP-dependent current is increased by uncouplers and decreased by inhibitors of glycolysis and oxidative phosphorylation. These data show that the cAMP effect can hardly be explained simply in terms of protein phosphorylation, but rather is related in some way to the rates of the oxidative and glycolytic reactions. The cAMP effect proved to be similar to the neuron reaction to mechanical distension of the neuron [3–7,12] and is reduced by the microtubule-destroying agent nocodazole [6]. We supposed [4,10] that in the neuron, cAMP interacts with the R subunit of protein kinase bound to microtubule-associated protein [13]. As a result of this interaction, a mechanical signal should arise. This signal spreads through the cytoskeleton to the neuron membrane and causes opening of the channels for Na^+ ions and closing of those for K^+. The energy released during metabolic reactions is needed to prevent fast fading of this signal. Measurements of the electric activity of single neuron ion channels described in this paper are consistent with this hypothesis.

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2. MATERIALS AND METHODS

Experiments were performed on neurons of suboesophageal ganglia of the land snail, *Helix lucorum* (Fig. 1). The neurons were isolated and placed in a 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. The cell was impaled by 2 microelectrodes. One electrode filled with 0.1 M cAMP was used for the intracellular injection of this drug by pressure impulse and for the measurement of potential differences on the neuron membrane. The second microelectrode filled with 0.5 M ATP was used for neuron membrane polarization and fixation of the membrane potential. Intracellular cAMP injection, fixation of the neuron membrane potential and measurement of the total current through the cell membrane were managed with a computer as described previously [12]. The current of a single ion channel was registered with standard methods [14]. Pressure was applied to the patch pipette by syringe and was monitored with a pneumatic transducer, Sapfir. All data were recorded by computer Eclipse (Data General).

3. RESULTS AND DISCUSSION

When the patch pipette was filled with 84 mM KCl, 7 mM $CaCl_2$, 5 mM $MgCl_2$, 5 mM Tris-HCl buffer (pH 7.5) and the potential difference on cell membrane was −50 mV, an inward current was recorded. Fig. 2A shows this channel activity during cAMP injection (time of injection is indicated by arrow). Total current, recorded simultaneously, is shown in Fig. 2B. One can see a significant decrease in channel activity when the electrical effect on the whole cell membrane reaches its maximum.

The recording in Fig. 2C was made with the same patch after restoring of channel activity. At the moment marked by arrow, negative pressure (−15 mm Hg) was applied to the patch pipette. Pressure change caused a fast decrease in channel activity.

The slope conductance of these channels was 40 pS. Zero-current potential was about 0 mV. Channels displayed burst activity. Pressure change and cAMP injec-

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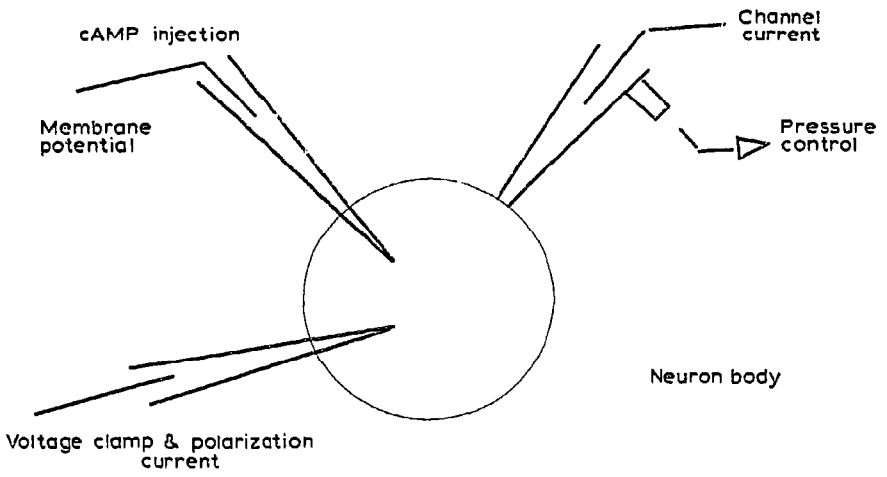

Fig. 1. The technique used for investigation of mechano- and cAMP-sensitive ion channels.

tion did not affect either mean open time or the closed time within the single bursts. Both cAMP injection and pressure increased the closed-state time between bursts.

Recordings were made after removing the patch from cell membrane (inside-out), when the inner side of the membrane was in extracellular solution containing 4 mM KCl. Under these conditions, a single channel current did not reverse up to 50 mV potential, showing ion selectivity of these channels for potassium. Channels in the excised (cell-free) patches did not display pressure dependence.

The fast fraction of cAMP-induced current is produced by the change in sodium permeation [4–6]. The same experiments as described above have been done with a patch pipette filled with extracellular solution for recording currents through sodium channels. But corresponding channels had too small an amplitude and a too short open-state time to be resolved with our technique. so that changes in their activity we recorded as changes in background noise. From the data recorded in this way, we came to the conclusion that the cAMP-dependent sodium channels are activated both by cAMP in-

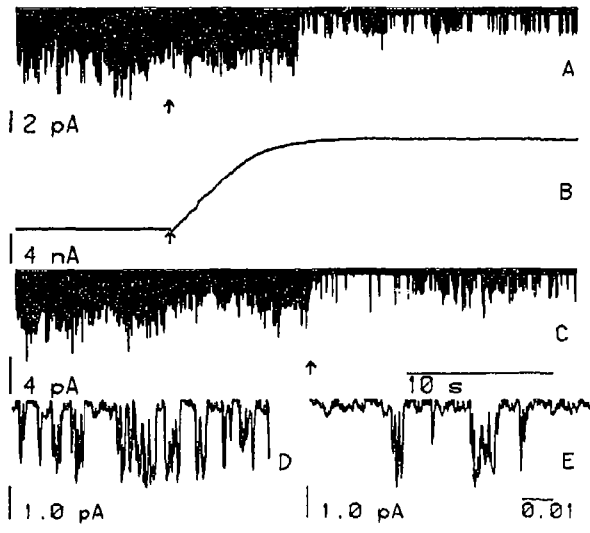

Fig. 2. Effect of intracellular cAMP injection (A) and negative pressure application to patch pipette (C) on a potassium channel. Whole cell current (B) recorded simultaneously with record (A). Arrows mark the time of cAMP injection and pressure application. Fragments of recording (C) before (D) and after (E) of pressure application.

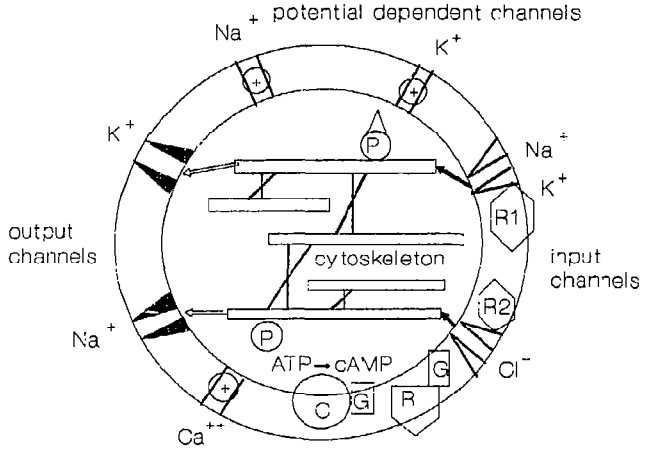

Fig. 3. Scheme of the input and output ion channels and the neuron calculating medium. Membrane input channels for Cl^-, K^+ and Na^+ are indicated as arrows directed inside the neuron. The open input channel (light arrows) sends a specific hypersound signal spreading through the calculating medium in the direction of the output Na^+ and K^+ channels (heavy arrows). Depending on the calculating medium structure, hypersound signals open or close output channels, causing neuron depolarization (generation potential) or hyperpolarization. The generator potential controls voltage-dependent Na^+, K^+ and Ca^{2+} channels, generating nerve impulses. The structure of the calculating media is created by the molecular computer of neuron [24]. This structure can be changed quickly by mediators which regulate the system of cAMP synthesis, that consists of a receptor (R), a regulatory protein (G), and adenylate cyclase (C).

jection and by application of negative pressure to the patch pipette.

Some influence of cAMP on single ion channel activity was described earlier [15–18]. Mechano-sensitive ion channels have also been found [19,20]. In this paper we present for the first time evidence on the existence of neuron ion channels which are sensitive to both cAMP and pressure change. Similarity of cAMP and mechanical effects is consistent with the idea of participation of mechanical processes in electrical response of the neuron to cAMP injection.

These data may be explained in the framework of a hypothesis about neuron as a quantum molecular computer [21]. We proposed [22] that the quantum computer of the neuron is organized by the cell cytoskeleton serving as a calculating medium and input ion channels sending a hypersound signal to observe this medium. The sound spreads over the medium passing via microtubules and microfilaments and switching between them via molecular bridges which serve as elementary switches. The whole system is assumed to work like a wave guiding net, connecting input ion channels (which generate different sound signals) and output ion channels (which are controlled by the processed sound signals). Thus the output of such systems depends on the input (controlled by synaptic activity) and on the construction and state of these calculating media (Fig. 3).

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