Cyclic Adenosine Monophosphate (cAMP)-Induced Potentiation of Synaptic Responses in Helix Neurons

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SUMMARY

1. The effect of intracellularly injected cAMP on the amplitude of excitatory postsynaptic potentials was studied using identified neurons of the snail Helix pomatia.

2. In 25% of the experiments, postsynaptic cAMP elevation caused a pronounced augmentation of the excitatory postsynaptic potential (EPSP) amplitude, lasting up to 15–30 min.

3. The results suggest that a cAMP increase in the postsynaptic neuron may be involved in the enhancement of synaptic efficiency.

Cyclic adenosine monophosphate (cAMP) is known to mediate different actions of neurotransmitters and hormones (Durmmond, 1983). Accumulating evidence suggests that cAMP is involved in the modulation of synaptic transmission (Shimahara and Tauc, 1978; Kandel and Schwartz, 1982; Occorr et al., 1985). As was shown for the Aplysia nervous system, short-term facilitation of synaptic transmission may be mediated by a cAMP increase in the presynaptic neurons resulting in the facilitation of transmitter release from the nerve terminals (Kandel and Schwartz, 1982). The influence of cAMP changes in the postsynaptic neuron on the efficiency of synaptic transmission has not yet been investigated. At the same time intracellularly injected cAMP has been shown to potentiate muscarinic responses in neuroblastoma cells (Tsunoo and Narahashi, 1987).

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The present study was undertaken to clarify whether an increase in the cAMP level in the postsynaptic neuron can modulate the amplitude of postsynaptic potentials. For this purpose we used the isolated CNS of a pulmonate mollusk, *Helix pomatia*. Molluscan neurons provide an excellent model for studying the cellular mechanisms of synaptic plasticity (Byrne, 1987), making it possible to use identified synaptic connections and allowing intracellular injections of substances thought to be involved in the modulation of synaptic efficiency.

The experiments were held on identified (Sakharov and Salanki, 1969) current-clamped neurons of the left parietal and visceral ganglia of the isolated CNS of *Helix pomatia*. The desheathed snail CNS was continuously bathed in saline of the following composition (mM): NaCl, 120; KCl, 5; MgCl₂, 3, 5; and CaCl₂, 6. The pH of the solution was adjusted to 7.5–7.8 with Tris·HCl. cAMP was injected intracellularly using the method of iontophoretic injections developed and introduced by Liberman *et al.* (1975). Seven-barreled microelectrodes (WPI, USA) were used, with three barrels filled with potassium citrate (2 M), the first one used for registration of the membrane potential, the second one for passing hyper- or depolarizing current through the cell membrane, and the third one as a compensation for microiontophoretic injections of cAMP and 5′-AMP. The remaining barrels were filled with 0.1 M sodium salt of cAMP (Sigma, St. Louis, Mo.) and 0.1 M sodium salt of 5′-AMP (Reanal). Both drugs were dissolved in deionized water. The resistance of the potassium citrate-filled barrels ranged from 10 to 15 MΩ. cAMP or 5′-AMP was injected iontophoretically by outward constant-current pulses of 2- to 15-sec duration with amplitudes ranging from 10 to 40 nA, the amplitude of the retaining current being 4 nA in all experiments. Synaptic activation was produced by anal nerve stimulation. Test stimuli were provided once in 5 min to prevent depression of synaptic potentials. Cells that did not display a stable excitatory postsynaptic potential (EPSP) amplitude were rejected. The neurons were usually hyperpolarized to -70 to -90 mV to prevent spontaneous spiking. Cell membrane resistance was periodically assessed by passing constant current pulses through a separate barrel of the microelectrode. The installation for the microelectrode investigations (Nihon Kohden) was used.

Stimulation of the anal nerve evoked monophasic EPSPs in LPa2, LPa3, V4, and F neurons and biphasic EPSPs in the V1 neuron. As the latency of the EPSPs did not change with increasing intensity of stimulation, we could conclude that at least the first component of the EPSPs was monosynaptic. After the 30-min control stimulation cAMP was injected. Intracellular injections of cAMP into the neurons tested invariably produced membrane depolarization (Fig. 1), which had a latency of 0.1–1 sec, a time of peak of 2–10 sec, and a duration of 10–30 sec. The extrapolated reversal potential of the cAMP response varied from -10 to -30 mV. A more detailed description of cAMP responses observed in our experiments is given elsewhere (Borisova and Solntseva, 1987). Injections of 5′-AMP under the same conditions were without effect. Our observations confirm the results of the initial experiments with multibarrel iontophoretic cAMP injections into *Helix* neurons which showed that cAMP had depolarizing effects (Liberman *et al.*, 1975). Similar data were obtained by other authors (Aldenhoff *et al.*, 1983; Konoenko *et al.*, 1983; Connor and Hockberger, 1984).