Cyclic Nucleotides Induce Long-Term Augmentation of Glutamate-Activated Chloride Current in Molluscan Neurons

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SUMMARY

1. Literature data indicate that serotonin induces the long-term potentiation of glutamate (Glu) response in molluscan neurons. The aim of present work was to elucidate whether cyclic nucleotides can cause the same effect.

2. Experiments were carried out on isolated neurons of the edible snail (Helix pomatia) using a two-microelectrode voltage-clamp method.

3. In the majority of the cells examined, the application of Glu elicited a Cl−-current. The reversal potential (E_r) of this current lied between −35 and −55 mV in different cells.

4. Picrotoxin, a blocker of Cl−-channels, suppressed this current equally on both sides of E_r. Furosemide, an antagonist of both Cl−-channels and the Na+/K+/Cl−-cotransporter, had a dual effect on Glu-response: decrease in conductance, and shift of E_r to negative potentials.

5. A short-term (2 min) cell treatment with 8-Br-cAMP or 8-Br-cGMP caused long-term (up to 30 min) change in Glu-response. At a holding potential of −60 mV, which was close to the resting level, an increase in Glu-activated inward current was observed. This potentiation seems to be related to the right shift of E_r of Glu-activated Cl−-current rather than to the increase in conductance of Cl−-channels. The blocking effect of picrotoxin rested after 8-Br-cAMP treatment.

6. The change in the Cl−-homeostasis as a possible mechanism for the observed effect of cyclic nucleotides is discussed.

KEY WORDS: cyclic AMP; cyclic GMP; potentiation; glutamate response; chloride current, molluscan neurons.

INTRODUCTION

Long-term potentiation (LTP) of glutamate (Glu) synapses is considered by many authors as a basis for learning and memory in animals of different species (Milner et al., 1998; Lewin and Walters, 1999; Lu et al., 1999; Chitwood et al., 2001; Antonov et al., 2003; Balaban et al., 2004). Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) play an important role in the mechanisms of this potentiation. It was shown that an increase in cAMP or cGMP level in postsynaptic...
neuron could cause the potentiation of Glu synapses in rat hippocampus (Milner et al., 1998; Yu et al., 2001; Lu and Hawkins, 2002).

The mechanism(s) of potentiation of Glu synapses in molluscan neurons evokes a lot of interest. Serotonin (5-HT), the neurotransmitter playing a critical role in behavioral sensitization, was found to cause long-term facilitation of Glu transmission in Aplysia and Helix neurons (Borisova and Skrebitsky, 1991; Milner et al., 1998; Chitwood et al., 2001; Antonov et al., 2003; Balaban et al., 2004). It was shown that mechanisms of this facilitation involve both the increase of neurotransmitter release from presynaptic terminal and the increase in sensitivity to Glu of postsynaptic membrane. At present, the explanations of the enhancement of Glu response during serotonin-induced facilitation is believed to be the insertion of additional AMPA-type Glu receptors into postsynaptic membrane (Chitwood et al., 2001). The participation of cAMP and cGMP both in presynaptic (Milner et al., 1998; Lewin and Walters, 1999; Antonov et al., 2003) and postsynaptic (Borisova and Skrebitsky, 1991) mechanisms of facilitation is well documented. However, the precise mechanisms of the cyclic nucleotides action on Glu response is poor understood.

Glutamatergic transmission in molluscan neurons has a number of characteristic features that make it different from this process in higher animals. In molluscan neurons, Glu can activate not only cation channels, but also anion (Cl\(^{-}\)) channels (Bolshakov et al., 1991; Dale and Kandel, 1993; Kehoe and Vulfius, 2000; Bravarenko et al., 2003). Glu-induced cationic current (\(E_r\) is close to 0 mV) causes an excitatory response (Dale and Kandel, 1993; Bravarenko et al., 2003), while the outward K\(^{+}\)-current (\(E_r = -85\) mV) hyperpolarizes the membrane and inhibits spike activity (Bolshakov et al., 1991). The \(E_r\) of Glu-activated Cl\(^{-}\)-current has been found to vary in molluscan neurons from \(-60\) to \(-41\) mV, and, therefore, this current can hyperpolarize or depolarize cell membrane, depending on whether \(E_r\) is negative or positive, respectively, to the resting membrane potential (Eusebi et al., 1978; Sawada et al., 1984; Bolshakov et al., 1991; Kehoe and Vulfius, 2000).

The question of possible involvement of Cl\(^{-}\)-component of Glu response in the mechanisms of synaptic plasticity in molluscan neurons was not examined yet. The present study was undertaken to clarify whether cyclic nucleotides can induce long-term potentiation of Glu-activated Cl\(^{-}\)-current in molluscan neurons.

**METHODS**

The experiments were performed on isolated neurons of the visceral ganglion and the left and right parietal ganglions of the land snail (Helix pomatia). Neurons were isolated with the help of perfect needles without any pretreatment of the ganglia with proteolytic enzymes. The neurons were pipetted into the recording chamber of about 1 ml volume and continuously perfused with a standard Ringer solution feeding by gravity. The flow rate of the perfusion was 0.6 ml/min.

Two microelectrodes voltage-clamp technique was used. The microelectrodes were filled with potassium citrate solution (2 M), and the microelectrode tip resistance was about 10 M\(\Omega\). The experiments were performed using a MEZ 7101 micro-electrode amplifier and a CEZ 1100 voltage clamp amplifier (Nihon Kohden, Japan).