Modulation of the Histaminergic Inhibitory Synaptic Potential in the *Onchidium* Neuron by Cyclic Nucleotides

Tsukasa Gotow

*Department of Physiology, School of Medicine, Kagoshima University, Kagoshima, Kagoshima, 890 Japan*

**Summary** In the identified molluscan neuron, the presynaptic stimulation evokes a histaminergic inhibition of long duration (HILD), resulting from an increase in K conductance followed by a conductance-independent hyperpolarizing process. The later component of HILD was enhanced by cyclic AMP but depressed by cyclic GMP. However, neither agent alone altered the resting membrane potential nor the conductance.

**Key Words:** molluscan neuron, histaminergic inhibition, cyclic nucleotides.

A brief application of exogenous histamine to an identified *Onchidium* neuron, Be-1, can induce a long-lasting hyperpolarization (Gotow et al., 1980a, b). This response is enhanced by cyclic AMP applied extra- or intracellularly, but is depressed by cyclic GMP applied similarly (Gotow and Hashimura, 1982).

Our recent study suggested that the histamine-induced response is equivalent to an inhibitory synaptic potential recorded from Be-1 in response to a physiological, presynaptic stimulation of the cardiac nerve (Gotow, 1984). Thus, the present study was conducted to examine the possibility that cyclic nucleotides may also be involved in modulation of this postsynaptic response of the Be-1.

The circumesophageal ganglia were dissected from *Onchidium verruculatum* weighing about 10 g, placed in a 1 ml bath, and perfused with saline at 20–24°C. The saline composition was (in mM): 450 NaCl, 10 KCl, 10 CaCl$_2$, 50 MgCl$_2$, and 10 tris-hydroxymethyl-aminomethane-HCl, adjusted to pH 7.8. A single-barrelled voltage electrode and a double-barrelled current electrode were inserted into the Be-1. The voltage electrode and one barrel of the current electrode were filled with 2.5 M KCl solution, and the other barrel with 2 M tetrabutylammonium (TEA)-Br, 0.5 M cyclic AMP, or 0.5 M cyclic GMP. TEA ions and cyclic AMP and GMP were injected intracellularly by passing appropriate currents between
Fig. 1. Effects of intracellular cyclic AMP or cyclic GMP on the HILD recorded from the same Be-1 cell. The HILD was produced by ten stimuli at 1.4 Hz applied to the cardiac nerve. In the left column, a three-step current pulse lasting 3.3 sec, composed of each 1.1 sec step pulse, was applied before and at three different times during an HILD as shown in trace e. a: control HILD. b, c: after intracellular cyclic AMP (b) or cyclic GMP (c) injection for 10 min with 1 Hz of 10-20 nA and 0.5 sec duration. Trace c was made after full recovery from the effect of cyclic AMP shown in trace b. d: recovery after 30 min wash. The time of presynaptic stimulation is shown in the bottom trace e. Top portions of spikes are cut in all illustrations. Initial membrane potential was between -45 and -47 mV through all records. Right column: current-voltage (I-V) characteristics for the resting state and three different times of the HILD. Each I-V line in Al-3 and Bi-3 is constructed from the corresponding three-step pulse shown in the left column. Phases 1, 2, and 3 in the right column correspond to each time of the second, third, and last three-step pulses during an HILD shown in the left column. Open and filled circles were obtained from the resting membranes before and after the injection of cyclic AMP (Al-3) or GMP (Bi-3). Open and filled triangles, squares, and rhombi in A1-B1, A2-B2, and A3-B3 show the I-V lines for each phase of the HILD before and after the injection of cyclic AMP or GMP, respectively.

Japanese Journal of Physiology
the barrels of the double electrodes. The cardiac nerve was sucked into a tight-fitting glass tube and stimulated with isolated pulses of 0.5 msec and 2-3 V.

The identified neuron, Be-1 located in the right pleuro-parietal ganglion, is characterized by a beating activity consisting of a sustained train of intrinsic spikes. The beating activity is monosynaptically blocked during a histaminergic inhibition of long duration (HILD) elicited by a brief train of stimulus applied to the cardiac nerve (Goto, 1984). Ten presynaptic stimuli to the cardiac nerve delivered at 1.4 Hz usually gave an HILD of about 10 mV lasting for 0.5–1 min. The stimulus condition used for the present experiments was the same as above unless otherwise described.

Figure 1 shows the effects of intracellular injection of cyclic nucleotides on HILD. In each trace of the left column, three-step hyperpolarizing constant current pulses were applied every 12 sec before and during an HILD in order to evaluate the changes in membrane resistance. The first three-step current pulses at the resting state were set so that the second three-step current came to approximately the peak (phase 1) of the HILD following the end of the stimulus train. The voltage-current (V-I) relationships thus obtained are shown in the right column of Fig. 1. Phases 2 and 3 correspond to the times of the third and fourth current pulses delivered during an HILD as shown in the left column. The reversal potential for phase 1 is shown to be −80.5 mV in both A1 and B1, whereas that for phase 2 is shown to be −97.5 mV in both A2 and B2. No reversal potential was estimated for phase 3 as shown in either A3 or B3. Such behavior of the reversal potential during the time course of HILD has been explained by a mechanism in which an increase in K conductance, dominant in phase 1 and 2, is accompanied by a simultaneous conductance-independent hyperpolarizing process during each phase, particularly dominant in phase 3 (Goto, 1984). After the injection of cyclic AMP, each V-I line for phases 1, 2, and 3 induced parallel toward more negative potentials with respect to the control before cyclic AMP injection, respectively (A1, A2, and A3). Inversely, cyclic GMP injected into Be-1 shifted each line for the three phases toward depolarizing levels in parallel with the control (B1, B2, and B3). These findings imply that cyclic AMP or GMP increases or decreases the amplitude and duration of HILD, respectively, without any effect on the K conductance mechanism during the HILD (phases 1 and 2). The effects of these cyclic nucleotides on HILD were reversible (d in the left column). In the resting state before the presynaptic stimulus train, the intracellular injection of cyclic AMP or GMP itself had little effect on the resting membrane potential, the membrane resistance, or the intrinsic beating activity, as shown in both left and right columns. Similar results were obtained from six experiments. Thus, the conductance-independent hyperpolarizing process of HILD appears to be enhanced by cyclic AMP but depressed by cyclic GMP.

In a recent study (Goto, 1984), intracellular TEA selectively eliminated the conductance-dependent component (in phases 1 and 2) without affecting the con-
ductance-independent component (dominant in phase 3) of HILD. Thus, cyclic nucleotides should still affect the TEA-resistant (ductance-independent) HILD even after injection of intracellular TEA.

When TEA was injected into Be-1 by current pulses (20 nA, 0.5 sec) of 1 Hz for 5 min, the presynaptic stimulation of cardiac nerve evoked a part of HILD without detectable change in input resistance (Fig. 2a), thus attenuating phases 1 and 2. This TEA-resistant component of HILD was enhanced by cyclic AMP (Fig. 2b), but depressed by cyclic GMP (Fig. 2c), as expected. These effects were reversible after 30 min washing as shown in Fig. 2d.
The above actions of cyclic AMP or GMP on HILD were mimicked by 2 mM dibutryl cyclic AMP or GMP perfused externally for 5–10 min, respectively. However, intracellular injection of other adenine nucleotides or guanine nucleotides into the same Be-1 had no effect on HILD.

In other molluscan neurons, cyclic AMP has been reported to mediate either a decrease in K conductance (DETERRE et al., 1981; SIEGELBAUM et al., 1982) or an increase in K conductance (DRUMMOND et al., 1980) elicited by a neurotransmitter serotonin. This supports the idea that some synaptic actions may depend on the involvement of the second messenger cyclic AMP (GREENGARD, 1976). However, in the Onchidium Be-1, the intracellular injection of cyclic AMP or GMP alone did not change membrane potential or conductance, suggesting that their actions are somewhat different from the second messenger stated above. On the other hand, the conductance-independent component of HILD was enhanced by cyclic AMP and depressed by cyclic GMP. This seems rather similar to reports (LIBET et al., 1975; KOBAYASHI et al., 1978) that the conductance-independent slow excitatory synaptic potential (sEPSP) in the rabbit sympathetic ganglion is enhanced by cyclic AMP, but its enhancement of sEPSP is antagonized by cyclic GMP.

I would like to thank D. Mrozek for reviewing the manuscript.

REFERENCES


