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# BRADYKININ AS A FACTOR REGULATING SYNAPTIC INPUT TO *Helix pomatia* NEURONS

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The effect of bradykinin on spontaneous unit activity was studied in the subesophageal ganglion of *Helix pomatia* by microiontophoresis. Bradykinin was shown not only to facilitate unit responses to synaptic activation, but also to prolonged action potential generation evoked by this activation. The effects of bradykinin were observed in experiments carried out during the spring.

KEY WORDS: bradykinin; neuronal oligopeptides; synaptic transmission.

An important role in the mechanisms of chemical interaction between neurons is played not only by the classical mediators (acetylcholine, noradrenalin, dopamine, serotonin), and amino acids, but also by certain oligopeptides (substance P, vasopressin, oxytocin, releasing factors). These peptides, it is held, can act in the CNS as mediators or modulators [1, 11]. It accordingly seemed very likely that these oligopeptides can actively influence the performance of their integrative functions by nerve cells [3, 12].

Sensitivity to physiologically active peptides such as oxytocin, vasopressin, angiotensin-II, and physalaemin has been discovered in molluscan neurons [3, 5, 12]. Several peptide factors which significantly change the physiological properties of the neuron in these animals have been isolated from an extract of the nerve tissue of mollusks [2, 10]. These findings indicate that molluscan neurons can be used as a model with which to study the mechanisms of action of oligopeptides on single neurons and to investigate interaction between these substances and other biologically active compounds in the formation of the integrative functions of central neurons.

In this investigation the effect of bradykinin, and endogenous oligopeptide inducing pain in man and animals on systemic injection [7-9], on spontaneous unit activity was studied in the subesophageal ganglion of *Helix pomatia*.

## EXPERIMENTAL METHOD

Experiments were carried out on the isolated circumesophageal nerve ring, placed in hemolymph, and a semi-intact preparation of the mollusk with nervous connections undamaged. The use of the semi-intact preparation enabled perfusion to be carried out with standard physiological saline for mollusks, pH 7.6. After removal of the membrane, a microelectrode

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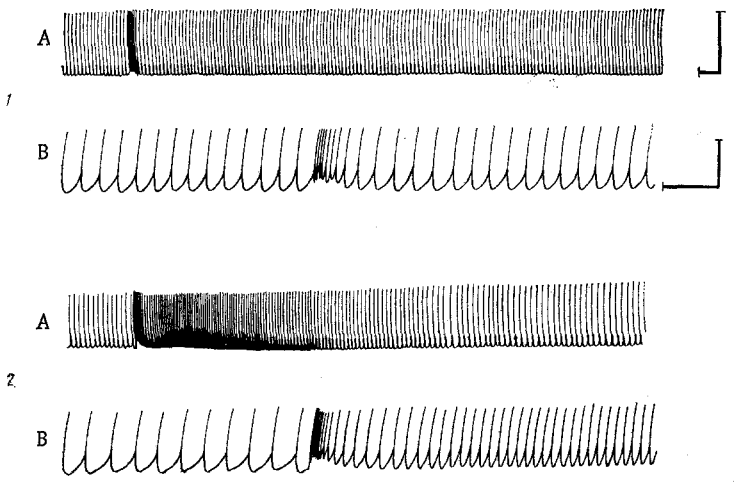


Fig. 1. Change in spontaneous unit activity in right parietal ganglion during microiontophoretic application of bradykinin. 1) Spontaneous unit activity before application of bradykinin by cationic current of 70 nA; 2) application of bradykinin. Calibration: in A) 50 mV, 5 sec; in B) 20 mV, 2 sec.

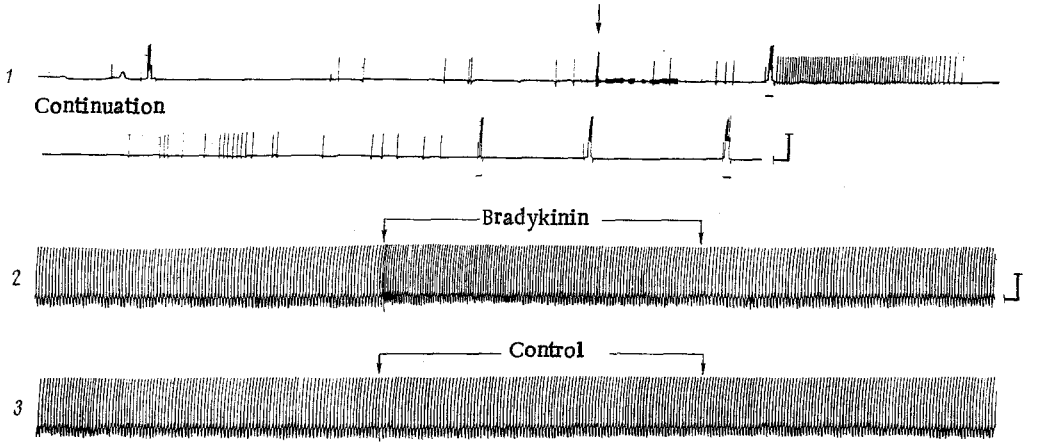


Fig. 2. Action of bradykinin on neurons of right parietal ganglion. 1) Effect of bradykinin on frequency of action potential generation after passage of depolarizing current (1 nA) through recording electrode. Moment of addition of bradykinin in concentration of $1 \cdot 10^{-4}$ g/ml to external solution marked by arrow. Horizontal line above trace of unit activity marks time of passage of depolarizing current; 2) increase in frequency of spike discharge during microiontophoretic application of bradykinin by cationic current of 80 nA; 3) absence of response of same neuron to application of control solution by current of same strength. Horizontal lines with arrows above traces of unit activity mark time of application. Calibration: in 1) 50 mV, 10 sec; in 2) 25 mV, 2 sec.

filled with a 2.5 M solution of potassium citrate, with a resistance of 15-20 MΩ, was inserted into the nerve cell to be studied. Bradykinin (1 mg/ml) was applied extracellularly, microiontophoretically from a buffered solution, pH 6.5. Cationic current with a strength of 40 to 100 nA were used. The duration of application varied from 40 sec to 2 min. Passage of a current of identical direction and strength through the second channel of a multichannel electrode, filled with buffered solution with addition of NaCl with the same pH, served as the control. In some experiments bradykinin was added directly to the solution bathing the semi-intact preparation. Unit activity was recorded by means of a type MZ-4 preamplifier and dual-beam oscilloscope, and also simultaneously on an NRF-3415 tape recorder and N-327-3 automatic

writer. Subsequently, recording was carried out with the necessary amplification and sweep on the automatic writer. Altogether 36 neurons in the right parietal ganglion were recorded (19 neurons between March through May and 17 cells from September through November).

## EXPERIMENTAL RESULTS

Analysis of the experimental data showed that between September and the end of November the nerve cells of the mollusks were insensitive to microiontophoretic application of bradykinin. In some cases, after application of bradykinin for 1-2 min phasic fluctuations of membrane potential, either upward or downward, were observed. Changes of this type also occured, however, after application of the control solution.

When the molluscan neurons were tested from March through May, changes in the character of the synaptic input were observed following microiontophoretic application of bradykinin.

A record of a neuron with spontaneous pacemaker activity, on which from time to time groups of synaptically evoked spikes are superposed, is shown in Fig. 1. On application of bradykinin, the synaptic response was facilitated, and for several tens of seconds thereafter there was a considerable increase in the frequency of pacemaker action potentials. Bradykinin thus not only facilitates the responses of nerve cells to synaptic activation, but also considerably prolongs action potential generation induced by this activation. In some neurons, on the addition of bradykinin to the solution bathing the mullusk preparation, a sharp increase in the frequency of action potential generation was observed after passage of a depolarizing current through the recording microelectrode (Fig. 2, 1). Bradykinin did not affect the membrane potential or spontaneous discharge frequency of these neurons. However, artificial depolarization of the cell to the action of bradykinin substantially modified its spike activity. Cells with a regular type of activity as a rule, it must be noted, did not respond to application of bradykinin, in agreement with the results of a study of the action of this oligopeptide on identified molluscan neurons [5, 12]. Nevertheless, in one neuron with a regular frequency of action potential generation, after microiontophoretic application of bradykinin there was some decrease in the membrane potential level and an increase in the frequency of spike generation (Fig. 2, 2).

The experiments thus showed that a characteristic feature of the action of bradykinin is facilitation of unit responses to synaptic activation and a marked increase in the period of action potential generation. The rapid development of the effect depends on activation of membrane processes and is evidently unconnected with the synthesis of new polypeptides or mediators.

The results indicate the presence of seasonal sensitivity to bradykinin and they suggest that this oligopeptide is a factor which modifies the responses of molluscan neurons to the synaptic input. Bradykinin evidently interacts primarily with the neuron membrane, and this leads to changes in processes participating in the response of the neuron to synaptic excitation.

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