EFFECT OF FMRF-AMIDE ON ACTIVITY OF DEFENSIVE BEHAVIOR COMMAND NEURONS OF FED AND HUNGRY SNAILS (*Helix pomatia*)

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An important task in the study of functions of the nervous system is the study of the role of neuropeptides in the neurophysiological mechanisms of goal-directed animal behavior. In recent years it has been shown by immunohistochemical and biochemical methods that the CNS of various animals (including mollusks and mammals) contains so-called cardioactive or FMRF-amide-like peptides [2, 3, 5, 7, 9]. The possibility of involvement of FMRF-amide in the regulation of both defensive and feeding behavior of various animals has been demonstrated experimentally. In particular, the modulating action of the peptide has been demonstrated during the study of sensitivity of rats to pain [10], of "thermal avoidance" by the snail *Cepaea nemoralis* [6], and of contraction of the gill of the marine mollusk *Aplysia californica* [11]. However, the authors cited above as a rule did not take account of the initial level of the animals' food motivation. Yet we know that close two-way relations exist between defensive and food-related forms of behavioral activity. In mollusks these relations are largely determined by the functions of so-called command neurons.

The aim of this investigation was to study the effect of FMRF-amide on command neurons of defensive behavior of hungry and fed snails.

EXPERIMENTAL METHODS

Experiments were carried out in April on 15 snails (*Helix pomatia*) weighing 25-30 g, caught in the neighborhood of Sukhumi. Before the experiments the mollusks of group I were kept in boxes in which their natural exposure to light and darkness, high humidity, and a temperature of 18-20°C were maintained. The snails had free access to water and food (carrot). Snails of group 2 were kept under the same conditions, but deprived of food for the 7 days before the experiments. The experiments were carried out on defensive behavior command neurons RPa2, RPa3, LPa2, and LPa3 of semi-intact preparations. Tactile stimulation was applied to the anterior part of the foot and mantle fold of the snail on the side ipsilateral relative to the neuron to be recorded. The duration of the stimulus was 20 msec and its intensity 50 m/mm^2. Intracellular potentials derived from the neurons by glass microelectrodes were led to the input of an M-707 microelectrode amplifier (WPI, USA). The diameter of the microelectrode tip was 1 μ and its resistance 5-30 MΩ. Potentials were recorded on a high-speed automatic writer of the N338-4p type and S1-102 oscilloscope. Spontaneous and evoked unit activity was studied for 30 min before and 60-120 min after application of FMRF-amide. Latent periods of onset of the excitatory postsynaptic potential (EPSP) and the first action potential (AP), their difference (prespike duration), the number of AP in the response, the thresholds of AP generation, the EPSP amplitude, and changes in resistance and excitability of the membrane were analyzed. Membrane resistance was determined by measuring the short-term shift of membrane potential toward hyperpolarization on passage of square pulses of current through the recording microelectrode. Membrane excitability was estimated from the number of AP generated during passage of a square depolarizing pulse of current with a duration of 10 sec through the recording microelectrode. The FMRF-amide (Phe-Met-Arg-Phe-NH_2) used was synthesized at the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Medical Sciences of the USSR, by Senior Scientific Assistant A. A. Shishkina. The peptide was applied to the neuron through an extracellular glass micropipet by compressed aid under a pressure of 1.5-2 atm by means of the Neuro Phore BH-2 apparatus (Medical Systems, USA) in the course of 10 sec. The diameter of the micropipet tip was 20-40 μ and the

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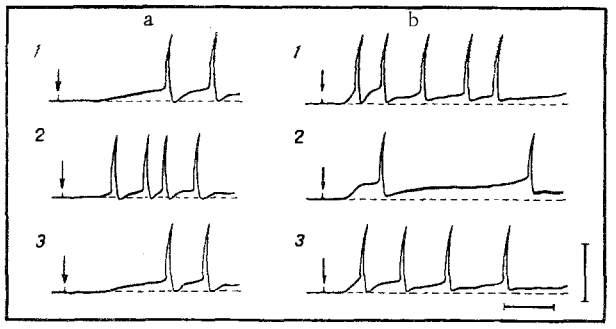

Fig. 1. Response of neuron RPa3 to tactile stimulation of mantle fold of fed (a) and hungry (b) snails. 1) Initial response: 2) response 10 min after application of 10^{-5} M FMRF-amide; 3) response 60 min after application of peptide. Arrow indicates application of stimulation. Amplitude of spikes not shown completely. Calibration: 60 mV, 100 msec.

concentration of the peptide in the micropipet was 10^{-5} M. To prevent any action of the peptide on the neuron by diffusion, the micropipet was applied to the soma of the cell immediately before application.

EXPERIMENTAL RESULTS

No significant differences in spontaneous spike activity, membrane potential level (60 ± 5 mV) and responses of the neurons tested to tactile stimulation of the body of the hungry and fed snails were observed.

Application of the peptides to the soma of the command neurons of defensive behavior of the fed snails evoked a depolarization shift of membrane potential in six of the seven cells by 2-4 mV. The input resistance of the membrane in most cases fell by 6-16% under the influence of FMRF-amide. These changes lasted 3-40 min. Pneumophoresis of the peptide to neurons of fed snails also led to an increase in intensity of responses of the neurons to tactile stimulation of different parts of the snails' body. For instance, Fig. 1a shows that neurons RPa3 of a fed snail responded initially to tactile stimulation of the mantle fold by generation of 2 AP. The threshold of generation of the first AP was 12 mV and the prespike duration was 130 msec. The number of AP in the response 10 min after pneumophoretic application of FMRF-amide increased to 4, and the threshold of generation of the first AP and the prespike duration decreased by 8 mV and 100 msec, respectively. Restoration of the original intensity of response was observed toward 55 min after application of the peptide. In other neurons tested an increase in amplitude of the evoked EPSP by 2-6 mV (30-90%), an increase in the frequency and number of AP in the response by 1.5-2 times, lowering of the threshold of AP generation by 2-11 mV (15-83%), and shortening of the prespike duration by 20-100 msec (18-90%) were found. These changes took place as a rule 2-8 min after application of the peptide and the return to the original value took place towards 30-50 min.

Application of FMRF-amide to eight command neurons of hungry snails led to hyperpolarization of the membrane potential by 1-5 mV in all cells tested. In half of the neurons the input membrane resistance was unchanged, whereas in the rest it was reduced by 7-30%. Excitability of the membrane either was unchanged (50% of cells) or was reduced by 20-50%. The effects discovered lasted 4-54 min. Application of FMRF-amide also reduces the intensity of neuronal responses to tactile stimulation of the snail's body. For instance, Fig. 1b shows that neuron RPa3 of a hungry snail initially responded to tactile stimulation of the mantle fold by generation of 5 AP. The threshold of generation of the first AP was 12 mV and the prespike duration 25 msec. The number of AP in the response 10 min after pneumophoretic application of FMRF-amide diminished to 2 AP and the threshold of generation of the first AP and the prespike duration increased by 6 mV and 50 msec, respectively. Recovery of the original intensity of the response was observed 60 min after application of the peptide. In another seven neurons the amplitude of EPSP fell by 1-6 mV (7-42%) and the frequency and number of AP in the responses decreased by 1.5-2.5 times. In 60% of neurons (three of five neurons which generated AP in their response) the threshold of AP generation was raised by 5-13 mV (17-44%) and the prespike duration was increased by 45-190 msec (by 2-6 times). These changes took place on average 5-15 min after application of the peptide and they lasted 40-70 min.

Thus FMRF-amide has an opposite action on spontaneous activity and on responses of neurons to tactile stimulation in hungry and fed snails.

The results suggest functional connections between command neurons of defensive behavior and neurons responsible for food-related behavior of the snail. This suggestion is con-

firmed by behavioral experiments in which the intensity of defensive response was shown to depend on the level of the snails' food motivation [8]. The possibility cannot be ruled out that the molecular factors modulating defensive behavior are peptides of the gastrin–cholecytokinin family. In his experiments, Bratyshev [1] showed that these peptides can modulate the food-related behavior of snails by a marked degree and in different directions. Gastrointestinal peptides probably reach the command neurons of defensive behavior by humoral or neuronal routes and specifically modify their metabolism and response to application of FMRF-amide.

Investigations at the cellular level revealed opposite effects of FMRF-amide on some parameters of bioelectrical activity: for example, in some neurons of the snail *Helix aspersa* the peptide induces depolarization, whereas in others it induces hyperpolarization; the ionic mechanisms of these effects, moreover, are different [4]. The action of the peptide in neurons S1 depended on the level of membrane potential: usually FMRF-amide induced hyperpolarization, but if the neuron was depolarized by intracellular injection of current, the behavior of the peptide led to further depolarization. The effects of FRMF-amide on command neurons of defensive behavior of *Helix pomatia* with different levels of food motivation, revealed by the present investigation, can evidently not be explained by the neurophysiological mechanisms described for S1 neurons, for no difference was found in the membrane potentials of command neurons of defensive behavior of fed and hungry snails.

The action of the peptide both on functions of the electrogenic membrane (membrane potential, input resistance and excitability of membranes), and on synaptic processes (EPSP, thresholds of AP generation, prespike duration) are evidence that the effects of FRMF-amide are realized on the postsynaptic neurons, but not on presynaptic endings. However, further explanation of the mechanisms of action of FRMF-amide require additional investigation.

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