PHYSIOLOGY

Cholinergic Sensory Inputs to Command Neurons in Edible Snail

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We studied cholinergic component of visceral sensory input to defensive behavior command neurons in edible snail. Nicotinic receptor antagonist tubocurarine and muscarinic receptor antagonist atropine reversibly decreased the amplitude of the total excitatory postsynaptic potential induced by electrostimulation of the peripheral region in the mechanosensory receptor field of command neurons on the surface of internal organs. Our results indicate that acetylcholine is involved in sensory signal transduction from the visceral sac to command neurons of snail parietal ganglia. The subsynaptic membrane of visceral synaptic input contains nicotinic and muscarinic receptors.

Key Words: *acetylcholine; tubocurarine; atropine; command neurons; edible snail*

Identified neurons are suitable objects for the studies the cellular mechanisms of behavior [6,10]. Giant neurons of parietal ganglia in edible snail serve as one of the common objects (LPa2 and LPa3 neurons; and PPa3 and PPa2 neurons) [2]. These neurons are command neurons of passive defensive response in snails [9,11]. Their functional and morphological characteristics were studied in details. However, the transmitter specificity of sensory inputs is unclear. It remains unknown whether acetylcholine (ACh) is involved in sensory signal transduction from various regions of the giant mechanosensory receptor field in command neurons.

Experiments with application of ACh showed that the membrane of command neurons in parietal ganglia contains subsynaptic and extrasynaptic cholinoceptors [4]. Extrasynaptic somatic cholinoceptors of the nicotinic and muscarinic types were pharmacologically identified. Muscarinic receptors differ from M1 and M2 subtypes [5]. Studies of the isolated soma of command neurons revealed cholinergic sensitivity of their somatic membrane [1]. Experiments with atropine and tubocurarine showed that the membrane of command neuron processes is also sensitive to ACh. There are published reports on cholinergic nature of synaptic transmission between identified sensory neurons and command neuron LPa3 [7]. Presynaptic command sensory neurons (LPa7 and LPa9) have local mechanosensory receptor fields that are localized in the visceral region of snail body surface (below the shell) [3]. The surface of internal organs serves as a peripheral region for giant receptor fields of parietal command neurons [8]. The intestinal nerve includes cholinergic fibers [12]. However, the role of ACh in sensory signal transduction from snail internal organs to command neurons remains unclear.

Here we studied the effects of antagonists of nicotinic and muscarinic receptors on the amplitude of the total excitatory postsynaptic potential (tEPSP) in command neurons of edible snail induced by electrostimulation of the surface of internal organs.

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MATERIALS AND METHODS

Experiments were performed on defensive behavior command neurons LPa2, LPa3, PPa3, and PPa2 [2] of the terrestrial lung mollusk *Helix lucorum* (Crimean population of edible snail). We used a semi-intact preparation of the central nervous system and visceral sac. The study was performed under conditions of stopped flow through a chamber with physiological saline (80 mM NaCl, 4 mM KCl, 7 mM $CaCl_2$, 4 mM $MgCl_2$, and 10 mM Tris-HCl; pH 7.8).

The semi-intact preparation (parapharyngeal ring of ganglia with nerve connection to the visceral sac) was obtained after exposure of the snail in a refrigerator at 2-5°C.

Bipolar electrostimulation of lung cavity surface with direct current for 50 msec served as the test stimulus. The intensity of the test stimulation did not exceed 5 V. It was adjusted so that the postsynaptic response of the command neuron did not reach the threshold of action potential generation ($^2/_3$ of the threshold value, Fig. 1). Stimulation was applied at 5-min intervals.

Cholinergic pathways were blocked with antagonists of nicotinic and muscarinic receptors (tubocurarine and atropine, respectively; Sigma). Solutions of cholinoceptor antagonists (50 μM) in physiological saline were prepared on the day of the study. We used 1 mM solutions that were stored at 2°C.

The study was performed in 2 series (control, 35 min; and experiment, 50 min). The amplitude of tEPSP was stabilized. Normal solution not containing cholinoceptor antagonist was substituted for normal solution (control series) and solution with tubocurarine or atropine (experimental series). After 35-min testing, cholinoceptor antagonist was washed out with 3-fold potrions of physiological saline. The testing was performed for additional 15 min.

Intracellular potentials of command neurons in parietal ganglia were recorded using glass microelectrodes filled with 2 M potassium acetate. Microelectrode resistance was 10-15 MΩ. The results were obtained in experiments with 30 neurons (12 LPa3, 10 PPa3, 5 LPa2, and 3 PPa2). In 6 trials, the recording was performed simultaneously with 2 command neurons. The mean value of the neuronal membrane potential in the control and experimental series was -56.08±0.44 mV.

tEPSP were amplified on a MSE-3R amplifier (Nihon Kohden), digitized, and recorded on a hard drive of a personal computer (DigiScope-99 software). The amplitude of tEPSP was measured using AxoTape v.2 software (Axon Instruments, Inc).

We measured the amplitude of the first peak and maximum amplitude of tEPSP. In the control series, the amplitude of tEPSP to the penultimate stimulus estimated after substitution of physiological saline in the chamber with the test preparation for the solution not containing cholinoceptor antagonist was taken as 100%. In the experimental series, the amplitude of tEPSP to the penultimate stimulus estimated before substitution of physiological saline for physiological saline with cholinoceptor antagonist was taken as 100%.

We calculated the arithmetic mean of the sample and standard error of the arithmetic mean. The results were analyzed by nonparametric Wilcoxon test (STADIA 6.2).

RESULTS

Electrostimulation of the visceral sac was followed by generation of tEPSP. The latency of tEPSP did not exceed 300 msec. The duration of tEPSP was <600 msec. tEPSP usually had 2 components (Fig. 1).

Preliminary analysis without pharmacological treatment revealed no spontaneous changes in tEPSP. Thirty minutes after substitution of physiological saline in the chamber with the test preparation for the solution not containing cholinoceptor antagonist, the amplitude of the first peak of tEPSP was 90.58±9.39% (n=11). The maximum amplitude of tEPSP was 103.46±8.78% (n=16).

Variations in the amplitude of the short-latency peak and maximum tEPSP differed after treatment with nicotinic receptor antagonist tubocurarine (50 μM, Fig. 2, *a*). Tubocurarine induced a progressive decrease in the amplitude of the short-latency peak of tEPSP (n=6). This parameter decreased 15 min after treatment and reached minimum by the 35th minute (64.67±5.31%, p<0.01). Tubocurarine had a biphasic effect on the maximum amplitude of tEPSP (n=7). We revealed a short-latency increase and subsequent decrease in the maximum amplitude of tEPSP. The maximum am-

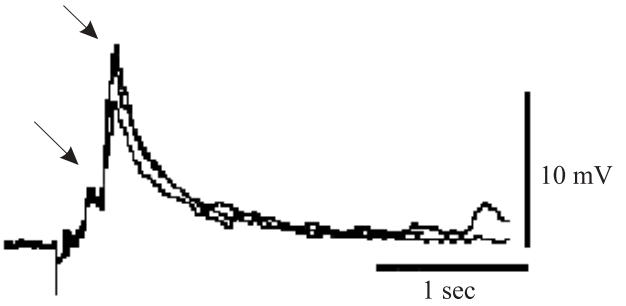

Fig. 1. Total EPSP of neuron PPa3 induced by electrostimulation of the surface of internal organs. Superposition of 3 tEPSP in normal physiological saline. Arrows: first peak and maximum tEPSP.

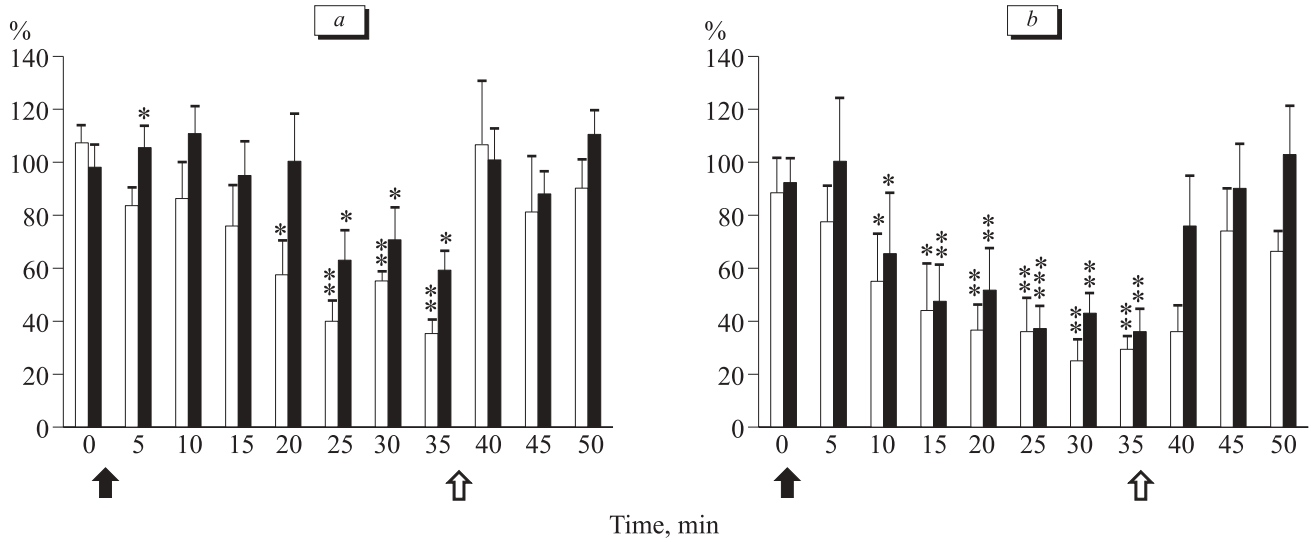

Fig. 2. Effects of tubocurarine and atropine on the amplitude of tEPSP in command neurons induced by electrostimulation of the surface of internal organs (visceral sac). Amplitude of tEPSP after treatment with 50 μM tubocurarine (*a*) and 50 μM atropine (*b*). Light bars: amplitude of the first peak of tEPSP; dark bars, maximum amplitude of tEPSP. The amplitude of tEPSP before pharmacological treatment is taken as 100%. Dark arrow: application of cholinoceptor antagonist; light arrow, washing out of the preparation with physiological saline. **p*<0.05, ***p*<0.01, and ****p*<0.001 compared to physiological saline.

plitude of tEPSP increased by 5.56±8.23% after 5 min ($p<0.05$). The maximum amplitude of tEPSP decreased by the 25th minute and reached minimum 35 min after treatment (40.66±7.25%, $p<0.05$). The amplitude of the first peak and maximum amplitude of tEPSP returned to normal 5 min after washout.

Muscarinic receptor antagonist atropine (50 μM) induced a similar decrease in the amplitude of the first peak ($n=6$) and maximum amplitude of tEPSP ($n=7$, Fig. 2, *b*). The amplitude of the first peak of tEPSP decreased by the 10th minutes and reached minimum 30 min after treatment (75.06±7.90%, $p<0.01$). The maximum amplitude of tEPSP significantly decreased by the 10th minutes and reached minimum 35 min after treatment (63.87±8.50%, $p<0.01$). The amplitude of the first peak and maximum amplitude of tEPSP returned to normal 10 and 5 min after washout, respectively.

Our results indicate that cholinergic synaptic inputs are involved in the formation of visceral tEPSP in parietal command neurons. Cholinergic inputs constitute a significant part of afferent influences in the visceral sac. The effect of cholinergic afferents is realized via nicotinic and muscarinic receptors. The short-term increase in the maximum amplitude of tEPSP under the influence of tubocurarine was probably related to the recurrent inhibition of sensory neurons via nicotinic receptors [7].

We previously identified extrasynaptic nicotinic and muscarinic receptors on the soma of command neurons LPa3 and PPa3 in edible snail [4,5]. These data indicate that nicotinic and muscarinic receptors are localized not only in the extrasynaptic zone of the membrane in command neurons of edible snail, but also in the subsynaptic region.

The study of the transmitter nature of afferent inputs in command neurons of edible snail is of considerable theoretical importance. Edible snail is a suitable object to study the molecular mechanisms of synaptic plasticity. Identification of ergically different afferent inputs from various regions of receptor fields in command neurons allows us to compare the role of various transmitters in various types of synaptic plasticity. It mainly concerns the identification of cholinergic and glutamatergic afferent inputs.

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