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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Dependence of Electric Activity of Motoneurons and Locomotor Behavior of *Lymnaea stagnalis* on Environmental Temperature

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Abstract—Experiments on serotoninergic motoneurons of the pedal A-cluster innervating the foot ciliated epithelium of *Lymnaea stagnalis* L. have shown that changes of the environmental temperature lead to a change of several electrophysiological parameters of these neurons. The firing rate of action potential (AP) progressively increases with elevation of the temperature up to the level of about 30°C, after that an abrupt drop of the rate occurs. The membrane potential and AP amplitude decrease as the temperature rises. The revealed changes may be explained by an increase of the membrane permeability, specifically, to sodium ions. The characteristic activity pattern of these neurons disappears in the temperature range of 34–36°C. The peculiarities observed in the neuronal activity at changes of temperature correlate with a change of characteristics of locomotor behavior of *L. stagnalis*.

INTRODUCTION

Studies on invertebrate neurobiology have one important advantage, a possibility of working on identified neurons. It becomes possible to trace precisely connections between a certain type of behavior and changes in parameters of activity of nerve cells, which control this behavior. The pond snail, *Lymnaea stagnalis* L., is one of the animals that have been studied the most comprehensively in neurobiological aspect. Studies performed for the recent years allowed identification of neuronal networks responsible for the respiratory [1, 2], alimentary [3], and other types of behavior. The locomotor behavior, as compared to the above-mentioned ones, has been studied to a much lesser extent. A change in temperature conditions produces an essential readjustment of many aspects of the life activity in *L. stagnalis*, including its locomotor activity.

In this connection the goal of the present work was to study the changes in the spontaneous electric activity of motoneurons controlling locomotion, which are observed at changes of the environmental temperature.

MATERIALS AND METHODS

The work was carried out on a representative of the order Basommatophora, *Lymnaea stagnalis* L. In the laboratory the animals were kept in an aquarium under standard conditions (water temperature of 16–18°C, lettuce and cabbage as food). Used in experiments were adult specimens weighing 1–4 g (the shell length more than 25 mm).

A standard electrophysiological procedure was applied using glass micropipettes filled with 2.5 M KCl solution, with resistance of 10–40 MOhm. To soften the perineuronal sheath, prior to microelectrode recording, preparations of the ner-

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vous system were treated with a pronase solution (Protease E, type XIV, Sigma, USA) at a concentration of 1 mg/ml in the normal saline for *L. stagnalis* [4]. Fluctuations of pH values at temperature changes did not exceed 0.02. Recorded were parameters of the spontaneous activity of neurons responsible for locomotion: firing rate and amplitude of action potential (AP) as well as membrane potential (MP).

A thermal cell specially designed on the basis of the Peltier element provided maintenance and change of the temperature ranges required (4–6, 14–16, 24–26, 34–36°C). Preparations of the nervous system were constantly washed with a fresh solution of the same temperature as in the experimental chamber of 200 μl volume.

Analysis of locomotor behavior was performed using visual control methods. The velocity of locomotion was recorded. For this purpose the animals were transferred to a flat vessel standing on a millimeter graph paper and filled with water of the corresponding temperature (4–6, 14–16, 24–26, 34–36°C) that was maintained with the aid of an ultrathermostat. In 30 min after placing the snails into new conditions the time necessary to pass a distance of 3 squares (1 × 1 cm each) was measured with a stopwatch. Based on the results obtained, the velocity of snail locomotion (mm/s) was calculated. The latent period of protraction was defined as time from the moment of placing the mollusc into new temperature conditions to the start of its movement.

The data obtained were processed by commonly accepted methods of variation statistics using Student's criterion [5]. All data are presented as $x \pm S_x$. Results were considered statistically significant at the level of significance (P) below 0.05.

RESULTS AND DISCUSSION

Neurons of the A-cluster innervating the ciliated epithelium of the foot are located, as a rule, symmetrically [6, 7] on the inner medial surfaces of the right and left pedal ganglia (Fig. 1). Their color is red and orange, their size is about 50 μm, and they contain serotonin [7, 8]. A weak electric connection is present between the symmetrically located neurons in the right and left pedal ganglia [7]. They have a characteristic pattern of the spontaneous activity, the so-called "A-cluster-rhythm" that allows their easy identification (Figs. 2a–2c).

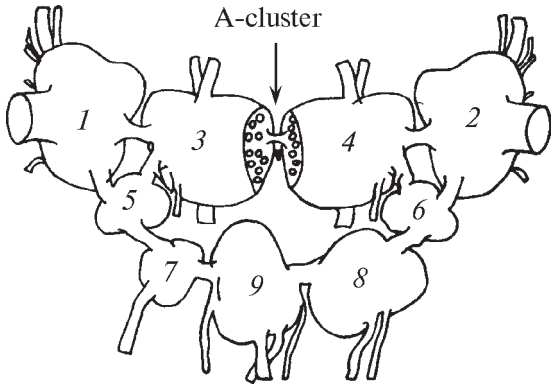

Fig. 1. Diagram of the CNS in *Lymnaea stagnalis*. Location of the studied neurons is shown. The view from the dorsal side. The buccal ganglia are removed. The cerebral commissure is cut. Ganglia: (*1*), (*2*) the left and right cerebral; (*3*), (*4*) the left and right pedal; (*5*), (*6*) the left and right pleural; (*7*), (*8*) the left and right parietal, (*9*) visceral.

In our preparations at 14–16°C the firing rate of AP was 1.1 ± 0.11 spikes/s (n = 14). The MP value was −52.3 ± 2.83 mV (n = 10) (Fig. 2b).

A decrease of temperature produced the following changes (Fig. 2a). A significant reduction of spike activity was observed: at 4–6°C the AP firing rate was 0.5 ± 0.07 spikes/s (n = 14). Under these conditions the Q_{10} coefficient was 2.3 ± 0.16 (n = 14). The studied neurons were hyperpolarized, the MP value being −66.9 ± 3.52 mV (n = 10). By the character of the cooling-produced changes in the AP amplitude the neurons of A-cluster were divided into two groups. In one group this amplitude rose by 12.2 ± 1.85% (n = 8), in the other one, on the contrary, it decreased by 22.3 ± 9.09% (n = 6). No change in the characteristic locomotor pattern was observed, neither was revealed the existence of the lowest temperature limit of the spontaneous activity. The neurons retained such ("restricted") work capacity down to 2.5°C (the technical limit of the lowest temperature value for the set-up) for no less than 30 min of observation. The return of the temperature to norm restored the parameters of functioning of the A-cluster neurons.

An elevation of the temperature (to 24–26°C) resulted in the following changes in the electric

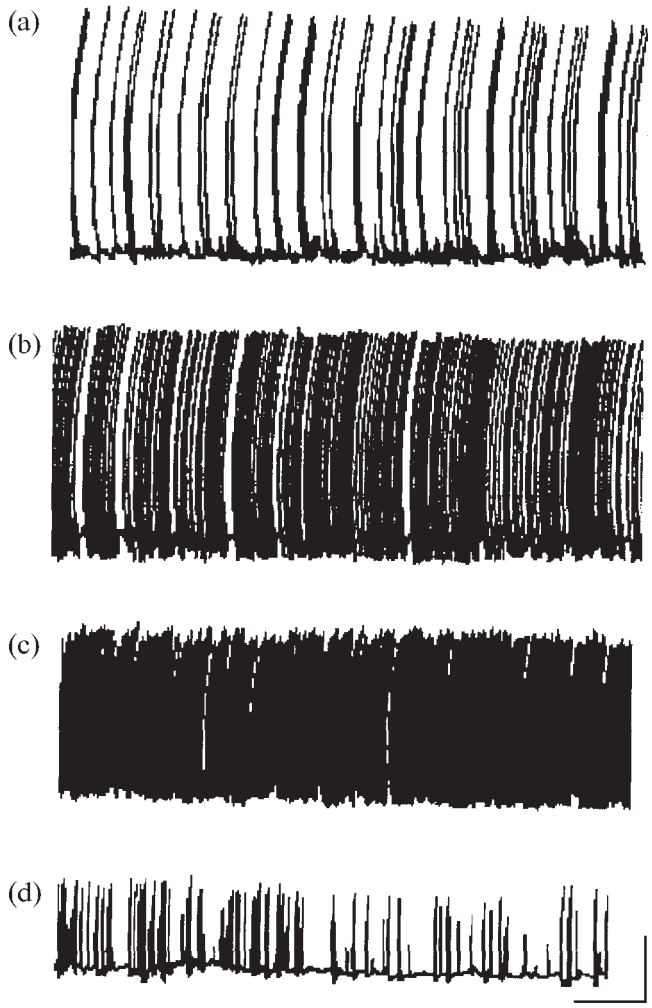

Fig. 2. Electric activity of an A-cluster neuron at different environmental temperatures. Temperature (°C): (a) 5, (b) 15, (c) 25, (d) 35. Bars: 20 s, 35 mV.

Effects of temperature on the locomotor behavior of *L. stagnalis* ($n = 15$)

Temperature, °C	Velocity of locomotion, mm/s	Latent period of protraction, s
4–6	1.4 ± 0.12*	20.5 ± 2.18*
14–16	2.8 ± 0.24	11.8 ± 1.13
24–26	5.4 ± 0.32*	16.6 ± 1.08*
34–36:		
for the first 10 min of observation	8.7 ± 0.56*	16.6 ± 1.08*
after 30 min	0.7 ± 0.14*	6.0 ± 1.04*

Note: Asterisks indicate a statistically significant difference from the parameters at 14–16°C.

characteristics of the neurons (Fig. 2c). The firing rate of AP was 1.5 ± 0.04 spikes/s ($n = 5$), with the Q_{10} coefficient value being 1.65 ± 0.07 ($n = 5$). The neurons were slightly depolarized; the MP value did not differ statistically significantly, whereas the AP amplitude was reduced by 20.0 ± 2.93% ($n = 6$) as compared to the parameters at 14–16°C.

Further elevation of the temperature (to the level of 34–36°C) abruptly decreases the AP firing rate to 0.41 ± 0.03 spikes/s ($n = 5$). The depolarization is increased by 59.6 ± 2.30%, while MP reaches the value of −36.5 ± 1.71 mV ($n = 6$). The AP amplitude falls even more, as compared to the temperature of 14–16°C. The characteristic activity pattern (A-cluster-rhythm) disappears, and there appear periods of irregular spontaneous activity (Fig. 2d).

Normalization of the temperature at the given stage of the experiment restored the electrophysiological characteristics of the studied neurons. On reaching temperatures of 40°C and higher the "heat" death of neurons occurred.

Study of the temperature effects on locomotor behavior showed the following (see table). A decrease of the temperature results either in the complete arrest of locomotor activity or in its considerable reduction. At elevation of temperatures the animals remain in the state of an increased motor activity for a long time (up to 2 h of observation). At 4–6°C, in case of a long experiment (more than 1 h), the molluscs gradually stop moving, stay for a long time at the same place and then occasionally perform slight excursions (no farther than 10 cm). However, soon, even these manifestations of activity stop (after 2 h and more after putting the snails into the conditions of the lowered environmental temperature). At water temperature of 34–36°C an unusually fast movement of molluscs is observed for the first minutes. The animals intensively explore all territory at their disposal by crossing it in all directions. After some time (no longer than in 30 min after the animals were placed into these conditions) a sharp decrease of the locomotor activity is observed. Probably, this is also due to a reduced adhesion of the foot to the substrate (a phenomenon clearly seen at 30°C and higher). The molluscs totally protruded from their shells

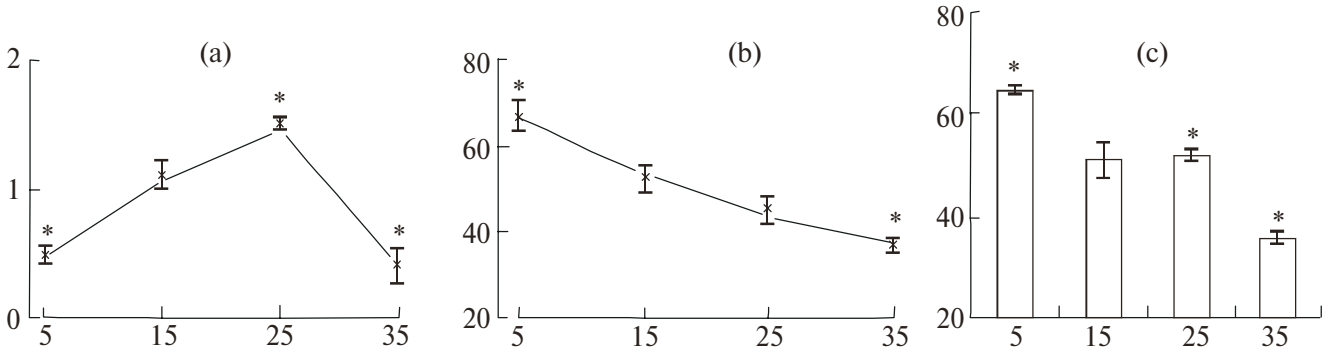

Fig. 3. Changes of electrophysiological parameters of A-cluster neurons at different experimental temperatures. *Ordinate*: (a) AP firing rate (spikes/s), (b) MP value (−mV), (c) AP amplitude (% of the value at 14−16°C (100%)); *abscissa*: temperature (°C). *Asterisks*—statistically significant differences ($p < 0.05$) as compared to the value at 14−16°C.

stop moving actively and do not react to tactile stimulation of tentacles. After normalization of the temperature the locomotor activity and the ability to react to external stimuli are restored.

When the temperature changed both to a decrease and to an elevation (in the range of 24−26°C), an elongation of the latent period of protraction was observed. The results at 4−6 and 24−26°C differed statistically significantly from those at 14−16°C, but not from each other. The minimal value of the studied parameter was revealed in the range of 34−36°C.

As it was already noted, the studied group of cells innervates the locomotor ciliated epithelium [7, 8], i.e., it is included in the locomotor network in the nervous system of *L. stagnalis*. Our experiments on velocity of locomotion demonstrated that the degree of its change is associated with the Q_{10} coefficient valued determined for the given group of neurons. This fact emphasizes again involvement of these neurons in this type of behavior. Hence, manifestations of life activity of molluscs are determined in many ways by the character of functioning of the appropriate neural elements. Thus, the heat coma temperature in Littorina approximately coincides with the temperature at which the heat block of the neuronal spontaneous activity in the nervous system occurs [9]. The heat block of endogenous activity of burst-type neurons was noted in Limax [10]. The temporary inhibition of the spontaneous activity in ganglia at an elevation of temperature is characteristic for several groups of invertebrate animals—cockroaches, crayfish, slugs [11].

The change of the AP firing rate is characterized by the curve (Fig. 3a) with the saturation plateau in the range of 24−26°C, after which it bends abruptly as a consequence of a reduction in the spike firing rate. It is to be noted that as the temperature rises, a decrease of the electric negativity of the membrane is observed (Fig. 3b). The MP value was minimal in the range of 34−36°C. Most likely, these facts are a consequence of changes of neuronal membrane permeability to cations. In particular, molluscan neurons were shown to increase their Na^+-permeability with heating [12]. As a result, a rise of the AP firing rate in A-cluster neurons and, as a consequence, of the velocity of movements of the whole animal seems to take place. With a further elevation of temperature, a prolonged depolarization develops to result in inactivation of Na^+-channels. For instance, the squid nerve fiber loses its excitability if MP becomes more positive than the −60—−50 mV level [13]. In A-cluster neurons of *L. stagnalis* this value seems to be at the level of about −40 mV. Indeed, a more substantial drop of the MP magnitude leads to an obvious disturbance of neuronal activity (Fig. 2d). A change of the characteristic activity pattern may indicate the direct adverse effect of high temperatures not only on A-cluster neurons, but also on modulatory interneurons that "stand" above the ciliated epithelium motoneurons and determine their activity (A-cluster-rhythm). These changes are reflected in the inhibition of locomotion, which also

may be promoted by a reduced adhesion of the foot to the substrate. A pronounced increase of locomotion at temperatures of 30°C and higher (practically immediately after placing the animals into these conditions) emphasizes again that such temperatures are very unfavorable for molluscs. A high movement velocity seems to be an intention to leave this temperature zone as soon as possible (i.e., a peculiar kind of "active" escape is observed). If these high temperatures are further maintained, the above-described changes in characteristics of the work of A-cluster neurons start to appear in a corresponding way (Fig. 2d).

Preservation of the work capacity at low temperatures in locomotor neurons, although to a rather limited extent, seems to be necessary for the mollusc to be able to find a suitable place to survive under the unfavorable conditions (the ability of animals to move is preserved) until the start of the hypobiotic state observed in this situation [14]. This is facilitated by a high MP value, which provides AP generation in spite of an increase in the threshold potential. The preservation of the neuronal activity indicates that interneurons determining it are not damaged, i.e., the temperature decrease is much more favorable for manifestations of the mollusc life activity, than its increase. By the character of change in the AP amplitude at 4–6°C, this group of neurons was not uniform (Fig. 3c). The changes had different directions. Thus, even within one homogeneous group, neurons may respond to a temperature change in a different way.

The increase in the latent period of protraction at 4–6 and 24–26°C might be explained as a manifestation of an exploratory reaction to new, comparatively comfortable conditions. Naturally, in this case some time delay should be observed as compared to control, particularly at a decreased temperature. The rapid protraction at 34–36°C reflects a behavioral reaction under extremely unfavorable conditions: the animal tries to leave this zone for the shortest time. Under conditions of temperature stress the initial defense reaction is quickly replaced by the exploratory behavior with the goal to avoid a dangerous situation. The exploratory behavior seems to be supported by the endogenous opioid system in the mollusc brain [15], which excites locomotor neurons and allows ignoring nociceptive stimuli. These behavioral reactions facilitate a rapid escape of animals from the zone with extremely uncomfortable temperature conditions before the onset of the heat coma.

Thus, at action of unusually high temperatures on the mollusc organism, a peculiar state develops, the heat coma, that is characterized by changes of the whole physiological state of the organism. Most likely, a process of a steady excitation (a phenomenon similar to N.E. Vvedenskii's parabiosis) is developed in the nervous system, this excitation being caused by a disturbance of the ionic mechanisms of conductivity of neuronal membranes, which results in inhibition of several physiological processes (including locomotion).

The data presented above allow concluding about a dependence of some characteristics of the locomotor activity in *L. stagnalis* on the environmental temperature, which is a result of effects on the corresponding neuronal structures controlling the given type of behavior. The function performed (locomotion) determines, in many aspects, the character of responses of neural elements to new temperature conditions.

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