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COMPARATIVE AND ONTOGENIC PHYSIOLOGY

Temperature Dependence of Monoamine-Induced Pulmonary Respiration in the Mollusc *Lymnaea stagnalis*

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Abstract—Pulmonary respiration (spontaneous and mediated by intracavitary administration of monoamines) has been studied in molluscs at different ambient temperatures (5, 15, and 25°C). Monoamines (dopamine, serotonin, and adrenaline) were established not to broaden the temperature diapason realization of the respiratory behavior. Microelectrode studies of the spontaneous electrical activity of the *Lymnaea stagnalis* respiratory network neurons (RPeD1, VD4, and Vi-cluster cells) revealed that both spontaneous and monoamine-induced respiration programs had been terminated under hypothermia conditions. The indicated effects are suggested to be due to the temperature dependence of the chemical, predominantly peptidergic, transmission of signal between neurons of the central respiratory rhythm generator in Lymnaea.

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Key words: central rhythm generator, dopamine, neuron, behavior, invertebrates.

INTRODUCTION

The effect of ambient temperature on the performance of vital activities by vertebrates and invertebrates does not cast doubts [1]. Temperature dependence and/or sensitivity of any biological process is traditionally considered to obey the van't Hoff–Arrhenius rule, according to which the rate of a chemical reaction changes 2–3 times when temperature increases or decreases by10°C. At the same time, most living systems function in a certain temperature regime restricted by the upper (heat) and lower (cold) threshold values. The mechanisms providing for these limits at the cellular level, particularly the neuronal one, may be diverse [1, 2]. They include, in particular, both deterioration of the membrane lipids [3] and a change of conformation of protein molecules (ion channels and ion pumps) responsible for maintenance of transmembrane ion gradients [1]. It has also been suggested that at disturbance of temperature conditions there is changed the receptor sensitivity to controlling factors [2]. This, in turn, may predetermine the "turning on" or "turning out" of a certain physiological function at reaching a certain temperature.

To study temperature dependence of the intercellular interactions based on the chemical way of the signal transmission, from the methodical point of view, it is quite convenient to use ectothermal invertebrates, i.e., the animals whose body temperature actually corresponds to the environmental temperature. Besides, for CNS of many of them, including several molluscan species, there are identified positions and connections neurocytes in nerve centers [4].

In the freshwater mollusc *Lymnaea stagnalis*, the best studied with respect to its cellular structure is the neuronal network controlling pulmonary respiration (Fig. 1). The presence of monoamines

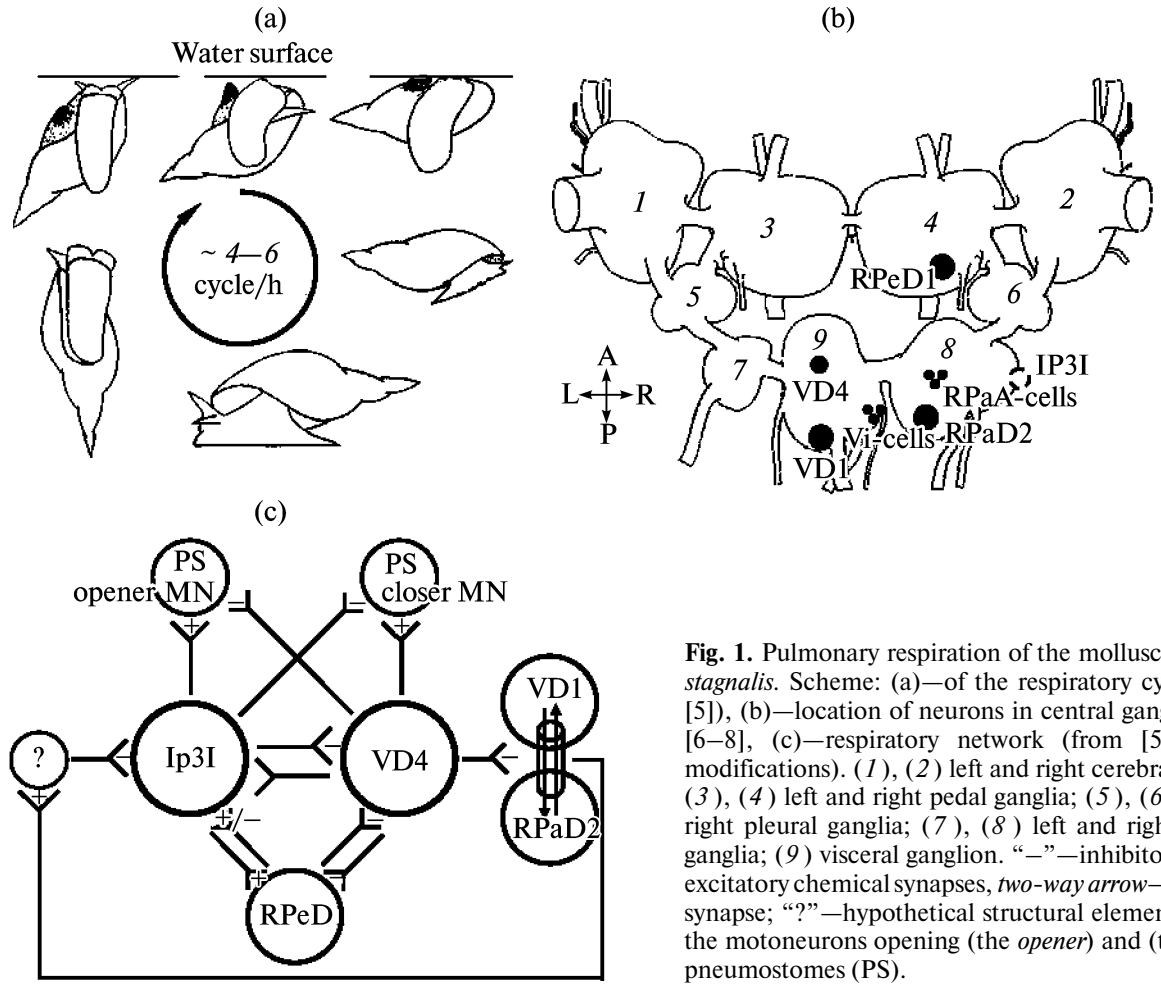

Fig. 1. Pulmonary respiration of the mollusc *Lymnaea stagnalis*. Scheme: (a)—of the respiratory cycle (from [5]), (b)—location of neurons in central ganglia (from [6–8], (c)—respiratory network (from [5–8] with modifications). (*1*), (*2*) left and right cerebral ganglia; (*3*), (*4*) left and right pedal ganglia; (*5*), (*6*) left and right pleural ganglia; (*7*), (*8*) left and right parietal ganglia; (*9*) visceral ganglion. "–"—inhibitory, "+"—excitatory chemical synapses, *two-way arrow*—electrical synapse; "?"—hypothetical structural elements; *MN*—the motoneurons opening (the *opener*) and (the *closer*) pneumostomes (PS).

and dopamine in particular in the central nervous system of the pond snail is known to be essential for the proper functioning of its respiration program [5, 6]. At the same time, changes in temperature conditions of existence also result in cardinal readjustment of pulmonary respiration [9], so that this process may be either intensified (within a certain temperature optimum) or terminated (at reaching the "heat" or "cold" boundaries). It can be suggested that the effect of monoamines is able to enlarge or to narrow the temperature diapason, in which the neuronal program of pulmonary respiration is realized. This paper is just dealing with the experimental checking of the above-indicated hypothesis.

MATERIALS AND METHODS

The work was carried out on a representative of freshwater molluscs, the pond snail *Lymnaea stagnalis* (L.). Animals were kept at the laboratory in aquariums at the temperature of 14–16°C, each animals getting at least 1 liter of water and free access to food (lettuce and dandelion leaves). Adult individuals weighing 3–4 g (with the shell length of 25–35 mm) were used in the experiments.

To study the respiratory behavior of the animals, they were transferred to the 0.5 liter vials half filled with water, 1 snail per vial. The number of respiratory acts (opening and closing of the pneumostome) within 1 h of observation and the duration of the respiration act were recorded. The study was performed at water temperatures of 5.0 ± 0.5, 15.0 ± 0.5, and 25 ± 0.5°C. The effect of chemicals of the monoamine family (serotonin, dopamine, and adrenaline; Sigma, USA) was analyzed as their fresh solutions were injected directly into the cephalopedal sinus of snails, the foot punctured with a syringe. These compounds were administered in the amount of 4 μg per 1 g of ani-

mal body weight in the volume of 0.1 ml of saline. Since the total hemolymph volume in the snails is about 1 ml, the resulted dilution of injection was 1:10. The animals administered with 0.1 ml of saline served as the control group. For the dopamine solution, ascorbic acid (50 μM) was added as an antioxidant, and ascorbate at the concentration mentioned above injected with saline was used for the control in this case. Tests were started 20 min after the drug administration.

Electrophysiological experiments were performed on isolated CNS preparations. They were pretreated for 5 min at 20°C with pronase solution (Protease E, type XIV, Sigma, USA) at a concentration of 1 mg/ml prepared on normal saline, for the perineural sheath to soften and microelectrodes to penetrate neurons more easily. Electrical activity of neurons was recorded after a pretreated preparation had been washed in the fresh saline for 30 min. Intracellular recording of neuronal electrical properties was performed by using Ag/AgCl electrodes. Micropipettes were filled with a 2.5 M KCl solution (microelectrode resistance 10–40 MOhm). As an indifferent electrode, the chlorinated silver wire was used. Amplified electrical signals were fixed on an H327-3 recorder band and displayed in parallel on the screen of a C1-74 oscilloscope.

For perfusion (0.1 ml/min) of isolated CNS preparations, the normal physiological solution was used which contained (mM): NaCl—44.0; KCl—1.7; $CaCl_2$—4.0; $MgCl_2 \cdot 6H_2O$—1.5; HEPES—10.0; pH 7.8 ± 0.03. Fresh monoamine solutions were applied directly on the dorsal surface of central nerve ganglia. The perfusion system was switched off temporarily, no longer than for 5–10 min, to maintain a steady-state concentration of a studied compound.

Neurons were identified by their size, location in the CNS, color, electrophysiological characteristics of action potentials, resting potential value, and pattern of spontaneous activity. The work was carried out on the CNS cells involved in pulmonary respiration of *Lymnaea stagnalis*, i.e., interneurons of the respiratory central pattern generator (CPG)—RpeD1 and VD4—and motoneurons controlling pneumostome movements (Vi-cluster cells) [6, 7].

A specially designed chamber based on the Peltier semiconductor element provided a possibility of maintenance and a change of the designed experimental temperatures in the diapason from 2 to 35°C with the ±0.1°C precision.

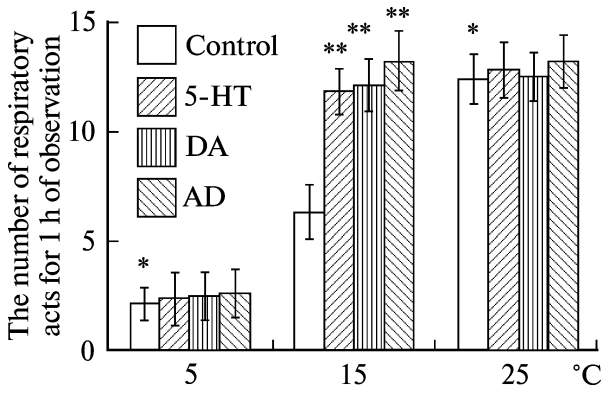

Fig. 2. Frequency of pulmonary respiration of the mollusc *Lymnaea stagnalis* under conditions of action of monoamines at different water temperatures. *—significant at $p < 0.05$; **—significant at $p < 0.05$ as compared with control at this temperature; 5-HT—serotonin; DA—dopamine; AD—adrenaline.

The experimental data were processed by the variation statistics methods [10]. The number of observations (n) is indicates separately for each experimental series. The data are presented as $\bar{x} \pm S_{\bar{x}}$. The significance of the obtained differences was evaluated by Student's t-criterion with $p < 0.05$ considered significant.

RESULTS

Effect of temperature on monoamine-induced respiratory behavior of Lymnaea stagnalis. The normal respiratory act of *Lymnaea stagnalis* consists in a complex of stereotypical movements (Fig. 1a). It begins from the snail approaching the water surface. When its lips touch the surface film, the counterclockwise movement of the shell is observed; then locomotion is inhibited and the pneumostome is protracted. The respiratory orifice opens and the respiration itself begins to take, on average, 45–60 s. After that the pneumostome closes and the snail as a rule leaves the water surface.

Changes in respiration rate under effect of monoamines at different water temperatures are presented in Fig. 2. At 15°C, the monoamine

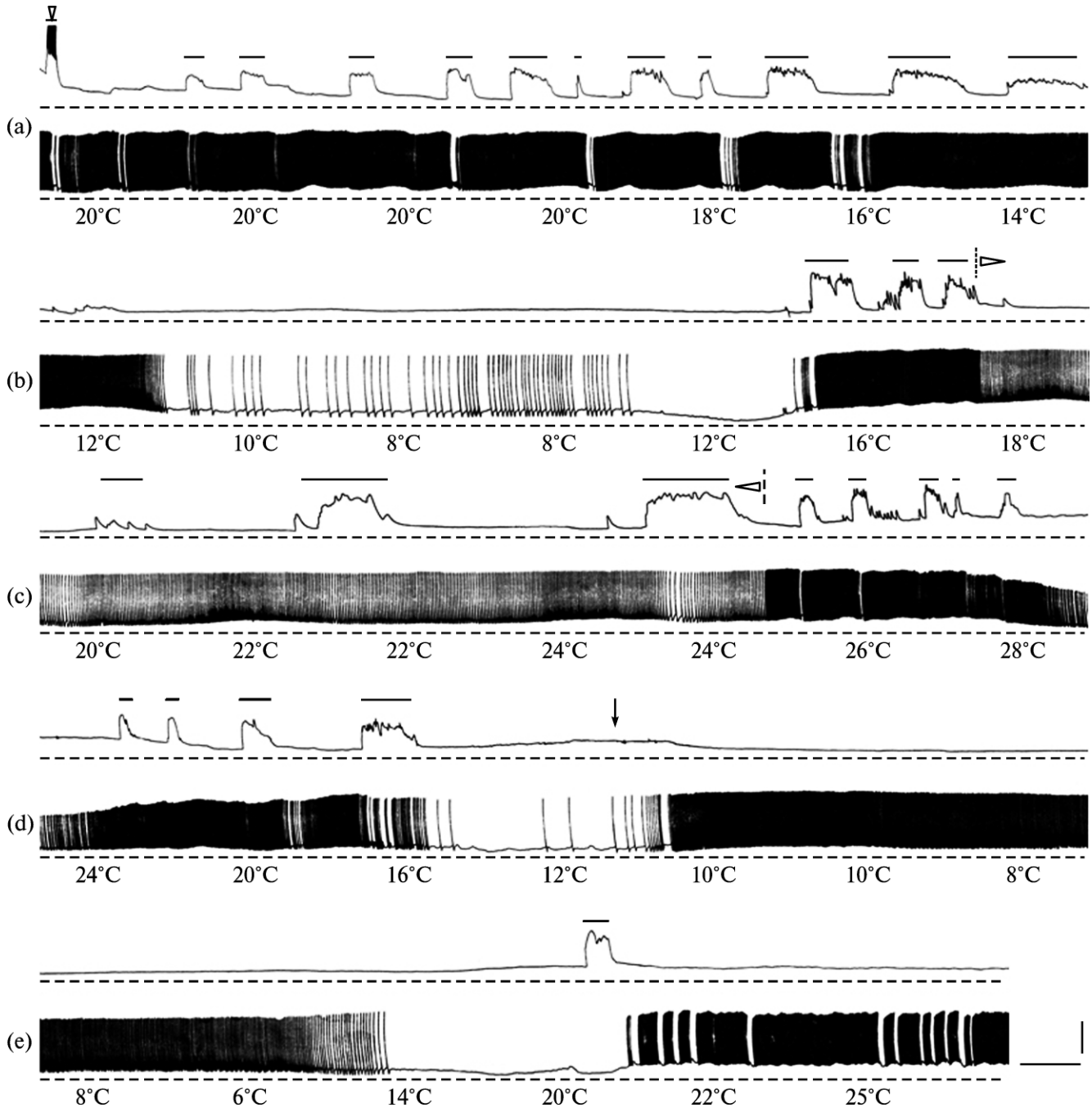

Fig. 3. Effect of temperature on spontaneous and monoamine-induced respiratory rhythms in the nervous system of *Lymnaea stagnalis*. Presented is the continuous record of electrical activity (a–e). *The upper line*—the VD4 membrane potential; *the lower line*—the RPeD1 membrane potential. Escape of the VD4 from the Ip3I inhibitory effect is designated by *dash*; adrenaline application (the final concentration of 10^{-6} M)—by *arrow*. Stimulation of VD4 by current impulse (part (a)) is marked by *dash with triangle*. *Horizontal broken lines* are presented for better illustration of the membrane potential changes. Time calibration—40 s. Amplitude calibration—60 mV (for VD4) and 50 mV (for RPeD1). For the neurogram area restricted by vertical broken lines with triangles (b, c), time calibration—20 s.

administration led to an intensification of respiration. The number of respiratory acts for 1 h of observation increased significantly ($p < 0.05$) as compared with control (6.3 ± 1.2, $n = 15$) and

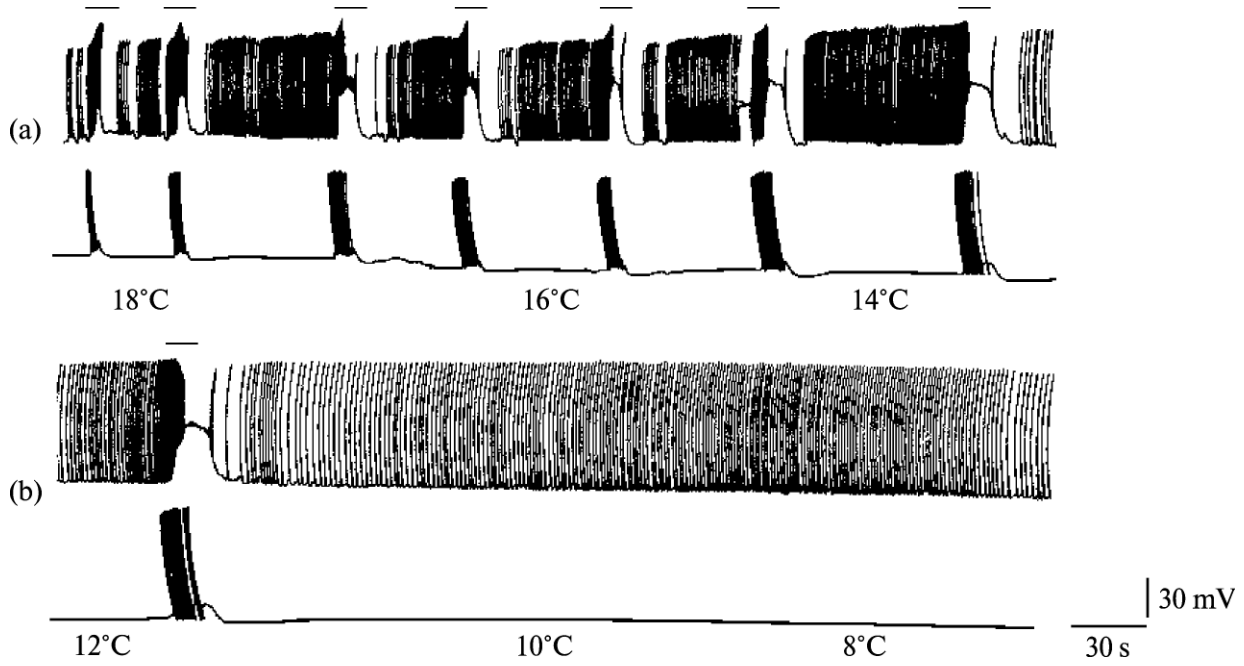

Fig. 4. Effect of temperature on the monoamine-induced respiratory rhythm in the nervous system of the mollusc *Lymnaea stagnalis*. The continuous record of electrical activity is presented. *The upper line*—the membrane potential of the neuron of the Vi-group; *the lower line*—RPeD1. The Ip3I activity (synaptic inputs) is designated by *dash*.

reached 12.1 ± 1.2, $n = 15$ (dopamine), 11.8 ± 1.0, $n = 15$ (serotonin), and 13.2 ± 1.3, $n = 15$ (adrenaline). At the same time, duration of the respiratory act did not change. At the lower and higher water temperatures, monoamines caused no increase in ventilation rate as comparative with control for this diapason and did not change the stereotypic complex of respiratory behavior. After normalization of temperature conditions, the monoamine-treated snails demonstrated the somewhat increased (1.4–1.5 times) respiratory activity as compared with animals of the control group. The latent periods of response to monoamines were minimal for serotonin (for the first 30 min) and more prolonged for dopamine and adrenaline (for 30–60 min).

Effect of temperature on electrical activity of the respiratory network neurons in Lymnaea stagnalis. The central respiratory pattern generator (CPG) represents the typical neuronal oscillator (Figs. 1b, 1c). In its structure, the VD4 and Ip3I neurons play a role of half-center cells. Electrostimulation of VD4 leads to the appearance of excitatory postsynaptic potentials (EPSP) in motoneurons innervating the pneumostome closing muscles and, simultaneously, to generation of inhibitory postsynaptic potentials (IPSP) in motoneurons innervating the pneumostome opening muscles. Electric stimulation of the Ip3I neuron causes opposite effects. Responsible for the initial activation of the neuronal ensemble are the RPeD1 neuron and the pair of electrically coupled neurons, VD1/RPaD2 [5–8].

Spontaneous neuronal activity of the respiratory CPG took place in about a half of the snails ($n = 15$) maintained at 15°C. In the indicated regime (Fig. 3a), frequency of alternated activity bursts of cycles Ip3I and VD4 amounted to 0.8 ± 0.1 cycles/min ($n = 10$). The lower temperature boundary (Fig. 3b) of the Ip3I spontaneous activity, at which this neuron exerts no detectable effect on neurons synaptically connected with it, was found to be 12.5 ± 0.4°C ($n = 10$). This is therefore the lower limit for the whole functioning of the respiratory CPG. During normalization of temperature conditions, the Ip3I activity became to be traced again on the respiratory network neurons that are synaptically connected with this half-center (i.e., RPeD1, VD4, and Vi-cluster cells). A temperature boundary for "recovery" of the synaptic

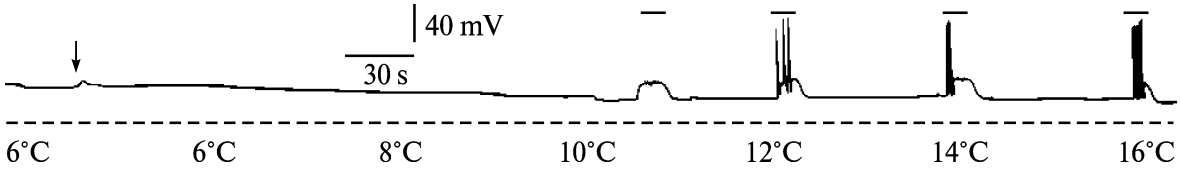

Fig. 5. The monoamine-induced rhythm in the nervous system of the mollusc *Lymnaea stagnalis* during normalization of temperature conditions. Neurogram of the RPeD1 electrical activity is presented. The Ip3I activity (synaptic inputs) is designated by *dash*, the dopamine application—by *arrow*. The *broken line* illustrates a change of the membrane potential.

component of the Ip3I activity was at 14.0 ± 0.1°C (n = 10) which is significantly different from the temperature of activity disappearance ($p < 0.05$) (Fig. 3b). The frequency of alternate spikings of the Ip3I and VD4 spontaneous activity increased with temperature rise by 10°C up to 25°C (Fig. 3c) and made up 1.4 ± 0.2 cycles/min (n = 10). The temperature quotient (Q_{10}) value calculated on the basis of these data was found to be 1.75 ± 0.12 (n = 10).

Under conditions of normothermia (15°C), the frequency of alternate Ip3I and VD4 activity bursts induced by monoamine application (10^{-4}–10^{-6} M) onto isolated CNS preparations of *Lymnaea stagnalis* differed significantly ($p < 0.05$) from the spontaneous one and reached 1.2 ± 0.1 cycles/min (Fig. 4a). Meanwhile, the lower temperature boundary for the monoamine-induced activity of the respiratory CPG neurons did not differ from that for spontaneous activity (Fig. 4b). Monoamine application did not change the pattern of the respiratory CPG neuron functioning in the nerve system of *Lymnaea stagnalis* after the temperature had been elevated by 10°C. The respiratory CPG activation under the monoamine effect was possible only within a certain temperature diapason. Thus, at a decrease of temperature below the limit of cessation of the Ip3I spontaneous activity, the monoamine application did not produce the characteristic pattern of respiratory rhythm (Fig. 5). The synaptic inputs caused by the Ip3I neuron activity appeared only at a rise of temperature to 13.9 ± 0.3°C (n = 6) and higher. Even in the presence of the initial spontaneous respiratory pattern in the CNS preparations at temperatures of 10°C and below, the exogenous monoamine application did not restore their respiration program (Fig. 3d). The characteristic respiratory pattern appeared only at normalization of temperature conditions (Fig. 3e).

DISCUSSION

The temperature dependence of the CRP functioning is characteristic of many representatives of invertebrates: molluscs [11], insects [12, 13], crustaceans [14], and annelids [15]. The observed changes in the neuronal patterns are often associated with a disturbance of ion homeostasis in CNS [13]. At the same time, it has been shown that an increase in concentration of biogenic amines (dopamine, octopamine) can serve a link for establishment of thermoprotector properties in respiratory neuronal networks in locusts [12].

Oscillations of the catecholamine level in the molluscan hemolymph and nervous tissue are noted at a change in environmental conditions including temperature. Meanwhile, alterations in dopamine concentration can occur in opposite directions. Thus, in some bivalves, its content in tissues has been found to decrease with rise of temperature, specifically in hemolymph of the scallop *Chlamys farreri* [16] and in CNS of the blue mussel *Mytilus edulis* [17]. On the contrary, the heat stress leads to increased dopamine concentration in hemolymph of the oyster *Crassostrea gigas* [18]. Fluctuations in the content of other biogenic amines (serotonin, adrenaline/noradrenaline) in tissues are, as a rule, opposite in their direction as compared with those of dopamine [16–18].

In *Lymnaea stagnalis*, under low water temperatures, the content of neurotransmitters (particularly that of dopamine and serotonin) in CNS also decreases, even in individual neurons [19]. This can be suggested to be accompanied by a reduction (a fall) of the efficiency of chemical synaptic transmission in monoaminergic cell contacts. With respect to the pond snail, this is to mean a restriction of pulmonary respiration, as the activity of the dopamine-containing RPeD1 neuron, which is

crucial for initiation of the entire respiration program in CNS of *Lymnaea stagnalis*. Thus, in preparations of the nervous system of the cold-adapted individuals, any functional synaptic connections between the respiratory CPG neurons (RPeD1, VD4) and the motor periphery are absent [20, 21]. Interestingly, the intracavitary administration of monoamines to snails does not affect frequency and duration of respiration at temperatures other than 15°C (normothermia). In other words, even excessive doses of a neurotransmitter are not capable of changing the respiratory pattern under conditions of hypo- and hyperthermia. Moreover, the dopaminergic neurotransmission is preserved, although in restricted form, in CNS of *Lymnaea stagnalis* under a short-term temperature decline by increasing progressively with subsequent elevation of temperature [20].

The situation with peptidergic transmission is different. Under conditions of hypothermia, the FMRFamidergic synaptic connections formed by the VD4 neuron are almost completely but reversibly inactivated, as shown by data on cold-adaptation in molluscs and at a short-term cold exposure [20]. This conforms well the observed restriction of pulmonary respiration in snails under conditions of hypothermia. In this connection it is worth mentioning the critical role of VD4 in performing pulmonary respiration in Lymnaea [22]. The temperature dependence of chemical synaptic transmission in CNS of Lymnaea is determined by the processes unrelated to disturbances in conductivity of the postsynaptic membrane. Thus, the ability of monoamines to selectively de- or hyperpolarize most neurons forming the respiratory network [5] is also preserved (Figs. 3d and 5) at lower temperatures (5°C). The same applies to postsynaptic effects of FMRFamide at 7–9°C [23]. In this connection it can be suggested that the thermodependent disturbances of chemical neurotransmission in CNS of Lymnaea are associated with a decline in neurotransmitter release into the synaptic cleft. As a result, inactivation of the synaptic translation between the respiratory CPG cells is responsible for disturbance of work of the entire neuronal oscillator. Obviously, under the formed conditions, the exogenous monoamine application alone is not sufficient for recovery of the functional activity of the respiratory network.

CONCLUSION

Thus, in the neuronal network controlling realization of pulmonary respiration in the mollusc *Lymnaea stagnalis*, the monoamine action does not lead to enlargement of broaden the temperature diapason of functioning of the respiratory program. Its temperature dependence is determined predominantly by physiological preservation of synaptic contacts (of the dopamine- and peptidergic nature) between neurons of the central respiratory pattern generator. It is this preservation that predetermines the character of interactions of the network cells.

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