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Effect of temperature on synaptic transmission between identified neurones of the mollusc *Lymnaea stagnalis*

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Abstract

The mollusc, *Lymnaea stagnalis*, has been used as a model to study the mechanisms of temperature-dependent processes in the central nervous system. Effects of temperature changes on transmission in monosynaptic connections, made by the FMRFamide-containing neurone VD4 and the giant dopaminergic neurone RPeD1 with follower neurones, were recorded with intracellular microelectrodes. In the temperature range of 4–6°C, inhibitory postsynaptic potentials (IPSP) in response to VD4 stimulation were not observed in postsynaptic cells while the IPSPs persisted in the RPeD1 followers. A temperature rise resulted in a sharp increase in the IPSP amplitude in followers of both VD4 and RPeD1. In isolated nervous systems taken from molluscs which have been kept at 4–6°C for 2 weeks and more, no coupling between VD4, RPeD1 and synaptically connected cells was seen in the full experimental temperature range. The synaptic coupling recovered only after maintaining the molluscs at a water temperature of 14–16°C for at least 2 days. The changes observed in synaptic responses to temperature alterations correspond to the behaviour of the molluscs.

Keywords: FMRFamide; Dopamine; Cold adaptation; Synapse; Gastropoda

Temperature is one of the most important environmental factors. Living organisms demonstrate different sensitivity to temperature changes. Conditions of natural habitation of the fresh-water pond snail *Lymnaea stagnalis* show a wide variation of temperature during the day and/or seasons. It can vary from 0°C (in winter some molluscs are even dwelling in the ice) to 35°C (on hot summer days). Apparently *Lymnaea* has special mechanisms for surviving in these varying conditions. Evidently, the central nervous system (CNS) must play an important role in these processes. Effects of temperature on *Lymnaea* neurones and their synaptic connections were reported only for a few identified neurones [3,10].

At present, there are a lot of questions concerning thermoregulation and thermoadaptation at the neuronal level both in vertebrates and invertebrates. It is presumed that changes in the functional condition of synapses determine behavioural adaptation, and molluscs may be useful models for investigation.

The FMRFamide-containing neurone VD4 and the giant dopaminergic neurone RPeD1 in the CNS of Lymnaea are interneurones involved in the respiratory central pattern generator (CPG) [11]. During temperature changes, life manifestation of *Lymnaea stagnalis*(respiratory behaviour in particular) undergoes reorganization. The aim of the present work is to find out what functional changes in synapses, formed by VD4 and RPeD1, occur under the conditions of temperature changes.

Specimens of *Lymnaea stagnalis* (L.) were collected locally, kept for up to 4 weeks in tap water at 16–18°C and fed on lettuce. All experiments were done on snails weighing 2–4 g. The animals were anaesthetized in 0.2 M $MgCl_2$ [4]. Isolated brains of molluscs were bathed in normal physiological saline (at 20°C) which consisted of (mM): NaCl 44.0, KCl 1.7, $CaCl_2$ 4.0, $MgCl_2$ 1.5, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] 10.0. The pH was adjusted to 7.5 with 0.1 M NaOH. Protease E type XIV (Sigma) was used to soften the perineurium (1.5 mg/ml for 10 min at 20°C). Individual neurones were impaled with glass microelectrodes filled with 2.5 M KCl; electrode resistance was 20–50 MΩ. Central neurones were identified according to their location, size, colour and electrophysiological characteristics (see the maps in Refs. [2,8]). A presynaptic cell was stimulated by injection of a depolarizing current (2 nA, 3 s) pulse, and the amplitude of

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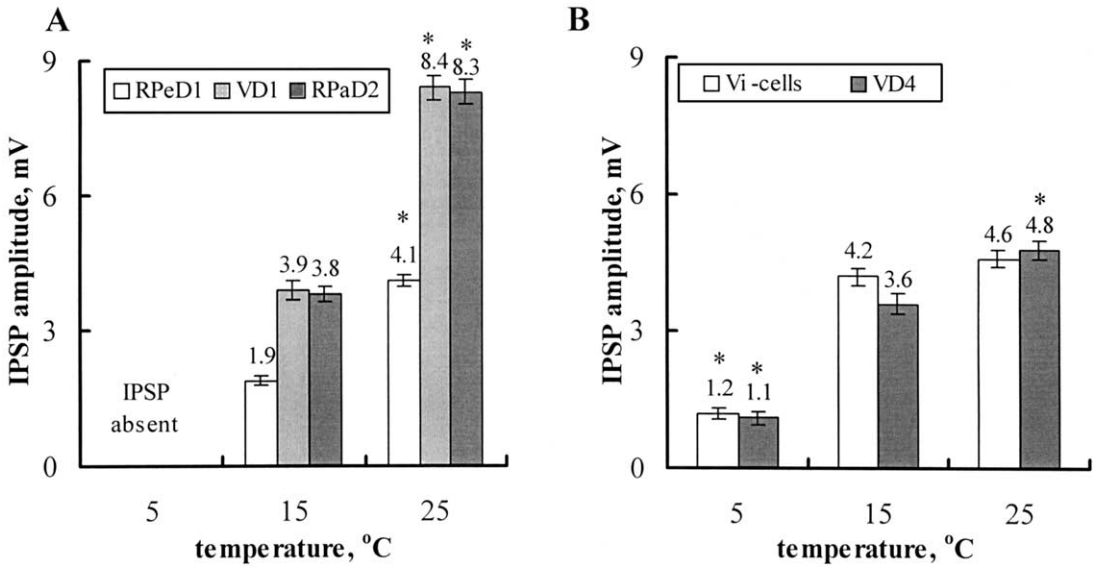

Fig. 1. IPSP amplitudes in neurones chemically connected with VD4 (A), and RPeD1 (B) at different temperatures. *Significant relative to the value at 15°C ($P < 0.05$). Data present the mean value (numbers above the columns) ± SEM ($n = 6$ for each synapse).

inhibitory postsynaptic potentials (IPSP) was recorded in synaptically coupled neurones. The stimuli elicit trains of action potentials in the presynaptic neuron with 3.0–3.5 Hz frequency (ten action potentials per burst). Electrophysiological signals were amplified, displayed on an oscilloscope and recorded by pen recorder. The temperature was maintained and changed using a laboratory-made thermostat based on a Peltier assembly. Q_{10} coefficients were determined using temperature rises from 15 to 25°C.

Interneurone VD4 is located on the dorsal surface of the visceral ganglion. Its soma (size about 50 μm) is white and contains FMRFamide [1]. VD4 plays an essential role in respiratory behaviour [13]. In all our isolated preparations this neurone was silent, without spontaneous spike activity. The giant dopamine cell RPeD1 is involved in many physiological processes in *Lymnaea*. It plays a key role in generating the respiratory rhythm in *Lymnaea*'s CNS [14].

We studied effects of temperature changes on synapses formed by VD4 with a neurone RPeD1, electrically coupled cells VD1/RPaD2 and also on synapses formed by RPeD1 with neurone VD4, cells from the Vi-cluster of the visceral ganglion. Their chemical nature and the monosynaptic origin of these connections has been shown previously [2,8,12]. In all these cells, the intracellular stimulation of VD4 or RPeD1 induced IPSPs of different amplitude.

The IPSP amplitudes in the neurones under study at different temperature are shown in Fig. 1. We did not observe IPSPs in response to VD4 stimulation at 4–6°C (Fig. 2A). However, in the same temperature range, in response to RPeD1 stimulation IPSPs were elicited in all neurones investigated (Fig. 3A). A temperature rise resulted in a sharp increase in the IPSP amplitudes in the neurones connected with VD4 (Fig. 2C) and RPeD1. The Q_{10} coeffi-

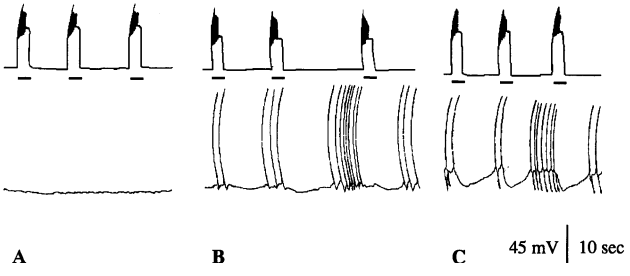

Fig. 2. Intracellularly recorded IPSPs in one neurone, chemically connected with VD4, at different temperatures. IPSPs in RPaD2 in response to VD4 stimulation at different temperatures, bottom trace. Top trace – membrane potential in VD4, above depolarizing current injected into VD4 marked by bar. Action potentials were elicited 1.5–2 s after stimulus beginning. (A) 4–6°C; (B) 16–18°C; (C) 26–28°C.

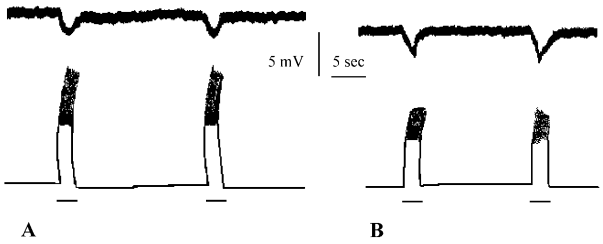

Fig. 3. Intracellularly recorded IPSPs in one neurone, chemically connected with RPeD1, at different temperatures. IPSP in VD4 in response to RPeD1 stimulation at different temperatures, top trace. Bottom trace – membrane potential in RPeD1, above depolarizing current injected into RPeD1 marked by bar. (A) 4–6°C; (B) 14–16°C.

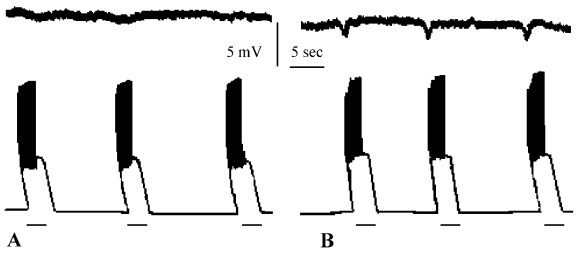

Fig. 4. Intracellularly recorded IPSPs in one neurone, chemically connected with RPeD1, at 16–18 °C (received from the nervous system preparations, stored for 24 h at 4–6°C). IPSP in VD4 in response to RPeD1 stimulation at different temperatures, top trace. Bottom trace – membrane potential in RPeD1, above depolarizing current injected into RPeD1 marked by bar. (A) Immediately after the normalization of temperature conditions; (B) after incubation of the preparations in a normal saline at 14–16°C for 2 h. Action potentials were elicited about 2 s after the start of the stimulus.

cient appeared to be 2.2 ± 0.20 ($n = 12$) for all VD4 followers cells. For the neurones making connections with RPeD1, the Q_{10} coefficient was a little bit lower: 1.3 ± 0.12 ($n = 6$) for VD4 and 1.03 ± 0.24 ($n = 6$) for Vi-cells. Returning to the normal temperature range of 14–16°C, the IPSPs recovered to the control amplitudes both in the VD4 and RPeD1 follower neurones.

In the isolated nervous system preparations ($n = 10$) from molluscs which had been kept at 4–6°C for 2 weeks and more, no coupling between the VD4 or RPeD1 and synaptically connected cells was revealed in all experimental temperature ranges. After the 4–6°C periods the coupling recovered only after maintaining molluscs at water temperature of 14–16°C for at least 2 days.

The high temperature adaptation of molluscs kept for 2 weeks and more at water temperature 24–26°C did not influence the pattern of transmission via the synapses formed by VD4 and RPeD1.

In isolated nervous system preparations ($n = 6$) stored for 24 h at 4–6°C immediately after normalization of temperature, no IPSPs were detected in response to VD4 and RPeD1 electrical stimulation, in all experimental ranges. After further incubation of the preparations in a normal saline at 14–16°C for 2 h, the synapses formed by VD4 or RPeD1 were functionally restored (Fig. 4B). The IPSP amplitudes, however, were somewhat lower (0.24 times) than those at 14–16°C in molluscs which had been kept at 14–16°C for a long time.

The size of the synaptic response increases with temperature [6,15]. Indeed, the IPSP amplitudes of the neurones connected with VD4 and RPeD1 depended on the temperature in which the recording took place. A relatively high Q_{10} (specially for VD4 followers) may indicate the dependence of chemical (particularly peptidergic) synaptic transmission on ambient temperature which is much higher than that of electrical coupling.

As mentioned above, VD4 is one of the important neurones in the respiratory CPG of *Lymnaea*. With a temperature decrease below a certain level for a rather long period of time, apparently active respiratory behaviour (lung ventilation) aimed at taking up oxygen ceased. These changes in behaviour may be due to cold inactivation of synaptic contacts, formed by VD4 with relevant neurones. Similar effects of temperature adaptation on chemical transmission through synaptic inactivation have been reported in cold adapted fish [9]. The mechanism may be seasonal (i.e. at different environmental temperatures) variability of neurotransmitter concentration in the brain of *Lymnaea*, as previously demonstrated for dopamine and serotonin [7]. In our experiments dopaminergic connections drop at low temperature only after a long period of time (hours) and were stable during temperature changes lasting only about 15 min. Vice versa, peptidergic synapses stop their functioning even during a brief temperature decrease. This can be explained by strong temperature dependence of FMRFamide release (postsynaptic effects of FMRFamide-related neuropeptide persist [5] at low (7–9°C) temperatures). We also hypothesize that disconnections between the CPG's neurones and its follower cells, observed in our experiments during temperature decrease, may be involved in the mechanism of *Lymnaea* hibernation during the cold months of the year.

The present data demonstrate that in the CNS of *Lymnaea stagnalis*: (i) chemical synapses formed by FMRFamide-containing neurone VD4 are inactivated at low temperatures (below 10°C); (ii) connections between dopaminergic neurone RPeD1 and its follower cells are observed at a wider temperature range (4–30°C); (iii) temperature determines the size of synaptic response. It is suggested that the synaptic response to temperature changes largely determines the behavioural pattern of the molluscs.

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