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ELECTRICAL COUPLING BETWEEN IDENTIFIED *LYMNAEA* NEURONS: NITRIC MONOXIDE AND TEMPERATURE ACTION

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Abstract. The isolated CNS of the freshwater mollusk *Lymnaea stagnalis* was used as a model to study the role of NO-mediated mechanisms. The coefficient of electrical coupling between two viscero-parietal peptidergic neurons (VDI/RPd2) was promptly increased by both DEA/NO (10^{-6} M) and 8-Br-cGMP (10^{-5} M), whereas 8-Br-cAMP (10^{-5} M) reduced the coupling. The coupling coefficient was also reduced during temperature rise. NO-donors effects on action potential frequency and membrane potential magnitude were closely mimic by 8-Br-cGMP (10^{-6} M). We suggest that gap junction proteins (connexins) are cGMP-gated ionic channels.

**Keywords**: Nitric oxide – cGMP - temperature - gap junction - *Lymnaea*

Introduction

The radical gaseous nitric oxide (NO) is a versatile neuromodulatory molecule widely distributed across the animal kingdom [1, 2]. In the CNS of the freshwater pulmonate snail, *Lymnaea stagnalis* the presence of NO synthase (NOS) activity was demonstrated in central nervous and peripheral tissues [3]. Putative nitricergic neurons are mainly located in the buccal ganglia; there are a relatively small number of these cells in the central ganglionic ring (i.e. in the cerebral, pedal, pleural, pedal, parietal and visceral ganglia). In *Lymnaea*, NO activates buccal motor patterns [4] and is considered as a mediator of chemosensory inputs to the feeding network [5]. Although in this and other grazer gastropod mollusks (Helix and Aplysia), NO acts via cGMP-dependent pathways [6]. The cAMP-dependent mechanisms can be also involved in intracellular signaling. In neuronal tissues cAMP and cGMP pathways are in reciprocally inhibitory connections and this systems might mediated many physiological processes on cellular level. Temperature is very important factor in mollusk life activity. It can mightly change the CNS’s functions by neurons and synapses activity modulation.

Here, we compare the action of NO-donors and temperature on electrophysiological characteristics of a pair of multifunctional peptidergic neurons (VDI/RPd2). We also investigate the effects of a cGMP and cAMP analogue on the electrical coupling between these cells.

Materials and methods

Specimens of *Lymnaea stagnalis* (2-4 g) were collected locally, kept for up to 4 weeks in tapwater at 14-16°C and fed on the lettuce. Freshly isolated CNSs were used in electrophysiological tests. Intracellular recording was performed by a conventional microelectrode technique. Glass microelectrodes (10-40 MOm) were filled with 2.5 M KCl. Central neurones were identified according to their location, size, colour and electrophysiological characteristics (see the map in [7]). The coupling coefficient (CC) was measured as $CC = \Delta V_{RPd2}/\Delta V_{VD1}$, where $\Delta V_{RPd2}$ and $\Delta V_{VD1}$ are changes in the membrane potential of RPd2 and VD1 respectively, and the hyperpolarizing current (0.5 nA) was injected in VD1. The temperature was maintained and changed using a specially made thermocell based on a Perlitt assembly.

NO donors (Diethylamine/nitric oxide sodium complex (DEA/NO), S-Nitroso-N-acetylpenicillamine (SNAP) - all from RBI, were prepared immediately before use in HEPES buffered saline for Lymnaea (in mM: NaCl -44, KCl - 2, MgCl2- 2, CaCl2-4, Hepes -10, pH=7.8). Membrane permeable cAMP and cGMP analogues (8-Br-cAMP and
8-Br-cGMP, respectively) were used. All drugs tested were added into the experimental chamber via a perfuse system, and all concentrations indicated in the text are the final concentrations.

Results

Firing rate of the investigated neurons is characterized by linear dependence in the temperature range 5 – 25 °C. At temperatures over this range spike frequency go down. Both cells were strongly depolarized during cooling and temperature rise. Membrane potential and firing rate dynamics presented at fig. 1.

![Graphs A and B showing firing activity and membrane potential vs temperature](image-url)

Fig 1. Firing rate (A) and membrane potential (B) of VD1/RPD2 neurons at different environmental temperatures. Data present mean value ± SEM. * P < 0.05 vs control (Student’s t-test), n=12.

Coupling coefficient between VD1 and RPD2 decreased during temperature rise up to 0.45±0.02 at 25 °C (fig. 2). Network input resistance was elevated to 20 % during heating.

![Temperature effects on neuronal activity and coupling](image-url)

Fig. 2. Effects of temperature (A. – 15 °C, B – 25 °C) on spontaneous neuronal activity and on the electrical coupling between VD1/RPD2. Top trace — VD1, bottom trace — RPD2. Hyperpolarizing current injected in VD1.

Both DEA/NO and SNAP at 10^-4 M increased the electrical coupling coefficient between VD1 and RPD2 and network input resistance by about 20 %. Investigated neurons also were depolarized: VD1 on 10.2±2.1 mV, n=4; RPD2 on 5.3±1.2 mV, n=4. NO-donors induced tonic increase in action potential rate on 23 % to control (see for details fig 3, 4).

There were no effects of NO-donors application on the *Lymnaea* neurons at 5 °C. Coupling coefficient also was without any changes. At 25 °C nitric oxide effects were more stable and prolonged. Coupling coefficient was about 26-28 % over control at same temperature. Moreover even after 20 minutes of washing in the Ringer solution the meaning was over the control on 8-10 %.
Fig. 3. Coupling coefficient between VD1/RPD2 at different temperatures (A) and after NO-donors (DEA/NO, SNAP, 10⁻⁴ M) application (B). Data present mean value ± SEM. * - P <0.05 vs control (Student’s t-test), n=5.

Fig. 4. Effects of NO-donors (DEA/NO, SNAP, 10⁻⁴ M) application (an arrow) on spontaneous neuronal activity and on the electrical coupling between VD1/RPD2. Top trace — RPD2, bottom trace — VD1. Hyperpolarizing current injected in VD1.

Cell membrane permeable analogue cyclic nucleotide 8-Br-cGMP (10⁻³ M) induced alterations in coupling ratio (fig. 5), steady-state depolarization of VD1/RPD2 and increase in network input resistance closely resembling those induced by NO. Vica versa, 8-Br-cAMP (10⁻³ M) induces decrease in coupling coefficient and deep waves of hyperpolarization intermittent with short periods of bursts.

Fig. 5. Coupling coefficient between VD1/RPD2 in control (A) and during 8-Br-cGMP action (B). Top trace — RPD2, bottom trace — VD1. Hyperpolarizing current injected in VD1.
Discussion

Rapid dynamics of nitric oxide and temperature action on electrical coupling ratio is the evidence that fast gap junctions proteins (connexins) conformation changes involved in this processes. Previously was shown the presence of temperature dependent domains in the structure of connexins of crayfish [8]. It is known, that fast cGMP gated channels is activated in retinal ganglion cells by nitric oxide donors [9]. It's common knowledge that soluble guanylate cyclase is a main intercellular target of NO action. The main pathway of cyclic nucleotide action is protein kinase phosphorylation. It can induce reversible changes in connexins conformation and increase the coupling ratio. But, this mechanism need an least 10 minutes for its maximum development. In case of nitric oxide action the neuronal effects were observed during first minute after NO-donors application. We speculate that connexins are the member of cGMP gated channels family and subjected direct activation by the cytosolic cGMP. Cyclic AMP acts contrary NO-donors and cGMP. All effects slowly developed (about 10 minutes). Probably, cAMP acts via cAMP-dependent phosphodiesterases which decrease cGMP level in cells. Lowering of cGMP intracellular concentration transfer connexins in non permeable state resulting in coupling coefficient reduction. Cyclic AMP-induced neuronal pattern, coupling decrease and input network resistance elevation are very close resembling those induced in the VD1/RPD2 neurons by 5-hydroxytryptamine (5-HT) [10], 5-HT is a powerful stimulator of cAMP production in Lymnaea's CNS [11]. So, 8-Br-cAMP mimics action of stressful elevation of temperature, whereas 8-Br-cGMP mimics action of nitric oxide, that to be supposed released in the state of feeding arousal [5]. In this case NO action also could prevent the disconnection between electrically coupled cells and neuronal ensembles of visceral and parietal ganglions at all, during temperature rise.

Thus, gap junction and its modulation by nitric oxide and temperature is an important nervous system function in different environmental and behavioral states.

References