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DOPAMINE SUPPRESSES NEURONAL ACTIVITY OF *HELISOMA* B5 NEURONS VIA A D2-LIKE RECEPTOR, ACTIVATING PLC AND K CHANNELS

L. R. ZHONG, L. ARTINIAN AND V. REHDER *

Department of Biology, Georgia State University, Atlanta, GA 30302, USA

Abstract—Dopamine (DA) plays fundamental roles as a neurotransmitter and neuromodulator in the central nervous system. How DA modulates the electrical excitability of individual neurons to elicit various behaviors is of great interest in many systems. The buccal ganglion of the freshwater pond snail *Helisoma trivolvis* contains the neuronal circuitry for feeding and DA is known to modulate the feeding motor program in *Helisoma*. The buccal neuron B5 participates in the control of gut contractile activity and is surrounded by dopaminergic processes, which are expected to release DA. In order to study whether DA modulates the electrical activity of individual B5 neurons, we performed experiments on physically isolated B5 neurons in culture and on B5 neurons within the buccal ganglion *in situ.* We report that DA application elicited a strong hyperpolarization in both conditions and turned the electrical activity from a spontaneously firing state to an electrically silent state. Using the cell culture system, we demonstrated that the strong hyperpolarization was inhibited by the D2 receptor antagonist sulpiride and the phospholipase C (PLC) inhibitor U73122, indicating that DA affected the membrane potential of B5 neurons through the activation of a D2-like receptor and PLC. Further studies revealed that the DA-induced hyperpolarization was inhibited by the K channel blockers 4-aminopyridine and tetraethylammonium, suggesting that K channels might serve as the ultimate target of DA signaling. Through its modulatory effect on the electrical activity of B5 neurons, the release of DA *in vivo* may contribute to a neuronal output that results in a variable feeding motor program.

Key words: dopamine, D2-like receptor, *Helisoma trivolvis*, PLC, K channels, excitability.

INTRODUCTION

Dopamine (DA) acts as a neurotransmitter and neuromodulator in the central nervous system, where it regulates a wide range of neuronal circuits in both vertebrate and invertebrate species (Harris-Warrick et al., 1998; Murphy, 2001; Bevan et al., 2006). How DA modulates the electrical excitability of individual neurons, alters the output of neuronal circuits, and finally affects an animal's behavior are central questions that are being addressed in many systems.

Gastropods have a relatively simple nervous system with identifiable neurons in different ganglia, providing a convenient model to study the role of DA in neuronal function. Previous studies have demonstrated that DA regulates various cellular and physiological functions in gastropods. DA plays critical roles in the initiation and regulation of respiratory and feeding central pattern generator activities in *Helisoma trivolvis* and *Lymnaea stagnalis* (Quinlan et al., 1997; Magoski and Bulloch, 1999). The DA-releasing right pedal dorsal 1 (RPeD1) neuron in *Lymnaea* promotes neurite extension in its *in vivo* target neuron, but collapses growth cones of non-target neurons (Spencer et al., 1996, 2000). Electrophysiological results have revealed that DA exposure alters neuronal electrical properties (Dobson et al., 2006). DA induces a sustained depolarization of the membrane potential in the *Helisoma* B19 neuron (McCobb et al., 1988b). On the other hand, a hyperpolarizing response induced by DA is shown in multiple *Lymnaea* neurons (de Vlieger et al., 1986; Audesirk, 1989; Magoski et al., 1995; Dobson et al., 2006). Considering the diverse roles that DA plays in gastropod nervous systems, it is important to develop a detailed understanding of which signaling pathways are activated by DA in particular neurons.

DA receptors are grouped into two subtypes: D1-like and D2-like receptors (Missale et al., 1998; Beaulieu and Gainetdinov, 2011). They are G-protein-coupled receptors, and separated based on their ability to modulate adenylate cyclase activity. D1-like receptors stimulate adenylate cyclase via G_s, whereas D2-like receptors inhibit it via G_i. In addition to the regulation of cyclic adenosine monophosphate (cAMP) signaling, DA receptors can also activate phospholipase C (PLC) via $G_{q/11}$ (Lee et al., 2004). These DA-activated intracellular signals have been reported to result in the modulation of a variety of ionic currents (Harris-Warrick et al., 1998). For example, an A-type K current has been shown to be

*Corresponding author. Address: Department of Biology and Neuroscience Institute, Georgia State University, P.O. Box 4010, Atlanta, GA 30302-4010, USA. Tel: +1-404-413-5412; fax: +1-404-413-5301.
E-mail address: vrehder@gsu.edu (V. Rehder).
Abbreviations: 4AP, 4-aminopyridine; cAMP, cyclic adenosine monophosphate; DA, dopamine; I_A, A-type K current; LP, lateral pyloric; PeA, pedal A; PLC, phospholipase C; RMP, resting membrane potentials; R_n, input resistance; RPeD1, right pedal dorsal 1; TEA, tetraethylammonium.

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regulated differentially by different DA receptors in the crustacean pyloric dilator and lateral pyloric (LP) neurons (Zhang et al., 2010). Therefore, a study of signaling pathways activated by DA in individual neurons is necessary to explain DA-associated effects on neuronal excitability.

B5 neurons in *Helisoma*, like their homologs in the closely-related species *Lymnaea* and *Clione*, innervate the esophagus and are involved in feeding behavior (Perry et al., 1998; Malyshev and Balaban, 2009). Excitation of the neurons in *Lymnaea* leads to contraction of the foregut, while their silencing results in relaxation (Perry et al., 1998). Immunohistochemistry shows the presence of dopaminergic neurons in the buccal ganglion, and DA is found to modulate the neural circuitry of feeding (Quinlan et al., 1997; Murphy, 2001). Here, we tested the effect of DA on the electrical activity of B5 neurons and investigated the signaling pathway activated by DA.

We report that DA hyperpolarized the membrane potential and switched B5 neuron from a state of spontaneous firing to being silent in both *in vitro* and *in situ* recording conditions. To assure that DA release from sources within the ganglion could not complicate the interpretation of the data, we performed all the following experiments on B5 neurons that had been physically isolated from the buccal ganglia and maintained in cell culture. Investigation of the signaling mechanism underlying the DA-induced hyperpolarization revealed that DA acts on a D2-like receptor, signals via PLC, and subsequently opens two types of K channels, a 4-aminopyridine (4AP)-sensitive K channel and a tetraethylammonium (TEA)-sensitive K channel. The modulatory effect of DA on the electrical activity of B5 neurons may contribute to the coordinated output of various neurons involved in the snail-feeding motor program.

EXPERIMENTAL PROCEDURES

Neuronal culture

Identified B5 neurons were removed from the buccal ganglion of the freshwater pond snail *H. trivolvis* and plated into Falcon Petri dishes as previously described (Rehder and Kater, 1992). Briefly, neurons were plated onto poly-L-lysine (hydrobromide, MW, 70–150 kDa, 0.25 mg/ml; Sigma, St. Louis, MO, USA)-coated glass coverslips attached to the bottom of 35-mm cell culture dishes (Falcon 1008). B5 neurons were kept in conditioned medium at room temperature. Conditioned medium was prepared by incubating two *H. trivolvis* brains per 1 mL of Leibovitz L-15 medium (Invitrogen, Carlsbad, CA, USA) for 3–4 days (Wong et al., 1981). Neurons were used for experiments 24–48 h after plating. The composition of L-15 medium was as follows (mM): 44.6 NaCl, 1.7 KCl, 1.5 $MgCl_2$, 0.3 $MgSO_4$, 0.14 KH_2PO_4, 0.4 Na_2HPO_4, 1.6 Na pyruvate, 4.1 $CaCl_2$, 5 HEPES, 50 μg/ml gentamicin, and 0.15 mg/ml glutamate in distilled water, pH 7.4.

Electrophysiology

Recordings from the *Helisoma* B5 neuron in whole-cell current-clamp mode were obtained as described previously (Artinian et al., 2010). The patch electrodes were pulled from borosilicate glass tube (outer diameter 1.5 mm; inner diameter 0.86 mm; Sutter instruments) on a Sutter instruments micropipette puller (P-87) and heat polished (Micro Forge MF-830; Narishige) with a resistance of about 3–8 MΩ. Neurons were recorded using Axopatch 200B and 700B amplifiers (Molecular Devices, Union City, CA) and analog-to-digital converters (Digidata 1322 and 1440). Data acquisition and analysis were performed using pClamp software version 10 (Molecular Devices). Current-clamp configuration was used to record membrane potential, firing properties, and input resistance. Leibowitz L-15 medium was normally used as extracellular recording solution. In some experiments, L-15 medium was replaced with normal saline containing in (mM): 51.3 NaCl, 1.7 $CaCl_2$, 1.5 $MgCl_2$, and 5 HEPES, pH 7.3–7.4 (127 mOsm). Intracellular recording solution contained (mM): 54.4 K-aspartate, 2 $MgCl_2$, 5 HEPES, 5 Dextrose, 5 ATP 0.1 EGTA (127 mOsm). TEA solution was made by replacing 20 mM NaCl with 20 mM TEACl. Low Cl solution was prepared by replacing 51.3 mM NaCl with 51.3 mM Na gluconate in the extracellular solution. Solution replacement was achieved through a gravity-based perfusion system (Warner Instruments). Membrane potentials were corrected for liquid junction potential caused by switching between solutions of different ionic composition. Resting membrane potential of spontaneous firing neuron was determined by measuring the value at the plateau of the depolarization phase before the membrane potential reached the threshold. Analysis of action potential properties was achieved by using the 'template search' function of Clampfit (pClamp 10, Molecular Devices). Initial broadening of action potentials induced by 4AP was measured as the action potential width at one quarter of action potential amplitude from the positive peak, where the effect of 4AP was most obvious. Measurement of input resistance was made by a series of hyperpolarizing current injections from −50 to −200 pA in steps of −50 pA for 3 s, and determined from the slope of a linear fit of the relationship between the peak change in membrane potential and the magnitude of the injected current (Robinson and Cameron, 2000).

For intracellular recordings from B5 neurons located within buccal ganglia, ganglia were pinned down in a dissection chamber containing normal saline. The ganglionic sheath in the vicinity of B5 neuron was cut open using a fine microknife. Neurons were impaled with sharp glass microelectrodes filled with 3 M KCl having resistances of about 20–50 MΩ (Sakurai et al., 2006). Neurons were recorded using Axoclamp 2B amplifiers (Molecular Devices, Union City, CA), and data acquisition and analysis were achieved with Spike2 software (Cambridge Electronic Design). Negative current (ranging from −0.2 nA to −1 nA, 1 s) was occasionally delivered to measure input resistance during recordings.

Pharmacological agents

All agents were purchased from Sigma. DA was dissolved in water to make a 100 mM stock solution. R(+)-SCH-23390 hydrochloride (D1 receptor inhibitor), (S)-(−)-Sulpiride (D2 receptor inhibitor), U-73122 hydrate (PLC inhibitor), U-73343 (inactive PLC inhibitor) were dissolved in dimethylsulfoxide (DMSO) to make 100, 100, 5, 5 mM stock solutions, respectively. For patch clamp experiments performed in cell culture, stock solutions were mixed with 50 μl of extracellular solution removed from the recording dish and directly and gently added back around the periphery of the dish for drugs to equilibrate to their final concentrations. For intracellular recordings from buccal ganglia, stock solutions were mixed in 500 μl of saline solution that was initially removed from the recording dish and then added back. The final concentration of DMSO in the extracellular recording solution was less than 0.1% and that concentration by itself had no measurable effect