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Effects of rolipram on induction of action potential bursts in central snail neurons

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Abstract

Effects of rolipram, a selective inhibitor of phosphodiesterases (PDE) IV, on induction of action potential bursts were studied pharmacologically on the RP4 central neuron of the giant African snail (*Achatina fulica* Ferussac). Oscillations of membrane potential bursts were elicited by rolipram and forskolin. The bursts of potential elicited by rolipram were not inhibited after administration with (a) calcium-free solution, (b) high-magnesium solution (30 mM) or (c) U73122. However, the bursts of potential elicited by rolipram were inhibited by pretreatment with KT-5720 (10 μM). Voltage-clamp studies revealed that rolipram decreased the total inward current and steady-state outward currents of the RP4 neuron. The negative slope resistance (NSR) was not detectable in control or rolipram treated RP4 neurons. TEA elicited action potential bursts and an NSR at membrane potential between −50 mV and −30 mV. It is suggested that the bursts of potential elicited by rolipram were not due to (1) synaptic effects of neurotransmitters; (2) NSR of steady-state *I–V* curve; (3) phospholipase activity of the neuron. The rolipram-elicited bursts of potential were dependent on the phosphodiesterases inhibitory activity and the cAMP signaling pathway in the neuron.

Keywords: Second messenger; Phosphodiesterase inhibitors; Snail; Central neuron; Rolipram; cAMP; Bursts of potential; Protein kinase

Introduction

Cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) regulated many biological animal behavior and cellular functions (Maurice et al., 2003). Elevation of cAMP in neurons that express PDE4s attenuated the rewarding properties of cocaine and morphine (Thompson et al., 2004). Elevated cAMP levels in dunce mutants with reduced phosphodiesterase activity cause enhanced nerve terminal arborization at larval neuromuscular junctions of *Drosophila* (Zhong and Wu, 2004). Phosphodiesterases (PDE) are responsible for hydrolysis of the cyclic nucleotides cAMP and cGMP and the enzyme also associated with the Alzheimer's disease brains (Perez-Torres et al., 2003). Rolipram, a selective inhibitor of phosphodiesterases (PDE) IV, attenuate the endogenous depression in the central nervous system (CNS) through elevation of intracellular cAMP and increasing synthesis and release of norepinephrine (Zhu et al., 2001). However, effect of rolipram on spontaneous action potential of central neuron was still unclear. Tetra-ethylammonium chloride (TEA), a blocker of the delayed outward K^+ current, elicited action potential bursts and negative slope resistance (NSR) in the RP4 neuron. The TEA-elicited action potential bursts were closely related to its NSR activity (Chen and Tsai, 2000). Whether rolipram can elicit NSR in the central neuron remained unknown. The aims of the study are to characterize the effects of rolipram on spontaneous action potential of the central snail neuron. We found that rolipram-elicited action potential bursts in the RP4 neuron and the effects were associated with phosphodiesterase activity and elevation of intracellular cAMP in the neuron and the effects were not associated with synaptic effects of neurotransmitters or NSR of steady-state *I–V* curve or phospholipase activity in the neuron.

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Materials and methods

Experiments were performed on the identified RP4 neuron from the subesophageal ganglia of the African snail, *Achatina fulica* Ferussac. The ganglia were pinned to the bottom of a 2 ml sylgard-coated perfusion chamber and carefully freed from the connective tissue sheath to allow easy identification and penetration by microelectrodes (Chen and Tsai, 1996, 2000; Lin and Tsai, 2003).

For intracellular recording, an Axoclamp 2A amplifier (Axon Instruments, Inc., USA) was used. Microelectrodes (5–6 MΩ) for recording membrane potentials were filled with 3 M KCl. The experimental chamber was perfused with control saline; composition (mM): NaCl, 85; KCl, 4; $CaCl_2$, 8; $MgCl_2$, 7; Tris–HCl, 10 (pH 7.6), at room temperature of 23–24, with perfusion speed of 6 ml/min. Calcium (Ca^{2+})-free saline was substituted with $CoCl_2$ for calcium ion in equimolar amounts (8 mM) (Kim et al., 1991). High-magnesium solution (30 mM) was prepared by either decreasing extracellular sodium ion concentration (Aldenhoff et al., 1983) or adding hyper-osmotically to control saline (Murakami and Takahashi, 1983). For the quality of neurons tested, neurons were studied only if they had resting membrane potentials more negative than −50 mV with the time constant at about 5–8 ms and the rate of rise of the action potentials at about 5–8 ΔV/s.

The same neurons were examined sequentially for control, drug treatments and in each of the tables (that is, tables present sequential experiments done on the same cells). The control result in physiological solution was recorded 60 min after electrophysiological recording on the RP4 neuron. The data for calcium-free solution were recorded 20 min after calcium-free solution treatment. The data recorded for rolipram in calcium-free solution was recorded in preparations pretreated with calcium-free solution for 20 min and further administration of rolipram (300 μM) for 20 min. To test the effect of synaptic transmission on the potential changes, preparation were perfused in calcium-free, high-magnesium solution (inhibition of transmitter releasing) for 20 min, and then, PDE inhibitors were further added in the perfusate on the RP4 neurons or as described in the text.

To test the role of phospholipase on the potential changes of the neuron, the U73122 (phosphoinositide-specific phospholipase C inhibitor) (Ellershaw et al., 2002) was added after PDE inhibitors elicit bursts of potential. To test the effects of protein kinase A activity on the neuron, KT-5720 (protein kinase A inhibitors) were added for 60 min before incubated with rolipram. The Control data in physiological solution were recorded 60 min after electrophysiological recording on the RP4 neuron. Data for rolipram (300 μM) and for KT-5720 (10 μM) were recorded 60 min after treatment. The data for KT-5720 (10 μM) control were recorded 60 min after KT-5720 (10 μM) treatment. The data for KT-5720 (10 μM) and rolipram (300 μM) were recorded in preparations pretreated with KT-5720 (10 μM) for 60 min and further administration of rolipram (300 μM) for 60 min (Table 2). To test the effect of adenyl cyclase on the potential changes in RP4 neuron, different concentrations of forskolin (30, 100 μM), an adenylate cyclase activator were examined.

The frequency of single spikes was presented as pulses per minute. The frequency of bursts was presented as bursts per minute. The bursts represent a change in the firing pattern from regularly spaced single spikes to one in which bursts of 2–20 action potentials were separated by large hyperpolarization of membrane potential (up to 6 mV) lasting 1–20 s. The resting membrane potentials, amplitudes of action potentials and the frequency of single spikes of action potentials were recorded 20–60 min after drugs administration or as mentioned in the text. Amplitude of action potential was measured from resting membrane potential to the peak of action potential. Phosphodiesterase inhibitors and other drugs were applied by extracellular incubation.

The ionic currents of the RP4 neurons were measured using two-electrode voltage-clamp method by means of a Gene clamp 500 amplifier (Axon Instrument Co.). All potentials and currents were recorded on tape via a digitalizing unit (Digidata 1200) and analyzed using a pCLAMP system (Axon Instruments, Inc., U.S.A.). For peak amplitude of total inward current, the currents were elicited by 70 ms depolarization of −50 to +30 mV from a holding potential of −60 mV. For steady-state outward currents, the currents were elicited by 5 s depolarization of −70 to +40 mV from a holding potential of −60 mV.

The amplitude and frequency of the action potentials, resting membrane potentials and currents after various treatments were compared with the pre-drug control by means of Student's two-tailed t test and paired t test. Student's t test was used when the samples in the control and experimental conditions were from different groups of preparation. Student's paired t test was used when the samples in the control and experimental condition were from the same groups of preparations. Differences were considered significant at $P < 0.05$.

Rolipram (PDE4 selective), TEA and U73122 (1-[6-[((17β)-3-methoxyestra-1,3,5[10]-trien-17-yl)amino]hexyl]-1*H*-pyrrole-2,5-dione) and forskolin were obtained from Sigma Chemical Company (St. Louis, MO, USA). KT-5720 was purchased from Calbiochem Ltd. (USA). TEA and forskolin were dissolved in distilled water. Rolipram, U73122 and KT-5720 were dissolved in dimethyl sulfoxide (DMSO). The concentration of DMSO we used (0.1%) did not affect the RMP, amplitude and frequency of spontaneous firing of action potentials in the RP4 neuron.

Results

The RP4 neuron of Achatina fulica Ferussac

The RP4 identified neuron had a resting membrane potential of −57.8 ± 1.7 mV (n = 10) and the neuron