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Different Concentration Selenium-modulated Action on Rat Dorsal Root Ganglion Neurons

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Abstract: In present study, a concentration-dependent effect of Selenium on rat dorsal root ganglion neurons was characterized using whole-cell patch-clamp recording. Among about 52% of the cells tested, the amplitude of membrane ion current increased, after administration of Selenium. In the presence of 0.001 nM (nanomolar) Selenium, the reversal potential and membrane conductance were changed. The above-mentioned actions suggest that selenium may play a physiological role in regulating the excitatory action on mammalian neurons.

Key words: Selenium, neuron, dorsal root ganglion, ion channel, membrane potential

INTRODUCTION

Selenium a trace mineral is an essential nutrient of fundamental importance to human kinds. Research in area has shown a hitherto unsuspected important role of this element to human health[1]. As a constituent of Selenoproteins, selenium has structural and enzymic roles, Selenium may be the most potent nutrient antioxidant, which has been shown to be a very potent anti-carcinogen and anti-mutagen. It also can reduce the risk of coronary disease at extremely low microgram doses. Selenium is a natural anti-inflammatory agent. In addition, various muscular wasting diseases such as myotonic dystrophy and lung diseases such as cystic fibrosis occur much more frequently in persons with Selenium deficiency. Several biochemical functions of selenium have been identified as a component of glutathione-peroxidase (GSHpx), phospholipid-hydroperoxide-glutathione-peroxidase (PH-GSHpx), iodothyrone 5'-deiodinase(5'-DI) and selenoprotein-P[2]. GSHpx is thought to regulate intracellular or extracellular hydroperoxide concentration[3]. PH-GSHpx is capable of reducing fatty acid hydroperoxide esterified to phospholipids[4]. Selenoprotein-P in plasma may be a free radical scavenger[5]. Selenium has additional important health effects particularly in relation to immune response and cancer prevention, which are almost certainly not exclusively linked to these enzymic functions. In recent study with PC12 cells, it has been demonstrated that overexpression of GPx in PC12 cells resulted in protection against METH cytotoxicity[6]. Selenium supplementation enhanced the element concentration in blood and seminal fluid but did not change the spermatozoal quality characteristics in subfertile men[7]. It has also been reported that Selenium has certain effects on animal reproductive organs fertility and indirect effect for a neural system[8-11], etc. Little is known, however, about the regulation of Selenium on neurons. We investigated the effect of Selenium on rat dorsal root ganglion neurons by the whole-cell patch-clamp technique and reported here modulation effects of Selenium at different concentration on neuron cells.

MATERIALS AND METHODS

Isolation of dorsal root ganglion neurons: 2-3-week-old Sprague-Dawley rats, irrespective of sex, were decapitated and the thoracic and lumbar segments of vertebrate column were dissected and longitudinally divided into two halves along the median lines on both dorsal and ventral sides. The rat Dorsal Root Ganglion neurons (DRG) together with dorsal and ventral roots and attached spinal nerves were taken out from the inner side of each half of the dissected vertebrate and transferred into Dulbecco's Modified Eagle's Medium (DMEM, Sigma) at pH = 7.4. After the removal of attached nerves and surrounding connective tissues, the DRGs were minced with iridectomy scissors and incubated with enzymes including trysin (type III, Sigma) 0.5 mg mL^{-1}, collagenase (type IA, sigma) 1.0 mg mL^{-1} and DNase (type IV, sigma)

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0.1 mg mL^{-1} in 5mL DMEM at 35°C in a shaking bath for 40 min. To stop the enzymatic digestion 1.25 mg mL^{-1} soybean trypsin inhibitor (type II-S1, Sigma) was added. The isolated neurons were transferred into a 35 mm culture dish and kept still for at least 30 min. All experiments were performed at room temperature (20-30°C)[12-18].

Solutions and electrophysiology: The external solution contained (in mM) NaCl 150, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1, HEPES 10, D-glucose 10, its osmolarity was adjusted to 340 m Osm with sucrose and pH was adjusted to 7.4 with NaOH. In voltage-clamp experiments, the patch-pipette (internal) solution contained 140 mM CsCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 11 mM EGTA, 10 mM Hepes, 2 mM ATP; the pH was adjusted to 7.4 with CsOH and the osmolarity was adjusted to 310 milliosmol. Solution of selenium was prepared daily in external solution, and selenium was added as Na_2SeO_3.

Current and voltage clamp experiments utilized the whole-cell recording configuration with a EPC-9 patch clamp amplifier (made in Germany). Pharmaceutical solutions were delivered by gravity flow from a linear barrel array consisting of fused silica tubes (i.d.=200 μm) connected to independence reservoirs; rapid solution exchanges (<50 m sec) were effected by shifting the electric-magnetic switch. Cells were constantly bathed in control solution flowing from one pipe barrel between Pharmaceutical applications. Culture dishes containing neurons were continuously perfused at 1-2 mL min^{-1} with normal external solution. Pipettes had resistances of 2-5 MΩ. Series resistances and junction potential were compensated. Membrane currents were filtered at 5 kHz and sampled at 0.5-5 ms intervals. Data analysis is based on IGOR Pro 4.03 (WaveMetrics, Inc), Statistical significance of results was assessed by using student's t tests or analysis of variance.

RESULTS

Figure 1A shows the variation in amplitude of membrane ion current that was modulated by the application of different Selenium concentration. In 41 of 78 neurons from rat dorsal root ganglion neurons, Selenium markedly increased the amplitude and decreased the desensitization rate of the current. Compared with control, in those neurons, 0.001 nM Selenium increased the amplitude of current by 22.5615±5.432% (n=10), 0.01 nM Selenium increased the amplitude of current by 17.6752±2.036% (n=20), 0.01 nM Selenium reduced the amplitude of current in 6 cells, 0.1 nM Selenium increased the amplitude of current by 13.1669±2.8997% (n=9), 0.1 nM Selenium reduced the amplitude of current by 14.1942±2.0953% (n=21), 1 nM Selenium increased the amplitude of current by 54.3427±7.7939% (n=2) and 1 nM Selenium reduced the amplitude of current by 48.8061±6.8477% (n=10). The decrease in desensitization rate appeared to be essential for the regulatory of Selenium, as Selenium greatly potentiated membrane ion current in 41 cells (of the 78) that exhibited desensitization. Details of the effects of Selenium on the desensitization of membrane ion current will be reported elsewhere. In Fig. 1B the dose-response curve of Selenium modulated membrane ion current was exponentially related. To determine whether Selenium alone (0.001-1 nM) modulated membrane ion current in other neurons, we also studied neurons from rat trigeminal ganglion and superior cervical ganglion. Similar results were obtained.

The current-voltage relation in Fig. 2A shows that 0.001 nM Selenium altered the reversal potential of membrane ion current. On average, the reversal potential of membrane ion current of the control was 45.33±1.5 mV and after adding 0.001 nM Selenium the reversal potential was 39.67±1.6 mV. This indicated that 0.001 nM Selenium enhanced membrane ion current and altered the reversal potential of membrane. Figure 2B shown the effect of membrane potential on Selenium-modulated membrane conductance. The effect of Selenium on membrane conductance had significantly different (analysis of variance; $p<0.005$; n=30) at membrane holding potentials between -80 and +40 mV. This suggested that the Selenium acted within the channel pore, while neither on nor near the exterior surface of the channel protein.

High concentration (>0.1 nM) of Selenium would inhibit excitation of neurons. To test this, two kinds of different concentrations of Selenium (0.1 nM (n=30) and 1 nM (n=12)) inhibited excitatory cells by 70 and 83.3%, respectively. Figure 3A indicated membrane ion current inhibited by 1 nM Selenium. Figure 3B indicated the membrane ion current inhibited by 0.1 nM and 1 nM Selenium. Data was obtained from a single dorsal root ganglion neuron with the membrane potential held at -60 mV. Traces were sequential (from left to right).

DISCUSSION

The observations reported indicated that 0.001 nM Selenium potentiated the excitatory action on neurons, 77% (n=26) cells excitation was potentiated by 0.01 nM Selenium. 70% (n=30) and 83.3% (n=12) cells excitation was inhibited by 0.1 and 1nM Selenium, respectively. Several lines of evidence suggested that effect of Selenium is attributable to within the channel pore rather

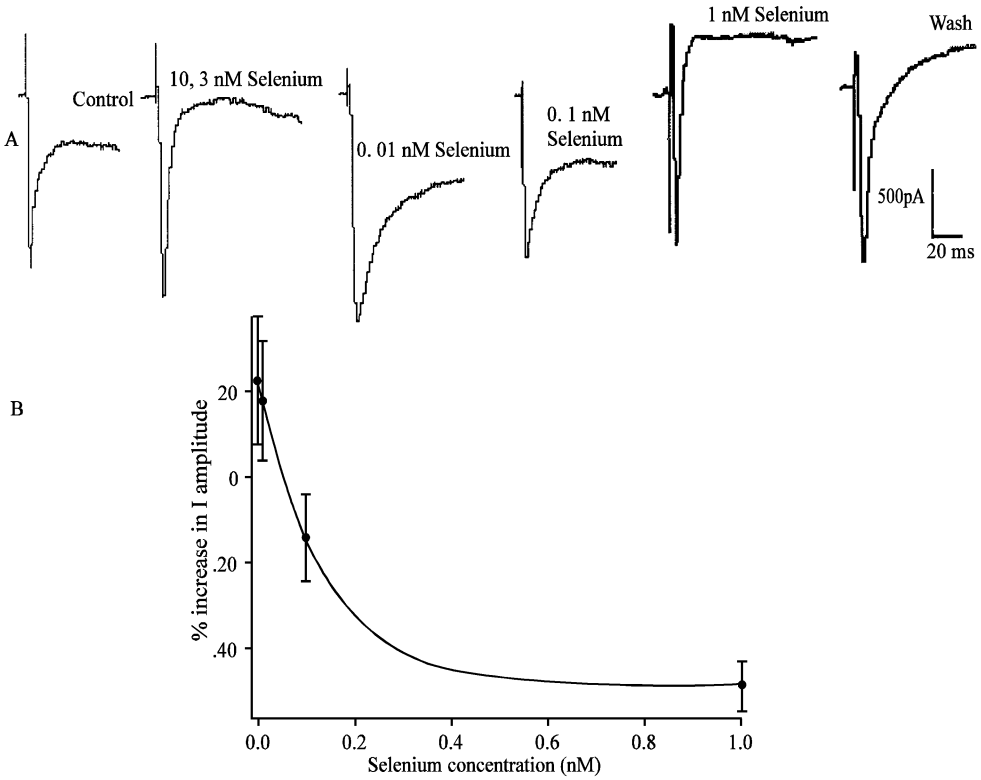

Fig. 1: Selenium modulated membrane ion current variation. Traces in (A) show the effects of different concentrations of selenium (1, 0.1, 0.01 and 0.001 nM) on membrane ion current. Records are sequential (from left to right). Current traces were obtained from a single rat dorsal root ganglion neurons voltage-clamped at -60 mV. (B) indicates I amplitude an decreasing current with the increase of Selenium concentration. Each point represents the Mean±SE of 10-26 rat dorsal root ganglion neurons voltage-clamped at –60mV; error bars not visible are larger than the size of the symbols. The curve shown fits in well with the exponential equation, $y=k_0+k_1*e^{-k2*x}$, where k_0=-48.8499±0.261, k_1=71.766±0.326, k_2=7.28942±0.0925

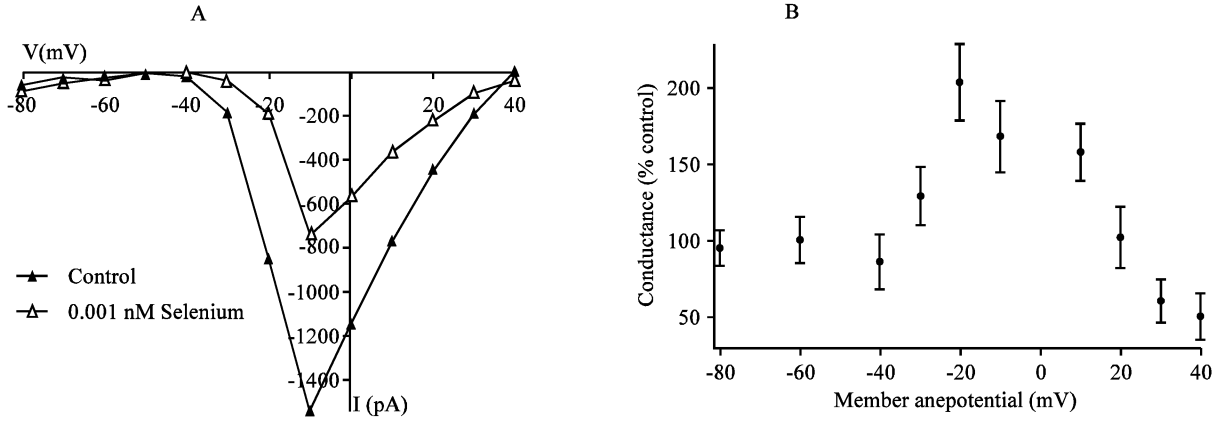

Fig. 2: 0.001 nM Selenium modulated membrane ion current has voltage-dependence. (A) Selenium alter the reversal potential of membrane ion current. The graph plots current (I) enhanced after adding 0.001 nM selenium (▲) and the control situation (△) at membrane potentials (v) between –80 mV and +40 mV in a single rat root ganglion neuron. (B) indicates the relation Membrane conductance and membrane potential. The plots percent conduction of control (G=I/V, where, I and V are current and voltage, respectively) by 0.001 nM Selenium modulation as a function of membrane holding potential in rat root ganglion neurons. Data points are given as means±SE (n=10). Membrane conductance was significantly different at holding potential between –80 and +40 mV (analysis of variance: $p<0.005$; n=10)

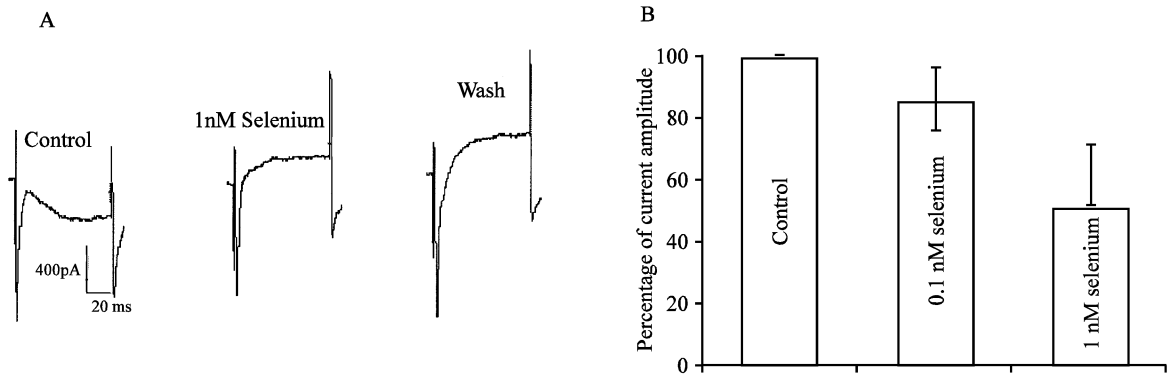

Fig. 3: 0.1 and 1 nM selenium inhibition membrane ion current. (A) Records are sequential (from left to right) current traces obtained from a single rat root ganglion neuron voltage-clamp at –60 mV, shown 1 nM concentration selenium alone inhibited membrane ion current. (B) percentage inhibition of membrane ion current by 0.1 and 1 nM Selenium

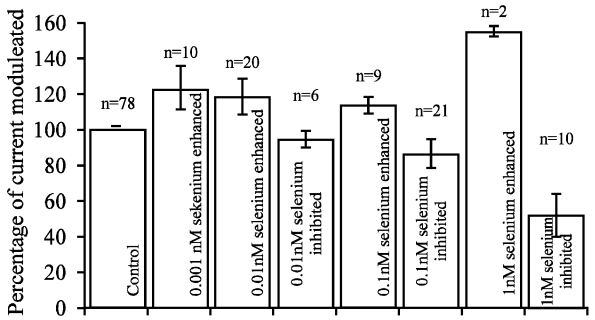

Fig. 4: Effects of enhancing and inhibiting Selenium at different concentrations and the number of neurons involved

than on or near the exterior surface of the channel protein. Firstly, the effect of Selenium observed showed that low concentration of Selenium markedly enhanced membrane ion current and high concentration inhibited excitatory neurons. Figure 4 summarized the inhibiting and enhancing action of different concentration of Selenium on excitatory neurons. Secondly, Selenium gave rise to membrane reversal potential change. Thirdly, Selenium led to membrane conductance variation, Supposed that membrane conductance did not change, membrane protein structure would not change. But membrane conductance, as a function of membrane holding potential in rat root ganglion neurons, showed an obvious variation, it suggested that Selenium changed ion channel protein structure.

Recent studies suggested that Selenium had great importance to human health[1]. At the same time, the effects of Selenium on central nervous system of S-D rats; provided evidences for its neurotoxicity. However, present studies shown that almost 74% cells excitation was inhibited by 0.1 and 1 nM Selenium, but 80% cells excitation was potentiated by 0.001 and 0.01 nM Selenium, thus Selenium (as a trace element) may have potential pharmacological effects on central nervous system.

Also recent evidences has reinforced the importance of adequate Selenium to health. Selenium intake at a much higher level than required to saturate the selenoenzymes appeared to be beneficial for cancer and AIDS treatment. Further research is needed to clarify the optimal nutrition at level of Selenium. In this context, the planned precisely and selected trials should give a definitive answer on the role of selenium to reduce nervous system disease management in the future.

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