The Inhibitory Effects of Nano-Ag on Voltage-Gated Potassium Currents of Hippocampal CA1 Neurons

Zhaowei Liu,1 Guogang Ren,2 Tao Zhang,3 Zhuo Yang1
1The Key Laboratory of Bioactive Materials, College of Medicine, Ministry of Education, Nankai University, Tianjin 300071, China
2Science and Technology Research Institute, University of Hertfordshire, Hatfield, Herts AL10 9AB, United Kingdom
3College of Life Science, Nankai University, Tianjin 300071, China

Received 20 November 2009; accepted 15 February 2010

ABSTRACT: The application of the nano-sized materials continues to grow at a rapid rate in the fields of medicine, biotechnology, and environmental technology. Voltage-gated potassium currents play a key role in excitable cellular viability and function, especially in the central nervous system. The aim of this study was to investigate the actions of silver nano-particles (nano-Ag) on voltage-activated potassium currents in hippocampal CA1 neurons using whole cell patch-clamp technique. The hydrodynamic mean diameter of nano-Ag (10−5 g mL−1) was 223.9 nm in artificial cerebrospinal fluid (ACSF). Both types, transient potassium (∣IA∣) and delayed rectifier potassium (∣IK∣) current amplitudes were inhibited by the nano-Ag (10−5 g mL−1). The nano-Ag particles produced a hyperpolarizing shift in the activation-voltage curve of ∣IK∣ and inactivation-voltage curve of ∣IA∣ and also delayed the recovery of ∣IA∣ from inactivation. The results suggest that nano-Ag may have potential to alter the excitability of neurons by depressing the potassium channels. © 2010 Wiley Periodicals, Inc. Environ Toxicol 00: 000–000, 2010.

Keywords: nano Ag; pyramidal neurons; transient outward potassium current (∣IA∣); delayed rectifier potassium current (∣IK∣)

INTRODUCTION

Silver nanoparticles (nano-Ag) can be applied to a range of healthcare products, such as broad-spectrum antimicrobial agents (Baker et al., 2005; Kim et al., 2008, 2009), biosensors (Sun et al., 2008), wound dressings for burns, scald, skin donor, and recipient sites (Chen et al., 2006; Muangman et al., 2006; Lu et al., 2008), contraceptive devices, coating of bone prostheses (Cohen et al., 2007). In daily life, consumers may have products including nano-silver in wash system, ink (Kim et al., 2007), clothing, underwear, and socks (Lee et al., 2007; Vigneshwaran et al., 2007).

Nanoparticles exposure caused a variety of impairments to neuron (Tang et al., 2008), microglia (Au et al., 2007) in normal animals, and aggravated the brain pathology (Sharma and Sharma, 2007). Though the neurological toxicity of silver is not clinically ascertained, several seizure cases have been related to exposure to silver or silver compounds (Ohbo et al., 1996; Mirschattari et al., 2004). In recent study about the neurotoxicity of nano-Ag, a neuroendocrine cell line (PC-12 cells) was exposed to silver nanoparticles. The results showed that nano-silver (5 × 10−3 g mL−1) reduced dopamine concentration, and the nanoparticles of
Ag were found probably more toxic than that of Mn nanoparticles (Hussain et al., 2006). These suggested that nano-Ag might have significant pathological consequences and risks to mammalian brain.

Voltage-gated potassium currents play crucial roles in modifying neuronal excitability and activity. The aim of the present study was to investigate the possible action of nano-Ag on voltage-gated potassium currents in hippocampal slices using whole cell patch-clamp technique.

**MATERIALS AND METHODS**

**Slice Preparation**

Male Wistar rats, bred in the Experimental Animal Center of Chinese Academy of Medical Sciences, were used on postnatal days 14–18. The experiments were conducted in accordance with the guidelines of the Medical Experimental Animal Administrative Committee of Nation. Horizontal slices that included the entire hippocampus and subiculum (400 μm in thickness) were prepared with a vibratome (VT1000M/E, Leica, Germany) and incubated with artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 1.25 KCl, 1.25 KH₂PO₄, 1.5 MgCl₂, 2.0 CaCl₂, 16 glucose. Slices were incubated in ACSF for at least 1 h before they were moved into the recording chamber. During recording/data logging, the slices were kept submerged in a chamber, which was perfused with ACSF. In the experiments, ACSF was saturated with 95% O₂ and 5% CO₂.

**Nano-Ag Particles and Solutions**

Nanoparticles of Ag were compounded at Research Institute of Science and Technology (RSTI), University of Hertfordshire, UK, using the raw materials originally obtained from QinetiQ Nanomaterials, produced through Plasma Nano-Technology (QNL TesimaTM) technology, England.

Microstructure of nano-Ag was obtained using transmission electron microscope (TEM, Tecnai G2 20 S-TWIN, FEI, USA). The purity of the prepared samples was studied by X-ray energy dispersive spectroscopy analysis (EDS).

The particle size of nano-Ag suspension (10⁻⁵ g mL⁻¹) in ACSF was characterized by dynamic light scattering (DLS) using a Zeta-PALS + BI-90Plus (Brookhaven Instruments, USA) at a wavelength of 659 nm. The scattering angle was fixed at 90°. The Zeta-potential of the nanoparticles (10⁻⁵ g mL⁻¹) was measured in ACSF with a combination of laser Doppler velocimetry and phase analysis light scattering (PALS) using Zeta-PALS + BI-90Plus. This technique uses a laser, which is being passed through the sample, to measure the velocity of the particles in an applied electric field of a known value.

Stock solution (10⁻⁵ g mL⁻¹) of nano-Ag (autoclaved) was prepared in Milli-Q water and dispersed by ultrasonic vibration for 20 min. Its suspension was stirred on vortex agitator before every use.

**Electrophysiological Recordings**

For whole-cell recording, slices were transferred into a recording chamber (1 mL volume) placed on the stage of a modified upright infrared DIC microscope equipped with Nomarski optics. Hippocampal CA1 neurons were visualized on a television monitor connected to a low light sensitive CCD camera. Recordings were performed using conventional patch-clamp techniques. Signals were filtered at 5 kHz and digitized at a sampling rate of 2 kHz. The series resistance was compensated at least 60%. Leakage and capacitive currents were subtracted off-line using a P4 subtraction procedure.

Data acquisition and analysis were performed on the computer using EPC10 patch-clamp amplifier (HEKA, Germany). After seal formation and membrane rupture, the cells were allowed to stabilize for 3–5 min before starting pulse protocols.

Two types of voltage-dependent potassium channels known as transient potassium currents (Iₖ) and delayed rectifier potassium currents (Iₖ; Klee et al., 1995) were studied in the present experiments. Iₖ was separated in presence of tetraethylammonium chloride (TEA-Cl, 25 mM). According to the different characterization of Iₖ and Iₖ, Iₖ can be obtained by inactivating the Iₖ from the total potassium current when neurons were held at −50 mV or more positive (Nunn et al., 1987; Hou et al., 2004). According to our previous experiment on voltage-gated potassium currents (Liu et al., 2007), neurons were held at −70 mV, current–voltage (I–V) and the steady-state activation curves of Iₖ current were obtained by applying hyperpolarizing prepulse to −110 mV for 180 ms and then depolarized from −50 to +90 mV for 450 ms in 10-mV increment. I–V and the steady-state activation curves of Iₖ current were obtained by applying hyperpolarizing prepulse to −110 mV for 1000 ms followed by a −50-mV pulse for 50 ms and then depolarized from −50 to +90 mV for 450 ms in 10-mV increment. The holding potential was −50 mV. All experiments were performed at room temperature (22–24°C). TTX (500 nM) was added to bath solution to block voltage-gated Na⁺ channels and CdCl₂ (200 μM) was added extracellularly to block Ca²⁺ current and Ca²⁺-dependent K⁺ current.

The steady-state inactivation curves of Iₖ were obtained using a double-pulse protocol, in which an 80 ms conditioning pulse from −110 to 0 mV was followed by a test pulse at +70 mV for 80 ms. To investigate the effects of nano-Ag on the recovery time course of Iₖ from inactivation, neurons were held at −70 mV, an 80 ms conditioning pulse to +50 mV was applied to inactivate the transient outward potassium channels fully, and then an 80 ms test pulse to...
+50 mV was applied after a series of −80 mV pulses in intervals varying from 10 to 260 ms (in 10-ms increments). All data in control and nano-Ag group were obtained before and after application of nano-Ag (10⁻²⁵g mL⁻¹), respectively.

**Data Analysis**

All data were analyzed by Clampfit 9.0 and figures were gotten by Origin 7.0. For activation, currents at each test potential were converted to conductance (G) using the following formula:

\[ G = \frac{I}{(V - V_h)} \]

where \( V_h \) is reversal potential. The peak conductance value for each test potential is normalized to \( G_{max} \) and plotted against the test potential to produce voltage–conductance relationship curves, which are fitted using Boltzmann function:

\[ \frac{G}{G_{max}} = \frac{1}{1 + \exp\left(\frac{V - V_h}{k}\right)} \]

where \( V_h \) is the voltage at which conductance is half-maximal, and \( k \) is slope factor.

Steady-state inactivation curves are fitted with the Boltzmann equations:

\[ \frac{I}{I_{max}} = \frac{1}{1 + \exp\left(\frac{V - V_h}{k}\right)} \]

where \( I \) is normalized current, \( V_h \) is the potential for half-maximal inactivation, and \( k \) is the slope factor. The time course of recovery of the \( I_A \) current from inactivation is fitted with a monoexponential function:

\[ \frac{I}{I_{max}} = A \times \left[1 - \exp\left(-\frac{\Delta t}{\tau}\right)\right] \]

where \( I_{max} \) is the maximal current amplitude, \( I \) is the current after a recovery period of \( \Delta t \), \( \tau \) is the time constant and \( A \) is the amplitude coefficient.

Data are presented as mean ± S.E.M. Statistical significance was assessed using a Student’s paired t-test, and \( P < 0.05 \) was considered significant. All data analyses were performed using the software SPSS 11.5.

**RESULTS**

**Characterization of Nano-Ag**

The EDS pattern of the obtained nano-Ag was shown in Figure 1. The surface-analysis of 10 µm × 100 µm showed the presence of Ag peaks only, without peaks of other elements. So it can be confirmed that the obtained nano-Ag is of high purity.
(10⁻⁵ g mL⁻¹, n = 7, P = 0.208), with a slope factor \(k\) of 48.52 ± 9.46 and 27.43 ± 2.04 (n = 7, P = 0.032), respectively [Fig. 4(A)]. Values of \(V_h\) were 14.81 ± 7.52 mV and 6.07 ± 6.92 for activation of \(I_K\) in control and nano-Ag (n = 7, P < 0.0001), with a slope factor \(k\) of 26.88 ± 2.40 and 25.58 ± 3.38 (n = 7, P = 0.1), respectively [Fig. 4(B)].

### Effects of Nano-Ag on the Voltage Dependence of Steady State Inactivation of \(I_A\)

The effects of nano-Ag on the inactivation kinetics of \(I_A\) were examined [Fig. 5(A)]. \(V_h\) for inactivation of \(I_A\) were -66.00 ± 2.27 mV and -70.35 ± 1.70 mV in control and nano-Ag (10⁻⁵ g mL⁻¹) group (n = 7, P = 0.009), and \(k\) was 25.68 ± 2.50 and 23.42 ± 2.60 (n = 7, P = 0.004), respectively [Fig. 5(B)].
values were 8.26 ± 0.62 and 8.86 ± 0.89 (n = 7, P = 0.082), respectively [Fig. 5(B)].

**Effects of Nano-Ag on the Recovery of I_A**

The effects of nano-Ag were examined for the recovery time course of I_A from inactivation [Fig. 6(A)]. The τ values were 28.60 ± 5.92 ms and 48.03 ± 7.09 ms (n = 7, P = 0.002) in control and nano-Ag (10^{-5} g mL^{-1}) group, respectively [Fig. 6(B)]. The results indicated that silver nanoparticles delayed the recovery of I_A from inactivation.

**DISCUSSION**

Small size also confers greater particle mobility both in the environment and in the body. It is expected that transport of nanoparticles go through the blood brain barrier. On the other hand, nanoparticle-induced drug delivery to the brain may impose risks to the patient (Sarin et al., 2008; Muthu and Singh, 2009). So it is necessary to investigate the neurotoxicity of nanoparticles.

The properties of ion channels serve as a subtle indicator of the condition and viability of the cells. The voltage-gated K^+ currents determine a large number of neuronal properties, such as action potential waveform, fire frequency, and

**Fig. 5.** Effect of 10^{-5} g mL^{-1} nano-Ag on the voltage dependence of steady state inactivation of I_A. Currents were elicited using a double-pulse protocol, in which a 80 ms conditioning pulse was followed from −110 to 0 mV was followed by a test pulse at +70 mV for 80 ms, and holding potential at −70 mV(A). Peak amplitudes for I_A currents were normalized and plotted versus prepulse potentials, and the data are fitted with Boltzmann function. Each point represents mean ± S.E.M (B) (n = 7).

**Fig. 6.** Effect of 10^{-5} g mL^{-1} nano-Ag on recovery from inactivation of I_A. The currents were obtained as followed protocol: holding potential at −70 mV, an 80-ms conditioning pulse to +50 mV was applied to inactivate the transient outward potassium channels fully, and then an 80-ms test pulse to +50 mV was applied after a series of −80 mV pulses in intervals varying from 10 to 260 ms (in 10-ms increments) (A). The peak value of I_A evoked by the conditioning pulse was designated as I_{max}, while the peak value of I_A evoked by the test pulse was designated as I. The ratio of I to I_{max} represents the recovery of I_A from inactivation. The plot of I/I_{max} versus the duration of the −80 mV intervals was fitted with a monoexponential function (B). Each point represents mean ± S.E.M (n = 7).
resting membrane potential. The currents are also known to play important roles in releasing neurotransmitters (Meir et al., 1999), hormones, and Ca\(^{2+}\)-dependent synaptic plasticity (Roeppe and Pongs, 1996; Fili et al., 2001; Muller and Bittner, 2002). \(I_A\) and \(I_K\) are the two main neuronal voltage-gated K\(^+\) currents. Therefore, the regulation of K\(^+\) channels is believed to have an effect on the overall neuronal response and activity.

According the previous studies, the concentration of 10\(^{-3}\)g mL\(^{-1}\) was used to investigate the toxicity of silver nanoparticles (Hussain et al., 2006). Nano silver at concentrations over 10\(^{-3}\)g mL\(^{-1}\) decreased proliferation of peripheral blood mononuclear cells (Shin et al., 2007) and had effects on the metabolic activity of mammalian stem cells (Braydich-Stolle et al., 2005). Thus, the concentration of nano-Ag (10\(^{-3}\)g mL\(^{-1}\)), employed in the present experiments, was to investigate whether the low concentration of silver nanoparticles could change the potassium channel function and kinetic character.

Our previous studies have shown that nano particles of CuO inhibited \(I_K\) in the concentration of 5 \(\times\) 10\(^{-3}\)g mL\(^{-1}\) (Xu et al., 2009), while nano-ZnO (10\(^{-3}\)g mL\(^{-1}\)) increased the amplitudes of \(I_A\) and \(I_K\) (Zhao et al., 2009). In the present study, we focused on the effects of nano-silver (10\(^{-3}\)g mL\(^{-1}\)) on both kinds of K\(^+\) currents of neurons. The results showed that nano-Ag inhibited the peak amplitudes of \(I_A\) and \(I_K\), which may result in accumulation of cytoplasmic K\(^+\) due to the decreased K\(^+\) efflux. Intracellular K\(^+\) can suppress the activation of apoptosis (Hughes et al., 1997), which is a physiological form of cell death. The higher concentration of K\(^+\) might disturb the process of apoptosis. \(I_A\) is one of the major outward currents responsible for repolarization of action potential, which is a fundamental determinant of neuronal excitability. Thus, the regulation of \(I_A\) by silver nanoparticles would make neurons display abnormal firing properties and neuronal discharge, which could be underlying mechanism for seizure generation related to exposure to silver.

According to previous reports, blocking currents with TEA (inhibitor of \(I_K\)) resulted in increasing action potential duration in neurons (Suppes, 1984), which suggested that the effects of silver nanoparticles might, at least in part, be due to the prolongation of action potential duration as a result of the \(I_K\) inhibition.

It has been shown that nano-Ag produced a hyperpolarizing shift in the activation-voltage curve on \(I_K\) currents without affecting the \(V_n\) of \(I_A\), which indicated that nano-Ag differentially affected on activations of the two kinds of K\(^+\) currents. The steady-state inactivation curve of \(I_A\) was also shifted toward more negative potential by silver nanoparticles, which caused a decrease in the population of channels in a resting state and available for activation by depolarization and normally would result in a decrease in current amplitudes. The increased time constants of recovery from inactivation of \(I_A\) suggested that nano-Ag retarded the change from the inactivation to the resting state of the \(I_A\) channel. The block of recovery of A-type K\(^+\) channels was also assumed to influence the firing behavior in neurons, which suggested the potential effect of nano-Ag on propagation of the action potential along axons. This action plays an important role in message transmission in neural network.

Blockage of K\(^+\) currents causes increased Ca\(^{2+}\) influx and accumulation of intracellular Ca\(^{2+}\) during synaptic stimulation (Jaffe et al., 1994). The increased intracellular Ca\(^{2+}\) accumulation can lead to the cell damage by multiple pathways. The inhibition of K\(^+\) currents by nano-Ag can lead to increased Ca\(^{2+}\) influx, thus beginning a cascade of subsequent cellular responses that eventually result in neuronal dysfunction and death.

It is well established that voltage-gated K\(^+\) channels play a role in neurotransmitter release (Meir et al., 1999). The effects of nano-silver on \(I_A\) and \(I_K\) means that modulation of either one may be able to produce significant functional effects at message transmission in CNS.

One possible mechanism postulated to be responsible for effects of nano-Ag on K\(^+\) currents is the action of phosphorylation cascades signal system. Subunits of voltage-gated K\(^+\) channel on hippocampal neurons presence multiple phosphorylation sites in hippocampus (Anderson et al., 2000; Park et al. 2006). On the other hand, many experiments confirmed that signal systems were involved in the effects on voltage gated K\(^+\) currents by small molecules on neuron (Mei et al., 2004; Gao et al., 2008). In our study, the effects of nan-Ag on K\(^+\) currents were observed within 2 min after nano-Ag application, reached a relative steady state in about 5 min, which suggested an involvement of signal systems.

The present study provides detailed analysis of nano-Ag modulation of voltage-gated potassium currents in rat hippocampal neurons that suggests silver nanoparticles may have potential danger on nervous system.

REFERENCES


INHIBITORY EFFECTS OF NANO-AG ON VOLTAGE-GATED POTASSIUM CURRENTS


