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Short Communication | Comunicación Corta

Negative inotropic and dromotropic actions of SiO_2 nanoparticles on isolated rat hearts: Effects on Na^+ and Ca^{2+} currents

[Acciones inotropo y dromotropo negativas de nanopartículas de SiO_2 en corazones aislados de ratas: Efectos sobre las corrientes de Na^+ y Ca^{2+}]

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Abstract

Context: SiO_2 nanoparticles (NP) are widely used in the industry and in varied biotechnological and medical applications. However, epidemiological studies suggest that pollution with fine particles (in which silica is an inorganic component) may increase morbidity and mortality from cardiovascular diseases, but little is known about their potential cardiovascular actions.

Aims: To study the actions of SiO_2 nanoparticles on the electrical and contractile activity of rat hearts and to identify the possible underlying cellular mechanisms.

Methods: Surface electrogram (ECG) and force of contraction (FC) was recorded in isolated rat hearts. Na^+ and Ca^{2+} currents (I_{Na} and I_{CaL}, respectively) were recorded, with the patch-clamp technique, in enzymatically isolated rat ventricular cardiomyocytes.

Results: SiO_2 NP (1-30 µg/mL) decreased the FC and markedly increased QRS duration and QT interval in spontaneously beating hearts. Electric stimulation (RR = 400 ms) partially restored the FC. In patch-clamp experiments NP (30 µg/mL) decreased I_{Na} in a use-dependent manner and increased I_{CaL}.

Conclusions: SiO_2 nanoparticles exert a negative inotropic action in rat hearts due, in part, to a decrease in the fast sodium current responsible for cardiac depolarization. SiO_2 nanoparticles are also able to increase the L-type Ca^{2+} current. These actions should be taken into account when analyzing the toxic effects of these nanoparticles.

Keywords: calcium channels; heart; nanoparticles; patch-clamp; silica; sodium channels.

Resumen

Contexto: Las nanopartículas de SiO_2 (NP) se utilizan ampliamente en la industria y en variadas aplicaciones biotecnológicas y médicas. No obstante, hay estudios epidemiológicos que sugieren que la polución con partículas finas (en las que la sílica es un componente inorgánico) pueden aumentar la morbilidad y mortalidad por enfermedades cardiovasculares, pero poco se conoce sobre sus potenciales acciones cardiovasculares.

Objetivos: Estudiar las acciones de nanopartículas de SiO_2 sobre las actividades eléctrica y contráctil de corazones de rata e identificar los posibles mecanismos subyacentes.

Métodos: Se registró el electrograma de superficie (ECG) y la fuerza de contracción (FC) en corazones aislados de rata. Las corrientes de Na^+ y Ca^{2+} (I_{Na} and I_{CaL}, respectivamente) se registraron, con la técnica de patch-clamp, en cardiomiocitos ventriculares de rata aislados enzimáticamente.

Resultados: Las NP de SiO_2 (1-30 µg/mL) disminuyeron la FC y aumentaron marcadamente la duración del QRS y el QT en corazones espontáneos. La estimulación eléctrica (RR = 400 ms), restauró parcialmente la FC. En los experimentos con patch-clamp, las NP (30 µg/mL) disminuyeron I_{Na} de manera dependiente del uso e incrementaron I_{CaL}.

Conclusiones: Las nanopartículas de SiO_2 ejercen una acción inotropo negativa en corazones de rata debido, en parte, a una reducción de la corriente rapida de sodio responsable de la despolarización cardíaca. Las NP de SiO_2 también aumentaron la corriente de Ca^{2+} tipo L. Estas acciones deben ser tomadas en consideración al analizar los efectos tóxicos de estas nanopartículas.

Palabras Clave: canales de calcio; canales de sodio; corazón; nanopartículas; patch-clamp; silica.

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INTRODUCTION

Nanoparticles (NP) of variable size and structure are widely used in industry, cosmetics as well as in medical and pharmaceutical products. Although there is a logarithmic increase in research on and applications of NP, including human health, it has been shown that NP may cause potential dangerous effects due to their high surface/volume ratio that makes them highly reactive or catalytic (Ying, 2001). NP can penetrate biologic membranes and cause toxic effects by interacting with different biological systems (Hanley et al., 2009). Yet, their biological actions are relatively not well understood even if nanotechnology foresee multiple applications of NP in medicine such as drug delivery (Hu et al., 2009), cancer therapy (Peer et al., 2007), biosensors (Lord and Kelley, 2009) and even nanoparticle drug-eluting stents (Yin et al., 2014). Thus, basic research is still required to evaluate potential toxicity issues related to the chemical properties of nanoparticle materials, as well as to their size and shape, but the wide variety of tissues, cells and cell membranes impose important hurdles to overcome in this promising field. Little is known about the mechanisms of action of NP on different biological systems (e.g. redox systems and metabolism; Fröhlich, 2013; Roy et al., 2014), as well as their action on voltage-dependent ionic channels (e.g. Liu et al., 2011).

Moreover, the environmental impact of nanomaterials is still under study. In this sense silicon dioxide (SiO_2; silica), a longstanding and widely used compound in industries, is known to be toxic and cause silicosis and bronchitis (*see: Center for Construction Research and Training - Work Safely with Silica: "What are the Health Effects?"* http://www.silica-safe.org). However, SiO_2 NP (10-15 nm diameter) are widely used in paints, rubbers, plastics, porcelain, batteries, adhesives, glass, steel, chemical fibers, plexiglass and aerogels (*see:* http://www.nanoparticles-microspheres.com/Products/). In addition SiO_2 NP are also used in different applications in biotechnology and medicine, such as medical diagnostics, drug delivery, gene therapy, biomolecules detection and bioimaging (Kumar et al., 2010; Lee et al., 2011; Barandeh et al., 2012; Li et al., 2012). Epidemiological studies link air pollution with fine particles (silica is an inorganic component) to increases in morbidity and mortality from cardiovascular diseases (Pope et al., 2004). However, there are only few studies of their potential cardiovascular actions (e.g. Duan et al., 2013). It was, thus, the purpose of the present investigation to study the actions of SiO_2 nanoparticles on the electrical and contractile activity of rat hearts and to identify the possible underlying cellular mechanisms.

MATERIAL AND METHODS

SiO2 nanoparticles and chemicals

SiO_2 nanoparticles (LUDOX® TM-40 colloidal silica; CAS Number 7631-86-9; Molecular Weight 60.08; PubChem Substance ID 24866350) and all other chemicals were from Sigma Aldrich.

Animals

Male adult (7 - 8 weeks) Wistar rats were obtained from the National Center for Laboratory Animal Reproduction (CENPALAB; La Habana; Cuba). Prior to experiment, animals were adapted for seven days to laboratory conditions (controlled temperature 25 ± 2°C, relative humidity 60 ± 10% and 12 h light/dark cycles). Tap water and standard diet for rodents supplied by CENPALAB were freely provided. All procedures were conducted according to the guidelines for the use and care of laboratory animals approved by CENPALAB.

Isolated hearts

Rat hearts were mounted on a Langendorff column and perfused at constant flow (10 mL/min) with a Tyrode solution of the following composition (mmol/L): NaCl 140, KCl 2.5, $MgCl_2$ 0.5, $CaCl_2$ 2, Tris-hydroxymethylaminomethane 10, Glucose 10 (pH = 7.4, gassed with O_2; T = 35°C). A bipolar platinum recording electrode was placed on the ventricular epicardium to record the surface electrocardiogram (ECG). Another bipolar platinum electrode was placed near the atrioventricular ring and was connected to an electronic stimulator. To record the force of contraction (FC), the cardiac apex was fixed to a force-displacement transducer with a surgical 6-0 silk thread. ECG and FC values were

recorded at the spontaneous heart rate and at a fixed stimulus rate (400-ms RR interval).

Isolated ventricular cardiomyocytes

Ventricular cardiomyocytes were isolated as previously described in detail (Alvarez-Collazo et al., 2012) and were kept in a K^+-Tyrode solution containing 1 mM Ca^{2+} at room temperature (21 ± 2 °C) and used for experiments for 6 h.

Patch-clamp recordings

Cardiomyocytes were patch-clamped as previously described (Alvarez-Collazo et al., 2012). Whole cell currents were filtered at 3 kHz and digitized at 50-µs intervals using the ACQUIS1 software (CNRS License). To study Na^+ (I_{Na}) and L-type Ca^{2+} (I_{CaL}) currents, K^+ currents were blocked by substituting all potassium by cesium in extracellular and "intracellular" solutions. The extracellular solution contained (in millimolars): 117 NaCl, 20 CsCl, 10 HEPES, 2 $CaCl_2$, 1.8 $MgCl_2$, and 10 glucose, pH 7.4. The standard pipette (intracellular) solution contained (in millimolars): 130 CsCl, 0.4 Na_2GTP, 5 Na_2ATP, Na_2-creatine phosphate, 2.0 $MgCl_2$, 11 EGTA, 4.7 $CaCl_2$ (free Ca^{2+} ≈ 108 nM), and 10 HEPES, pH 7.2 with CsOH.

Pipette resistance was 1.0 - 1.2 MΩ. Membrane capacitance (Cm) and series resistance (Rs) were calculated as previously described (Alvarez-Collazo et al., 2012) and their average values were 154 ± 17 pF and 3.5 ± 0.3 MΩ, respectively (N = 10). Rs could be electronically compensated up to 50 %. Liquid junction potential was compensated before establishing the gigaseal.

For routine monitoring of I_{Na} and I_{CaL} a double pulse voltage-clamp protocol was used: from a holding potential (HP) of -80 mV every 4s the cell membrane was depolarized by a prepulse to -40 mV for 50 ms to activate I_{Na}. From this membrane potential a 200-ms test pulse to 0 mV evoked I_{CaL}. The inactivation time courses of I_{Na} and I_{CaL} were fitted to double exponentials using the fitting procedures of the ACQUIS1 software.

Statistical analysis

Results are expressed as means and standard errors of means. Statistical significance was evaluated by means of Student's t test. Differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

Effects of SiO_2 nanoparticles on electrical and mechanical activities of isolated hearts

At concentrations of 1, 3 and 30 µg/mL, SiO_2 nanoparticles (NP) induced a marked decrease in the force of contraction (FC) of isolated rat hearts; this effect was stable in ≈ 5 min (Fig. 1). The decrease in FC was variable and a concentration dependence could not be established. Pooled results of the three concentrations used in five hearts yielded, however, a statistically significant 64.1 ± 10.3% decrease in FC. The decrease in FC was accompanied by statistically significant increases in QRS and QT interval from 8.8 ± 0.4 ms and 58.8 ± 10.8 ms to 26.2 ± 6.2 ms and 127 ± 22.7 ms, respectively (Fig. 1). The RR interval showed a tendency to increase (423.3 ± 56.7 ms to 471.6 ± 55.8 ms) but without statistical significance. The marked increase in QRS duration strongly suggests that NP could be acting on the fast Na^+ current (I_{Na}) responsible for the depolarization phase on the cardiac action potential. Although the negative inotropic action of the NP could be via their action on any of the mechanisms that lead to an increase intracellular Ca^{2+} during the excitation-contraction coupling (EC) such as Na^+ and Ca^{2+} channels, the Na-Ca exchanger, the ryanodine receptor, the Ca-ATPase (Bers, 2001), the increase in QRS duration, and therefore the dispersion of the depolarization wave front (negative dromotropic effect) and desynchronization of the contraction in the whole heart, could be also responsible for the decrease of FC. Indeed, when hearts were electrically stimulated (~ 20 pulses, twice the threshold) at a 400 ms interval (not significantly greater than the control RR interval), the QRS was shortened and the FC could be partly restored (Fig. 1) indicating that the negative inotropic effects of NP was partially due to a dispersion of the depolarization (activation) wave front. However, effects on other major protagonists of the intracellular Ca^{2+} increase during the EC, specifically on the L-type Ca^{2+} current I_{CaL}, cannot be ruled out. We must also point out that the NP seem to affect the repolarizing current system (mainly K^+ currents) since the QT in-

terval was significantly increased by an amount that cannot be only explained by the marked increase in the QRS duration. Two hearts developed arrhythmias such as extrasystoles with wide QRS complexes. The effects of NP on ECG and FC were only partially reversed upon returning to control solution.

Effects of SiO_2 nanoparticles on Na^+ and Ca^{2+} currents of isolated ventricular cardiomyocytes

Because in the experiments with isolated hearts we could not find a dependency of the NP action with the concentration, in the patch-clamp experiments we chose to work with the maximum concentration (30 µg/mL). Under control conditions I_{Na} and I_{CaL} densities at -40 and 0 mV were 79.3 ± 2.7 pA/pF and 8.1 ± 0.5 pA/pF, respectively (frequency of 0.25 Hz; N = 6). The corresponding times to peak currents were 0.9 ± 0.1 ms and 4.3 ± 0.2 ms. The inactivation time course of both currents could be fitted to two exponentials (τ_{fast} and τ_{slow}) with values of 0.9 ± 0.04 ms and 5.7 ± 0.7 ms and 12.1 ± 1.4 ms and 50.3 ± 4.9 ms for I_{Na} and I_{CaL}, respectively. As expected, both currents responded differently to the increase in stimulation frequency in control conditions (Fig. 2). Currents were stabilized at 0.25 Hz and stimulation stopped. After a rest period of one minute, the increase in the rate of voltage clamping to 1 Hz provoked no changes in I_{Na}. However, I_{CaL} responded with a typical increase-decrease or "facilitation" (Fig. 2; see also Alvarez et al., 2004). When the same stimulation protocol was applied in the presence of NP at the maximal concentration of 30 µg/mL, I_{Na} showed a marked "use-dependence" decrease (pulse-to-pulse decrease after restoring stimulation) that could be fitted to one exponential with a time constant of 54.8 ± 2.1 sec. At the steady-state at high frequency, I_{Na} was significantly inhibited by 48.2 ± 9.2 % while at the steady-state at the control frequency (0.25 Hz) I_{Na} was also significantly inhibited by 34.0 ± 7.5 % (see Fig. 2). At 0.25 Hz NP significantly increased the time to peak I_{Na} to 1.9 ± 0.03 ms while τ_{fast} and τ_{slow} reached values of 0.94 ± 0.15 ms and 4.7 ± 0.8 ms, respectively (not statistically significant). The significant decrease in I_{Na} by NP at both rates predicts that the maximal rate of depolarization of the ventricular action potential will be reduced and the conduction of excitation, at the whole heart level, will be slower (Carmeliet and Vereecke, 2002). This would create a greater dispersion of the depolarization wavefront and a desynchronization of the whole heart contraction giving as a result a negative inotropic action of NP. Indeed, as described above, when hearts were stimulated the FC was partially recovered. These results, however, do not rule out changes in intracellular Ca^{2+} load (via the Na^+ - Ca^{2+} exchanger) (Bers, 2001) or even at the connexins level.

One major protagonist of the cardiac excitation-contraction coupling and source of intracellular Ca^{2+} is the L-type Ca^{2+} current (Bers, 2001); therefore, another possible explanation for the negative inotropic effect of NP would be a decrease in I_{CaL}. However, this was not the case. NP did not alter the frequency response of I_{CaL} but rather increased I_{CaL} at both control and high frequencies (Fig. 2). Peak I_{CaL} at high frequency (during "facilitation") was variably but significantly increased by 182 ± 90 % and steady-state I_{CaL} at 0.25 Hz was significantly increased by 43.1 ± 5.9%. Time to peak I_{CaL}, τ_{fast} and τ_{slow} were not significantly modified by NP reaching values of 4.3 ± 0.3 ms, 15.5 ± 1.8 ms and 50.3 ± 4.9 ms, respectively. It is to note that the effects of NP on both I_{Na} and I_{CaL} were rapidly reversible (30-40 sec for I_{Na} and 10-15 sec for I_{CaL}; "off" effect) upon washout with control extracellular solution.

Due to the size of SiO_2 particles (approximately 10 nm in diameter) it is difficult to foresee that a direct interaction between NP and specific amino-acid sequences ("sites") within the Na^+ and Ca^{2+} channels subunits (as with pharmacological agents), are responsible for the effects we describe here. One may hypothesize that by interacting with membrane lipids SiO_2 NP may alter lipid microdomains (rafts) that are known to regulate ion channel function either by direct protein-lipid interaction or by modifying the lipid bilayer and ion channel environment (Maguy et al., 2006; Dart, 2010; Morris et al., 2012; Poveda et al., 2014). Other possibilities (Head et al., 2014) could be that NP modify the interaction of the lipid rafts with the cytoskeleton, a well-known modulator of ion channels activities (Calaghan et al., 2004), or any steps in intracellular signaling cascades such as the CaMKII-dependent phosphorylation of the L-type Ca^{2+} channel that determines I_{CaL} "facilitation" (Bers and Morotti, 2014). However, due to the fast washout of NP actions on both I_{Na} (less than 40

sec) and I_{CaL} (10-15 sec) effects on intracellular signaling pathways should be considered with caution.

Use-dependent block of I_{Na} by local anesthetics or arrhythmic drugs has been interpreted in terms of "modulated" or "guarded receptor" hypotheses that consider state-dependent interaction of the drugs with specific sites within the Na^+ channel (see for review Wang and Strichartz, 2012). Due to the size of SiO_2 NP, it seems challenging to explain the use-dependent action of NP in terms of specific site affinity according to Na^+ channel conformation. Our results might suggest that other "non-specific" actions should be also considered to explain use-dependent effects on I_{Na}.

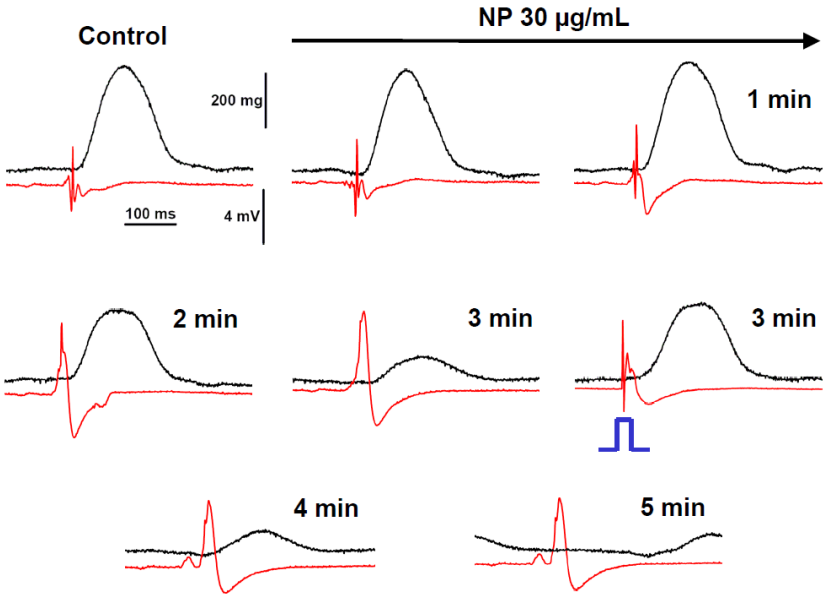

Figure 1. Effects of SiO_2 nanoparticles (30 µg/mL) on the ECG (red lower trace) and force of contraction (black upper trace) of a spontaneously beating rat heart.

Traces were selected in control conditions and at every minute under NP perfusion. After three minutes perfusion with NP, the heart was electrically stimulated (400 ms interval, ~20 pulses) with a suprathreshold stimulus (square pulse in blue).

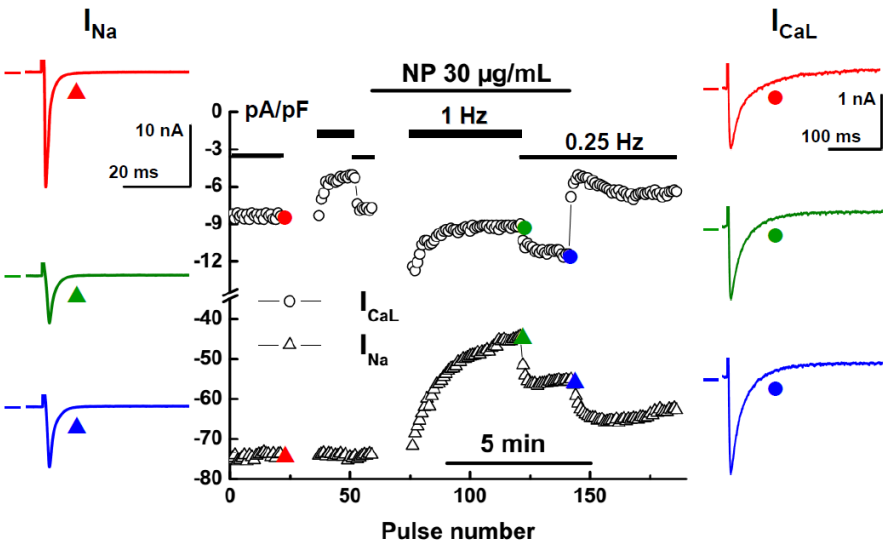

Figure 2. Effects of SiO2 nanoparticles (30 µg/mL) on Na^+ (I_{Na}) and Ca^{2+} (I_{CaL}) currents simultaneously recorded on a single rat ventricular cardiomyocyte.

The cell was patch-clamped with a double voltage pulse as indicated in Materials and Methods at 0.25 Hz (thin horizontal lines). At different times during the experiment (both in control and in the presence of NP) stimulation was stopped for one minute and reinitiated at 1 Hz (thick horizontal lines). After stabilization of current level, the rate of stimulation was returned to 0.25 Hz. The insets show I_{Na} and I_{CaL} recordings at different times during the experiment marked with the corresponding colored symbols.

CONCLUSIONS

SiO_2 nanoparticles exert a negative inotropic action in rat hearts. A decrease in the fast sodium current responsible for cardiac depolarization partially explains this negative inotropism. SiO_2 nanoparticles are also able to increase the L-type Ca^{2+} current. These actions should be taken into account when analyzing the toxic effects of these nanoparticles.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Contribution	Álvarez-Collazo J	Galán-Martínez L	Fleites-Vázquez A	Sánchez-Linde A	Talavera-Pérez K	Álvarez JL
Concepts or ideas	X	X			X	X
Design	X	X				X
Definition of intellectual content	X	X		X	X	X
Literature search	X	X	X	X	X	X
Experimental studies	X	X	X	X		X
Data acquisition	X	X	X	X		X
Data analysis	X	X	X	X		X
Statistical analysis	X	X	X	X		X
Manuscript preparation	X	X				X
Manuscript editing				X	X	X
Manuscript review	X	X	X	X	X	X

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