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Interaction of SiO_2 nanoparticles with neuronal cells: Ionic mechanisms involved in the perturbation of calcium homeostasis

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ABSTRACT

SiO_2 nanoparticles (NPs), in addition to their widespread utilization in consumer goods, are also being engineered for clinical use. They are considered to exert low toxicity both in vivo and in vitro, but the mechanisms involved in the cellular responses activated by these nanoobjects, even at non-toxic doses, have not been characterized in detail. This is of particular relevance for their interaction with the nervous system: silica NPs are good candidates for nanoneuromedicine applications. Here, by using two neuronal cell lines (GT1–7 and GN11 cells), derived from gonadotropin hormone releasing hormone (GnRH) neurons, we describe the mechanisms involved in the perturbation of calcium signaling, a key controller of neuronal function. At the non-toxic dose of 20 $\mu g\,mL^{-1}$, 50 nm SiO_2 NPs induce long lasting but reversible calcium signals, that in most cases show a complex oscillatory behavior. Using fluorescent NPs, we show that these signals do not depend on NPs internalization, are totally ascribable to calcium influx and are dependent in a complex way from size and surface charge. We provide evidence of the involvement of voltage-dependent and transient receptor potential-vanilloid 4 (TRPV4) channels.

1. Introduction

SiO_2 nanoparticles (NPs), in addition to their widespread utilization in consumer goods, are also being developed and engineered for clinical use (van Schooneveld et al., 2008). While they are considered to be among the most biocompatible NPs (Huang et al., 2005; Izak-Nau et al., 2014; Kim et al., 2006), extensive knowledge of the cellular responses elicited by their interaction is still incomplete. This is of particular relevance for the nervous system (NS): nanoneuromedicine is rapidly gaining momentum (Gendelman et al., 2014), and silica NPs are good candidates for applications in this field, ranging from drug delivery (Rosenholm et al., 2011) to cell tracking and subcellular imaging (Montalti et al., 2014). They can pass the blood brain barrier (Kim et al., 2006); moreover, protocols for direct access of NPs to the cerebrospinal fluid have been proposed (Papisov et al., 2013). On the other hand, neurons are particularly delicate and sensitive cells, and even small functional perturbations that can be noninfluential in other cell types may have severe outcomes in the nervous tissue. In this perspective, the understanding of the mechanisms activated by the interaction between NPs and neuronal cells is of primary relevance for the rational design of better and safer nanoparticles, particularly for long-term administration to the nervous system.

Cytosolic free calcium concentration, $[Ca^{2+}]_i$, is a highly relevant parameter, influencing both life and death of cells, and

Abbreviations: $[Ca^{2+}]_i$, cytosolic free calcium concentration; DLS, dynamic light scattering; DMEM, Dulbecco's modified Eagle's medium; EGTA, ethylene glycol tetraacetic acid; ELS, electrophoretic light scattering; FBS, fetal bovine serum; GnRH, gonadotropin hormone releasing hormone; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NPs, nanoparticles; PM, particulate matter; QDs, quantum dots; RR, ruthenium red; SOCE, store-operated calcium entry; TRPC, transient receptor potential canonical; TRPV, transient receptor potential-vanilloid; VDCCs, voltage dependent calcium channels.

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neurons are particularly sensitive to its changes, even subtle ones. These changes are involved in normal neuronal function, such as information coding; loss of control of $[Ca^{2+}]_i$ can lead to neuronal death (Arundine and Tymianski, 2003). During development, its changes are crucial in controlling proper differentiation and the correct establishment of neuronal connections. By interacting with both plasmamembranes and membranes of intracellular organelles, NPs can alter the delicate set of mechanisms controlling influx, efflux and sequestration of calcium ions. Apart from silica NPs (Ariano et al., 2011), data on the perturbations of calcium signaling mechanisms induced by NPs are still scarce (see e.g. Huang et al., 2010; Koeneman et al., 2010) and this is particularly true for neuronal cells (Guo et al., 2013; Haase et al., 2012; Nyitrai et al., 2013; Tang et al., 2008a, 2008b), as recently reviewed (Lovisolo et al., 2014).

Moreover, for potential applications in nanomedicine, it is relevant to investigate these mechanisms at non-toxic doses. To this purpose, we have taken advantage of the properties of silica NPs (both bare and hybridized in the bulk with organic fluorophores) previously shown (Miletto et al., 2010) to be non-toxic at the same size and dose used in a preliminary paper on calcium signals, performed with similar silica NPs (Ariano et al., 2011). They can be safely incorporated into neuronal (Miletto et al., 2010) and other cell types (Accomasso et al., 2012), and, because of their high fluorescence efficiency are quite suited to be used in cell tracking and subcellular imaging. By means of this tool, we have correlated their interaction and internalization in neuronal cells with the time course of the calcium signals they elicit and investigated the molecular targets involved in the perturbation of calcium homeostasis.

2. Methods

2.1. Materials

Both lab-made SiO_2 NPs and spherical commercial SiO_2 micro- and nanoparticles were used (Corpuscular Inc., www.microspheresnanosphere.com). These latter will be hereafter referred as 2000-SiO_2 and 500-SiO_2, because of their diameter of 2.0 or 0.5 μm, i.e. 2000 and 500 nm (see below). Lab-made silica NPs were prepared using the reverse microemulsion technique, following the procedure previously reported (Alberto et al., 2009; Miletto et al., 2010). Details are in Supplementary Materials (hereafter SM), Methods. These NPs exhibit a mean diameter close to 50 nm and then they will be referred to as 50-SiO_2.

Lab-made silica NPs carrying at their surface amine groups were also prepared, with mean diameter 55 nm (see below) and then referred to as NH_2-55-SiO_2. For preparation protocol and data dealing with sample characterization see SM, Methods and Fig. S1.

A third type of lab-made SiO_2 NPs was constituted by fluorescent nanoparticles (hereafter referred to as FL-50-SiO_2) resulting from the hybridization of silica with a fluorescent cyanine dye. The cyanine-silane derivative was used together with TEOS for the preparation of hybrid dye-SiO_2 NPs following the same procedure reported above (see SM, Methods and Fig. S2). Previous studies (Alberto et al., 2009) indicated that the procedure adopted resulted in the presence in each NP of ca. 110 cyanine molecules, all entrapped within the silica matrix.

All reagents and solvents used for the preparation of nanoparticles and for cell cultures were highly pure Sigma–Aldrich products and were used as received, unless otherwise mentioned.

2.2. Physicochemical characterization of NPs

Transmission electron microscopy images were obtained with a 3010 Jeol instrument operated at 300 kV. For the measurements, a droplet of the suspensions of lab-made silica nanoparticles was spread on a copper grid coated with a lacey carbon film, and then the liquid was allowed to evaporate. Histograms of the size distribution of NPs were obtained by measuring ca. 200 particles, and the mean particle diameter (d_m) was calculated as $d_m = \Sigma d_i n_i / \Sigma n_i$, where n_i was the number of particles of diameter d_i. The results are indicated as $d_m \pm$ standard deviation.

For dynamic light scattering (DLS) measurements (90Plus Particle Size Analyzer, laser wavelength 660 nm, detection angle 90°, $T = 20\,^{\circ}C$), the materials were suspended (0.1 mg mL^{-1}) in water, DMEM and Tyrode solution (see below for the composition); for each sample three measurements were performed. In presence of large agglomerates in the micrometer range, in some cases significant differences among repeated measurements occurred, because of the much more complex scattering behavior (Orts-Gil et al., 2011).

Hydrodynamic diameters are reported as mass distributions (SM, Fig. S3). The same samples were used for ζ-potential measurements by electrophoretic light scattering (ELS), using a Zetasizer Nano-ZS (Malvern Instruments). Results are reported as mean value ± standard deviation of five separate measurements each resulting from 10 runs.

2.3. Calcium imaging

For ratiometric measurements of $[Ca^{2+}]_i$ GT1–7 cells, an immortalized line derived from highly differentiated mouse gonadotropin-hormone releasing hormone (GnRH) neurons (generously donated by Prof. P.L. Mellon), were plated on glass coverslips (32 mm diameter) coated with poly-L-lysine (100 μg mL^{-1}) at densities of 10,000 cells cm^{-2}. The cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Lonza), gentamycin (50 μg mL^{-1}), and 2 mM glutamine at 37 °C, in a humidified atmosphere of 5% CO_2 in air and then switched for 4–5 days to 0.5% FBS supplemented with B27 (Invitrogen), to improve survival and differentiation. GN11 cells, another cell line derived from GnRH neurons at an early developmental stage (Radovick et al., 1991), were plated with the same protocol as for GT1–7 cells but kept in 10% FBS DMEM and used after 1–2 days in culture.

Cells were loaded with the Fura-2 acetoxymethyl ester (2.5 μM, 45 min, 37 °C) and subsequently shifted to a standard physiological Tyrode solution of the following composition (in mM): NaCl, 154; KCl, 4; $CaCl_2$, 2; $MgCl_2$, 1; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 5; glucose, 5.5; and NaOH (pH 7.35). The NPs were dispersed in the Tyrode solution at the required concentration. The solution was applied with a microperfusion system; for calcium-free conditions, the $CaCl_2$ salt was omitted and the calcium chelator ethylene glycol tetraacetic acid (EGTA; 0.5 mM) was added. Cells were imaged every 3 s at 37 °C using a monochromator system attached to an inverted microscope with a 20× objective (SFluor, Nikon). For experiments lasting several hours, in order to avoid excessive photodamage, after the initial phase of stimulation with the NPs, acquisition time was one every 5 min; control recordings were performed to check the stability of the fluorescence at the isosbestic point. Images were acquired using an enhanced CCD camera (PCO) and the Metafluor software (Universal Imaging Co.).

2.4. Wavelet analysis of calcium signals

To provide a quantitative evaluation of the changes in the oscillatory pattern of calcium signals following administration of SiO_2 NPs and of the calcium channel blockers, an approach based on wavelet analysis was employed, using KYM 0.5 software (http://sourceforge.net/projects/kym/) and according to the formulation