Vascular Pharmacology 72 (2015) 190–196

Contents lists available at ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Plasmonic gold nanoparticles possess the ability to open potassium channels in rat thoracic aorta smooth muscles in a remote control manner

Anatoly Soloviev [a,*], Alexander Zholos [b], Irina Ivanova [a], Tetiana Novokhatska [a], Sergei Tishkin [a], Alexandra Raevska [c], Alexander Stroyuk [c], Vladimir Yefanov [c]

[a] Institute of Pharmacology and Toxicology, National Academy of Medical Science of Ukraine, Kiev, Ukraine
[b] Taras Shevchenko Kiev National University, Kiev, Ukraine
[c] NanoMedTech LLC, Kiev, Ukraine

ARTICLE INFO

Article history:
Received 23 December 2014
Received in revised form 26 May 2015
Accepted 30 May 2015
Available online 2 June 2015

Keywords:
Aurum nanoparticles (AuNPs)
Smooth muscle (SM) relaxation
Plasmon resonance
Potassium channels
Remote control

ABSTRACT

Colloidal gold nanoparticles (AuNPs) of ~5 nm core size and Zeta-potential of −35 mV, having absorption maximum and plasmon resonance in the range of 510–570 nm, were studied as a potential K^+-channel opener in vascular smooth muscle (SM) cells. Experimental design of the study comprised SM contractile recordings.
When externally applied to the organ bath, AuNPs ($10^{-6} - 3 \times 10^{-4}$ M) led to decrease in amplitude of norepinephrine-induced contractions in a concentration-dependent and endothelium-independent manner in SM thoracic aorta, with mean value of pD_2 ($-\log EC_{50}$) 4.2 ± 0.03, $E_{max} = 55 \pm 4\%$. Being added to the bath solution in concentration of 10^{-4} M, AuNPs significantly increased whole cell peak outward current at +70 mV from 32 ± 2 pA/pF to 59 ± 5 pA/pF ($n = 14$, $P < 0.05$). External irradiation using a 5 mW/532 nm green laser, to facilitate plasmon resonance, led to an increment in the AuNPs-induced macroscopic outward potassium current (I_K) from 59 ± 5 pA/pF to 74 ± 1 pA/pF ($n = 10$, $P < 0.05$). Paxilline (500 nM), when added to the external bathing solution, significantly decreased AuNPs-induced increment of I_K in SM cells. Single channel recordings provided a direct confirmation of BK_{Ca} activation by AuNPs at the single-channel level. Application of AuNPs to the bath potentiated BK_{Ca} activity with a delay of 1–2 min, as was seen initially by more frequent channel openings followed by the progressive appearance of additional open levels corresponding to multiple openings of channels with identical single-channel amplitudes. Eventually, after 10–15 min in the presence of AuNPs and especially when combined with the green laser illumination, there was a massive increase in channel activity with >10 channels evident. When irradiated by laser, AuNPs significantly increased the amplitude of maximal AuNPs-induced relaxation from 55 ± 5% to 85 ± 5% ($n = 10$, $P < 0.05$) while the sensitivity of SM to AuNPs was without changes.
In summary, plasmonic AuNPs possess the ability to activate BK_{Ca} channel opening in vascular SM. Laser irradiation facilitates this effect due to local plasmon resonance that, in turn, further increases BK_{Ca} channel activity causing SM relaxation.

1. Introduction

Many types of nanomaterial are now widely used in medicine and biology as biosensors, biomaterials, drug-delivery systems etc. [16] but not as drugs. It is clear that the properties of nanoparticles (NPs) can seriously differ from those demonstrated by their bulk forms. These new physical and chemical properties are evident in unexpected biological or even cytotoxic effects [12]. Taking into account the high reaction ability of NPs to interact with cell proteins, combined with their extremely small size and thus increased ability to penetrate into cellular structures, it becomes clear that understanding of how NPs interact with living cells is essential for both theoretical and clinical medicine and biology.

Smooth muscle (SM) cells contractile function is extremely important for all aspects of human physiology, since they represent the main effector element of hollow organs, including blood vessels. Therefore, SM plays a key role in the maintenance of human life in health and disease since they provide an adequate organs supply with blood and oxygen.

The most important and widely occurring cardiovascular diseases, such as arterial hypertension [1,11], radiation [14], and diabetes-

* Corresponding author at: Department for Experimental Therapeutics, Institute of Pharmacology and Toxicology, National Academy of Medical Science of Ukraine, 14 Eugene Pottier Str., 03057 Kiev, Ukraine.
E-mail address: tonysolpharm@gmail.com (A. Soloviev).

http://dx.doi.org/10.1016/j.vph.2015.05.016
1537-1891/

induced angiopathies [8,10] have been suggested to be associated with reactive oxygen species (ROS) overproduction and related protein kinase C activation. The underlying mechanisms include impaired vasodilator potential and decreased endothelium-dependent vasorelaxation [5,15], and suppression of ionic currents through potassium channels ([6,7,13,14]). Finally, all these factors taken together promote vascular malfunction and decreased tissue blood supply.

It is well known that SM contractility is closely coupled to its membrane potential, which, in turn, is determined predominantly by K^+ membrane conductance. Thus, it is generally accepted that potassium conductivity plays an important role in the regulation of the membrane potential of SM cells and therefore vascular tone regulation. Four main types of K^+ channels have been described in SM cells: voltage activated K^+ channels (Kv) which are encoded by the Kv gene family, inward rectifiers (KIR) which are encoded by the Kir2.0 gene, ATP-sensitive K^+ channels (K_{ATP}) which are encoded by Kir6.0 and sulphonylurea receptor genes, and large conductance Ca^{2+}-dependent K^+ channels (BK_{Ca}) which are encoded by the *Slo1* (KNCMA1) gene. At the molecular level, BK_{Ca} channels are composed of the pore-forming α-subunits and regulatory β_1-subunits. The presence of the β_1-subunit confers a higher Ca^{2+} sensitivity of the voltage dependent BK_{Ca} channel, which makes this channel an efficient modulator of vascular SM function in health and disease. The role of ionic channels in SM contractility is usually evaluated using pharmacological tools y and patch-clamp technique in different modifications.

It has been shown that BK_{Ca} channels play an important role in both essential arterial hypertension [7] and radiation-induced vascular hypertension [6]. The data obtained clearly indicate that decrease in outward current density in both endothelial and SM cells combined with diminished BK_{Ca} mRNA expression led to vascular hypercontractility and hypertension development [13].

It is important to note that despite wide use of NPs, including AuNPs, in biology and medicine there is a lack of evidence of any direct effects of NPs on effector and regulatory elements of the vascular system, i.e. SM cells and endothelium.

The aim of this study was to investigate whether AuNPs possess the ability to relax vascular SM and to shed light on the mechanisms underlying possible changes in vascular SM outward ionic currents and contractility.

2. Materials and methods

2.1. Preparation of Au nanoparticles

Colloidal gold nanoparticles (AuNPs) having plasmon resonance of 532 nm were synthesized via reduction of sodium tetrachloroaurate by sodium ascorbate in aqueous solutions at room temperature. Then prepared colloids were neutralized to pH 6–7 by acetic acid. A dynamic light scattering study of colloidal Au solutions (200 mg/L, i.e. ~10^{-3} M or 6.02 × 10^{20} particles/L) showed that the average hydrodynamic size of gold nanoparticles was around 5 nm (Fig. 1). This result is supported by SEM measurements (see a fragment of SEM microphotograph in the inset to Fig. 1). The surface of Au nanoparticles stabilized by ascorbate anions supposed to be negatively charged (Zeta-potential of −35 mV). The colloidal solutions were stable for months and did not show any appreciable temporal changes in the size and size distribution of Au nanoparticles.

2.2. Ethical approval and animals

All animal studies were performed in accordance with the recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and approved by the Institutional Animal Care and Use Committees. Experiments were performed on 6–8 weeks male Wistar rats (weight 250–300 g) housed under controlled environmental conditions (21 °C, 12 h–12 h light–dark cycle) and free access to water and standard rodent chow.

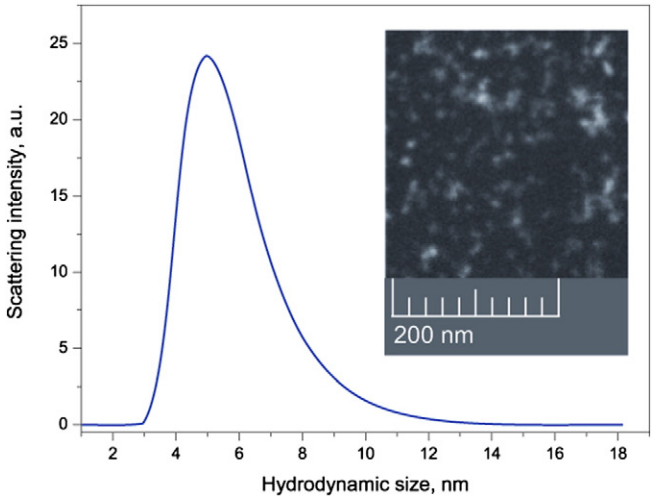

Fig. 1. Distribution of colloidal ascorbate-stabilized gold nanoparticle by the hydrodynamic size. Inset: SEM microphotograph of Au nanoparticles.

2.3. Isolation of rat thoracic aorta smooth muscle cells

Smooth muscle cells were isolated from rat thoracic aorta by collagenase and pronase treatment. Briefly, the rats were anesthetized with ketamine (37.5 mg/kg b.w., IP) and xylazine (10 mg/kg b.w. IP) and approximately 1.0–1.5 cm of the thoracic aorta was excised and cleaned of connective tissue. The aorta was then cut into small pieces (1.5 × 1.5 mm) in a cold low-Ca^{2+} solution containing (in mM): NaCl 140, KCl 6, $MgCl_2$ 3, D-glucose 10, HEPES 10 (pH 7.4) for 15 min. The vascular tissues were transferred to a fresh low-Ca^{2+} solution containing: 2 mg/mL collagenase type IA (417 U/mg), 0.5 mg/mL pronase E type XXV, and 2 mg/mL bovine serum albumin. The tissues were then incubated for 30 min at 37 °C. After incubation, the tissues were washed (2–3 min) twice in a fresh low-Ca^{2+} solution to remove the enzymes. Cells were dispersed by agitation using a glass pipette, and then were placed in a modified Krebs bicarbonate buffer. Aliquots of the myocytes were stored at +4 °C and remained functional for at least 4 h.

2.4. Electrophysiology studies

The whole-cell patch clamp technique in the amphotericin B (250 μg/mL) perforated-patch configuration was used to study whole-cell outward potassium currents (under voltage clamp mode). Data acquisition and voltage protocols were performed using an Axopatch 200B Patch-Clamp amplifier and Digidata 1200B interface (Molecular Devices, Sunnyvale, CA, USA) interfaced to a PC the pClamp software (version 6.02, Molecular Devices, USA). Membrane currents were filtered at 2 kHz and digitized at a sampling rate of 10 kHz. The reference electrode was an Ag–AgCl plug electrically connected to the bath. Macroscopic current values were normalized by the cell membrane capacitance and expressed as pA/pF. The membrane capacitance of each cell was estimated by integrating the capacitive current generated by a 10 mV hyperpolarizing pulse after electronic cancelation of pipette-patch capacitance using the Clampfit software (version 6.02, Molecular Devices, USA). All electrophysiological experiments were carried out at room temperature (20 °C).

Patch pipettes were made from borosilicate glass (Clark Electromedical Instruments, Pangbourne Reading, England) and backfilled with intracellular solution (in mM): KCl 140, NaCl 10, $MgCl_2$ 1.2, HEPES 10, EGTA 2, Na_2ATP 1, and $CaCl_2$ 1, adjusted to pH 7.2 with KOH, resulting in a free [Ca] of approximately 170 nM, D-glucose 11.5,