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Review Article

Do Plasmon Gold Nanoparticles Really Capable of Remotely Opening Ion Channels? Is it Fact or Fancy?

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Abstract

It was established that plasmonic gold nanoparticle (Au NPs) possess the ability to open Maxi-K (BKCa) channels in smooth muscle (SM) cells obtained from different tissues in a remote-control manner. The aim of this study was also to clarify the intrinsic mechanisms of this type channels activation in myocytes isolated from rat pulmonary artery and mouse ileum were recorded in the whole-cell and cell-attached configurations of the patch-clamp techniques and changes intracellular calcium concentration ($[Ca^{2+}]_i$) were monitored using confocal microscopy. First of all we have compared the effects of AuNPs on Maxi-K channel activity under weak and strong intracellular Ca^{2+} buffering (Ca^{2+} "clamp" at 100 nM). When weak Ca^{2+} buffering, Maxi-K current density was increased by AuNPs applied, and then further increased by green laser irradiation (GLI, 5 mW, 532 nm). The potentiating effect of AuNPs alone was due to an increase in maximal conductance (Gmax) by about 50% without any shift of the activation curve of K^+ conductance. GLI in the presence of AuNPs produced an additional effect increasing the maximal K^+ conductance, but instead shifted the potential of half-maximal activation (V1/2) value negatively by about 10 mV. In contrast, under conditions of strong intracellular Ca^{2+} buffering, no effect of AuNPs alone or AuNPs/GLI on Maxi-K currents was observed. It is clear that the effects of AuNPs/GLI were due to a significant increase in channel open probability, while single channel conductance remained unchanged. It is interesting that the deviation from independence of channel gating was observed, suggesting that surface plasmon resonance may not similarly affect all channels present in the membrane patch. This type of regulation is fundamentally different from other common types of drug action. We conclude that AuNPs activate Maxi-K channels via both Gmax increase and a negative V1/2 shift. Moreover, this channel opening effect of AuNPs/GLI is clearly calcium-dependent, as they are able to produce oscillations in $[Ca^{2+}]_i$ and it could be completely abolished by "clamping" the intracellular Ca^{2+} concentration.

Keywords: *Plasmon; Gold Nanoparticles; Ion Channels; Smooth Muscle (SM); Ca^{2+} Buffering; $[Ca^{2+}]_i$*

Introduction

How does a voltage-gated ion channel work? The potential sensor responds to a change in the level of polarization of the plasma membrane and, depending on whether it is depolarization or hyperpolarization, initiates the process of channel activation or deactivation. If

the channel is under double control (as, for example, Maxi-K), then to activate the channel, a concomitant increase in the intracellular concentration of calcium ions is also required. Moreover, changes in the intensity of the influence of one of the regulatory mechanisms affect the effectiveness of the other.

What leads to a change in the level of membrane polarization or calcium concentration and, accordingly, in the activity of the ion channel? This is a huge amount of biologically active substances. And what is most important, all of them have to properly carry out "docking" either directly with one or another subunit of the channel or with a receptor conjugated to it. Thus, is direct interaction a prerequisite? Yes, most of the time.

We suppose that plasmonic gold particles work differently. On their surface, there is a cloud of electron gas that forms an electromagnetic field. The strength of this field is such that it can activate the potential sensor of the ion channel at a distance of up to 1 μm. This effect can be enhanced by using the phenomenon of plasmon resonance. The field voltage can be increased by irradiating nanoparticles with a laser with a wavelength that coincides with the maximum absorption spectrum of nanoparticles. For 5 nm gold nanoparticles, this is 532 nm - a green laser.

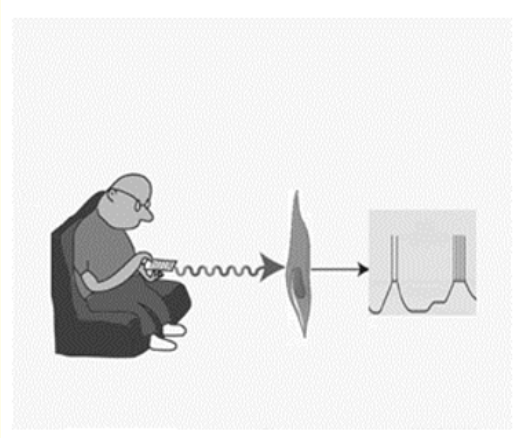

Figure 1: *Remote control of ion channels.*

Results and Discussion

Contractile studies: This is how it works. In experiments with SM of rat thoracic aorta, pre-activated with norepinephrine (NE, 10^{-6} M), it was shown that plasmonic gold nanoparticles 5 nm in size (AuNPs) in the concentration range 10^{-6} - 3 x 10^{-4} M have the ability to significantly reduce the level of tonic tension, caused by NE (pD2 = 4.2 ± 03, Emax = 55 ± 4%), i.e. AuNPs exhibit pronounced vasodilatory activity. Irradiation with a green laser (GLI, 5mW, 532 nm) increased the amplitude of smooth muscle relaxation from 55 ± 4% to 85 ± 5.0% (n = 10, P < 0.05). Endothelial injury did not significantly affect AuNP-induced SM relaxation. Similar data were obtained on fragments of the rat pulmonary artery - Emax = 89 ± 5% (n = 8, P < 0.05), pD2 = 4.2 ± 0.1 (n = 8, P > 0.05).

Electrophysiological studies: Electrophysiological studies showed, что both original traces of I_K and *I-V* relation curves obtained for SM cells from thiracic aortf treated with AuNPs (10^{-4} M) demonstrated a significant increase in outward K^+ current density amplitude from 32 ± 2 pA/pF to 59 ± 5 pA/pF at +70 mV, respectively (P < 0.05, n= 10). External green laser irradiation increased AuNps-induced increment in I_K from 59 ± pA/pF to 74 ± 1 pA/pF (n = 10, P < 0.05).

Current-voltage (I-V) relation curves measured also in isoalted pulmonary artery SM cells in control and in the presence of AuNPs. At maximum depolarization of +70 mV the net outward K^+ (I_K) current density was 49.7 ± 4.9 pA/pF (n = 10). Application of AuNPs (10^{-4} M) produced a significant enhancemnet of I_K density (to 130.2 ± 5.7 pA/pF). GLI in the presence of AuNPs further increased I_K density to 205.3 ± 7.6 pA/pF (n = 10, P < 0.05).

Plots of mean steady-state activation were fitted with a Boltzmann distribution giving voltage for half-maximal activation, i.e. significant increasing in $V_{1/2}$ values under AuNPs and AuNPs plus GLI treatment, ($V_{1/2}$ = 8.5 ± 0.5 and 14 ± 0.8 mV, for control and AuNPs, respectively, n = 10, P < 0.05). Laser irradiation further enhanced this effect ($V_{1/2}$ = 16 ± 0.8 mV, n = 10, P < 0.05), suggesting that this conductance increasing most likely related to the changes of unitary conductance or single channel open time. The data indicate a significant conductance increasing due to K^+ channel activation above -50 mV.

To investigate the phenomenon further we have performed experiments using single channel recordings using on-cell patches formed on rat aorta, pulmonary artery and mouse ileal myocytes. In on-cell patches formed on rat aortic myocytes, activity of 3-5 BK_{Ca} channels was usually observed at potentials from -20 to 40 mV. Depending on the extent of channel activity in each individual membrane fragment the most appropriate holding potential was set within this range in order to have the initial channel activity (commonly expressed as NPo) not too high, e.g. about 0.05 - 0.1. Application of AuNPs to the bath potentiated BK_{Ca} activity with a delay of 1 - 2 minute (Figure 2A), 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 current amplitudes (Figure 2A, middle panel). Eventually, after 10 - 15 minutes 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 (e.g. Figure 2B, top trace at about 35 minutes of the recording). Precise measurement of NPo at such high channel activity became even problematic because of the noisy appearance of the trace, but as an estimate a 5 - 10 fold increase in NPo values was observed in all 3 patches recorded.

Similar results were obtained for BK_{Ca} channels expressed in mouse ileal myocytes indicating that AuNPs act as BK_{Ca} "openers" both in vascular and visceral smooth muscles.

These experiments have revealed that the potentiating effects of AuNPs were due to a significant increase in channel open probability (NPo), while unitary current amplitude at the same test potential remained constant. It is conceivable that AuNPs and the energy of their plasmonic resonance do not directly interfere with the ion conductance pathway, or channel pore, rather they act as gating modifiers that affect kinetics of transitions between closed and open states of the channel and, ultimately, channel open probability. Such mechanism of regulation is common for most pharmacological modulators of ion channels. However, in sharp contrast to other drugs, which typically access and similarly modulate the activity of all channels present, we have occasionally observed an unusual channel behavior during AuNPs/GLI, as illustrated in figure 2. This phenomenon could be described as deviation from the principle of independent channel gating. Briefly, when multiple channels (N - their total number) are present in a membrane patch all having similar open probability P_O, the probability of seeing all channels closed or 1, 2, 3…X channels simultaneously open (P_X) is given by the binomial distribution:

$$P_X=\{N!/X!(N-X)!\}\ P_O^{\ X}\ (1-P_O)^{N-X}.$$

In control, all channels gated independently as confirmed by closely similar measured and calculated according to the binomial distribution P_X values (Figure 3). With AuNPs/GLI probability of seeing one channel open was unusually high, right panel) as if only one out of four channels was activated. Indeed, the experimentally observed probabilities of seeing all channels closed or 1, 2, 3 or 4 channels simultaneously open did not confirm to the principle of independent channel gating. The deviation was especially clear for $P_{X=0}$, $P_{X=1}$ and $P_{X=2}$.

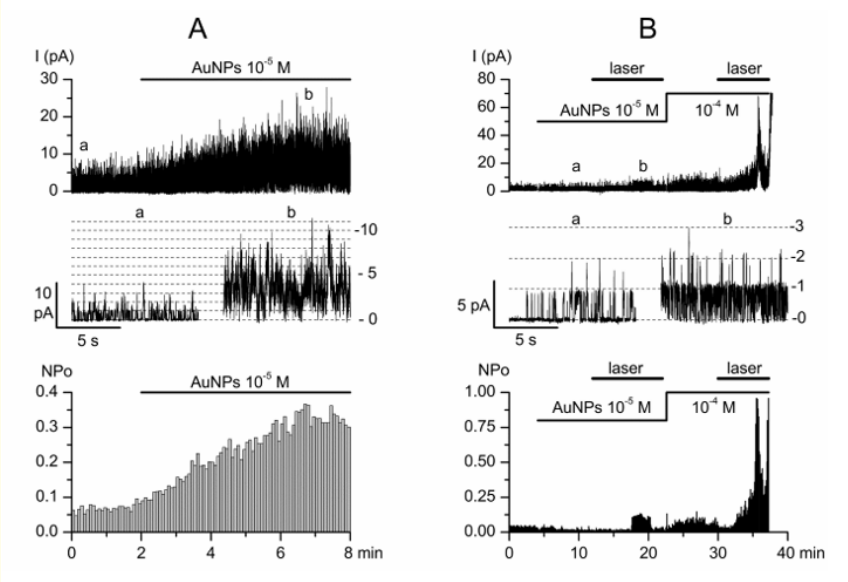

Figure 2: *Potentiating effect of AuNPs on single BK_{Ca} channel activity in rat aortic myocytes. A, BK_{Ca} channel activity was recorded at -20 mV (top) before and after AuNPs application at 10^{-5} M, as indicated by horizontal line (top). Two 10s duration segments of the current trace are illustrated on the expanded time scale in middle panel, taken at time points marked a and b on the whole time-course trace (top panel). Up to 11 active channels were evident in this membrane fragment at full activation, as evident from multiple openings shown by the horizontal dotted lines. Bottom panel shows NPo measured over consecutive 5s time intervals. B, in a different patch held at 40 mV AuNPs were applied initially at 10^{-5} M, followed by 10^{-4} M, as indicated by the thinner horizontal lines, while green laser illumination was additionally used (thicker horizontal lines). Otherwise, the data were analysed and illustrated similar to panel A.*

Role of intracellular Ca^{2+}: It is interesting that under conditions of strong intracellular Ca^{2+} buffering using 10 mM BAPTA/4.6 mM Ca^{2+} mixture, no effect of AuNPs on current density was observed (54 ± 4 pA/pF in control vs 55 ± 4 in the presence of 10^{-4} M AuNPs; $p > 0.05$, n = 6) (Figure 3A). Gmax and the $V_{1/2}$ values also remained largely unchanged. Thus, the mechanism of activation of the potassium channel under the action of gold nanoparticles is calcium-dependent.

The situation was further complicated by experiments with the measurement of intracellular calcium in pulmonary artery cells under the influence of gold. SMCs freshly isolated from pulmonary artery, were preloaded with the high affinity Ca^{2+} indicator fluo-3 and bathed in modified Krebs solution. Intact cells were tested with application of 5 mM caffeine**.** After being washed with the modified Krebs solution, 10^{-4} M AuNPs were added to the pulmonary artery cell suspension. Spatio-temporal patterns of AuNPs-induced $[Ca^{2+}]_i$ oscillations are illustrated at figure 4 showing the results obtained in 3 different cells. Depending on the cell, 3 - 5 minutes after application of 10^{-4} M AuNPs, we observed an appearance of $[Ca^{2+}]_i$ oscillations with a certain periodicity. Each subsequent oscillation was of a smaller amplitude, i.e. progressively decreased in amplitude, which probably corresponded to a decrease in the Ca^{2+} content of the SR. It thus seems to

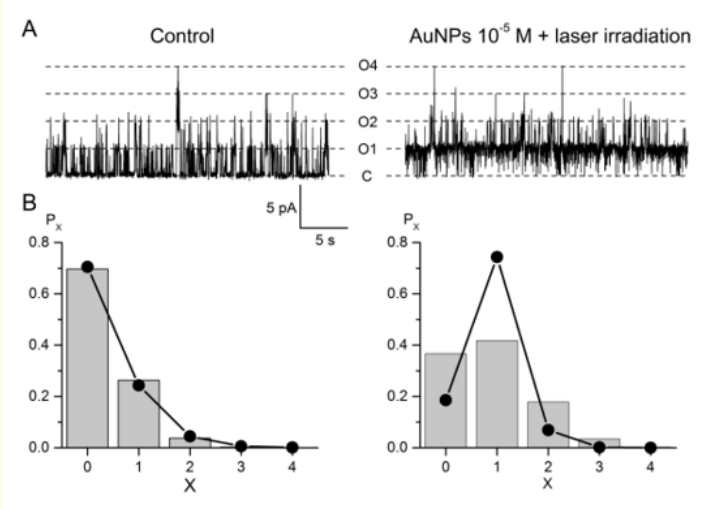

Figure 3: *AuNPs-induced BK_{Ca} channel activation is associated with deviation from the principle of independent ion channel gating. A, simultaneous openings of up to 4 BK_{Ca} channels were observed in a membrane patch both in control and with AuNPs/GLI. These current levels are denoted O1-O4, corresponding to probabilities $P_{X=1}$, $P_{X=2}$, $P_{X=3}$ and $P_{X=4}$ in panel B, while C denotes closed level, e.g. all channels are closed ($P_{X=0}$). B, experimentally found P_X values (closed circles) and those predicted by binomial distribution (columns) assuming N = 4, P_O = 0.086 in control and N = 4, P_O = 0.222 in the presence of AuNPs/GLI. The P_O values were obtained from the measured NP_O values, 0.345 and 0.887 in control and with AuNPs/GLI, respectively.*

be related to the SR Ca^{2+} release, whereas activation of Ca^{2+} entry across the plasma membrane efficiently re-stores Ca^{2+} transients, which indicates effective Ca^{2+} store re-filling. Thus, it seems likely that the effect of AuNPs is calcium-dependent but precise mechanisms of the genesis of AuNPs-induced $[Ca^{2+}]_i$ oscillations in vascular myocytes await further investigations.

Conclusion

First, the effect of AuNPs was observed in the cell-attached configuration, whereby the exterior of the membrane is not directly accessible to any molecules in the bath solution, it is known that even small neurotransmitter molecules cannot act on ligand-gated ion channels, when they are isolated from the bath by the tight gigaseal. This indicates that either AuNPs can cross the membrane and act on the intracellular part of the channel, or that they can somehow alter membrane properties in such a way, that this signal (most likely surface plasmon resonance energy) can be transmitted within the membrane plane.

Second, single channel current amplitude during AuNPs application remained constant indicating that these nanoparticles do not interfere with the ion conductance pathway, or channel pore. Instead activation of the whole-cell currents at the single channel level can be explained by the significant NP_0 increase as the main determinant.

Third, although we could not analyse open and closed times, as multiple channels were present in all patches, at early times NP_0 increase by AuNPs seemed to be due to more frequent channel openings, rather than increase in channel open time. Understanding the exact

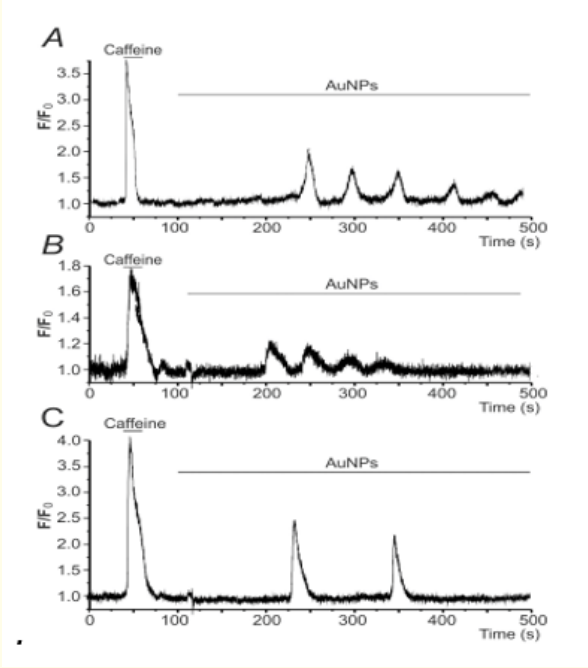

Figure 4: *Spatio-temporal patterns of AuNPs-induced $[Ca^{2+}]_i$ oscillations after control caffeine application recorded in 3 different representative cells to illustrate similarities (e.g. oscillatory nature) and variability (e.g. amplitude and frequency of $[Ca^{2+}]_i$ rises) of the responses. For each cell (A-C), the time course plot of the normalized fluo-3 fluorescence intensity was averaged within the total optical slice of the SMC, where Ca^{2+} waves induced by 10^{-4} M AuNPs) occurred.*

mechanism of channel activation requires additional experiments, but it seems that AuNPs may modulate BK_{Ca} channels one by one and with variable time delays, which is different from the action of most other known channel chemical modulators.

Are we right or wrong? In any case, we are open to discussion and would be grateful for any criticisms or advices.

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