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Zinc nanoparticles interact with olfactory receptor neurons

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Abstract Zinc metal nanoparticles strongly enhance odorant responses of olfactory receptor neurons. Olfactory receptors belong to the large superfamily of G-protein coupled receptors. A theoretical model based on experimental results explains a stoichiometry of metal nanoparticles receptor interaction. The model is similar to that used by A.V. Hill for the binding reaction between hemoglobin and oxygen. The model predicted that one metal nanoparticle binds two receptor molecules to create a dimer. This result is consistent with the evidence that many G-protein-coupled receptors form dimers or larger oligomers.

Keywords Zinc metal clusters · Smell · Olfaction · Receptors · G-proteins · Oligomers

Introduction

The fine properties of the olfactory system results from physical and biochemical events that occur at the olfactory epithelium of the nasal cavity where olfactory receptor neurons interact with odorants. Then the information received from the sensory neurons is transferred to the secondary neurons in olfactory bulb, and it is further sent to the cortex where the information is used for the discrimination of many odors (Zufall et al. 1994).

The initial events in olfaction take place in an olfactory neuroepithelium situated in the posterior nasal cavity. Olfactory receptor neurons have a few dozen hair-like cellular structures, called olfactory cilia. Cilia harbor the sensory apparatus (olfactory receptor proteins and other components) that converts and amplifies the physical–chemical signal of odorant molecule into electric current (Pace et al. 1985). Olfactory receptors have been found to belong to the large superfamily of G-protein coupled receptors (Buck and Axel 1991). Olfaction begins with sniffing that transports odorant molecules into the nose and delivers them to the mucus layer covering the olfactory epithelium. The binding of the odorant by a receptor protein initiates an intracellular cascade of signal transduction events, including the G-protein-dependent production of second messenger molecules, leading to opening of ion channels and passing of ion currents.

It was demonstrated earlier (Viswaprakash et al. 2009a) that zinc metal nanoparticles in picomolar concentrations strongly enhance odorant responses of olfactory sensory neurons. One to two nanometer metallic particles contain 40–300 zinc metal atoms, which are not in an ionic state. We exposed rat olfactory epithelium to metal nanoparticles and

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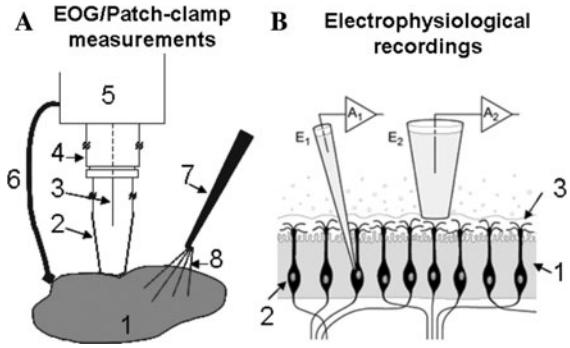

Fig. 1 Electrophysiological set up. **A** EOG/patch-clamp. 1 olfactory tissue; 2 glass micro-electrode; 3 silver/silver-chloride wire; 4 glass micro-electrode holder; 5 head stage; 6 ground wire; 7 multibarrel odor applicator/puffer; 8 odor stream; **B** 1 olfactory epithelium, 2 olfactory receptor neuron, 3 cilia in mucus layer; E_1, and E_2—patch clamp and EOG electrodes, respectively; A_1 and A_2—patch clamp and EOG amplifiers, respectively

measured odorant responses by electroolfactogram and whole cell patch clamp (Fig. 1). A small amount of zinc nanoparticles added to an odorant or an extracellular/intracellular particle perfusion strongly increased the odorant response in a dose-dependent manner (Fig. 2). It was further demonstrated that zinc nanoparticles alone produced no odor effects. Gold or silver nanoparticles did not produce effects similar to those of zinc. Though copper nanoparticles did not enhance olfactory response, they assisted to maintain a stable level of odorant responses for a long time. If zinc nanoparticles are replaced by Zn^{2+}-ions in the same concentration range, we observed a reduction of the olfactory receptor neuron odorant response. Based on these observations, we hypothesize that zinc nanoparticles are closely located to the interface between the G- protein and the receptor proteins and are involved in transferring signals in the initial events of olfaction. In the present work we built a theoretical model that allowed us to characterize a stoichiometry of odorant and zinc interactions with receptor and G-protein.

Theoretical model

Stoichiometry of odorant and zinc binding

If olfactory receptors, odorant molecules and metal nanoparticles interact we can write kinetic equations (Segel 1975; Giraldo 2008) as shown in Fig. 3. The equations are similar to those used by A.V. Hill for the binding reaction between hemoglobin and oxygen. In Fig. 3 we denote R—receptors, M—metal nanoparticles, n, m—numbers of nanoparticles and odorant molecules, respectively.

The association constants k_m and k_o for the above reactions are:

$$k_m = \frac{[R][M]^n}{[RM_n]} = \frac{[RO_m][M]^n}{[RM_nO_m]} \tag{1}$$

$$k_o = \frac{[R][O]^m}{[RO_m]} = \frac{[RM_n][O]^m}{[RM_nO_m]} \tag{2}$$

For the ion currents (I) evoked by the odorant in the presence of metal nanoparticles we can write:

$$\frac{I}{I_{MAX}} = \frac{I}{C_R} = \frac{[RM_nO_m]}{[R] + [RO_m] + [RM_n] + [RM_nO_m]} \tag{3}$$

where C_R is a total concentration of receptors, $[R]$, $[RO_m]$, $[RM_n]$ are concentrations of free receptors and receptors bound with odorant and metal nanoparticles, respectively.

From Eq. 3 we can derive:

$$[RO_m] = \frac{[R][O]^m}{k_o} \tag{4}$$

$$[RM_n] = \frac{[R][M]^n}{k_m} \tag{5}$$

$$[RM_nO_m] = \frac{[M]^n[RO_m]}{k_m} = \frac{[M]^n[O]^m}{k_mk_o} \times [R] \tag{6}$$

$$\frac{I}{I_{MAX}} = \frac{[M]^n}{k_m + [M]^n} \times \frac{[O]^m}{k_o + [O]^m} \tag{7}$$

If $[O]$ = constant

$$\frac{I}{I_{MAXo}} = \frac{[M]^n}{k_m + [M]^n}, \tag{8}$$

$$\text{where } I_{MAXo} = I_{MAX} \times \frac{[O]^m}{k_o + [O]^m} \tag{9}$$

If a fraction of receptors bound by metal nanoparticles, $Y_m \cong \frac{I}{I_{MAXo}}$ then from Eq. 8 we can determine a ratio of free and bound by metal nanoparticles as following:

$$\frac{Y_m}{1 - Y_m} = \frac{1}{k_m} \times [M]^n \tag{10}$$

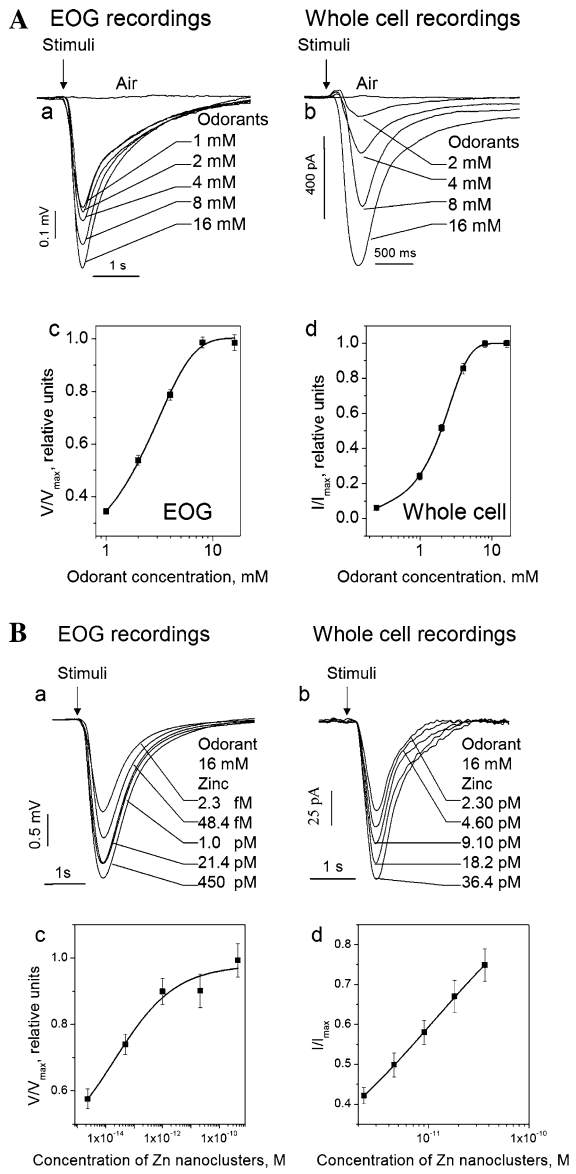

Fig. 2 Electrophysiological recordings from rat olfactory epithelium and a single olfactory neuron. The stimulus was a 0.25-s pulse of air, odorant mixture, or odorant/zinc nanoparticle mixture. An odorant mixture contained 16 mM each ethyl butyrate, eugenol, and (+) and (−) carvone. Odorants vapors were collected above the odorant or odorant/zinc suspensions (in water). Panel **A**: (*a*) EOG traces induced by a pure air and odorant mixture at different concentrations (no zinc added). The representative set of traces was obtained from 4 tissues, 10 contacts, and 34 EOG recordings. (*b*) Current traces from whole-cell recordings of olfactory neurons with holding potentials of −70 mV at different odorant concentrations (no zinc added). Downward direction indicates inward current. This is a typical representation of 23 whole cell recordings. Each recording comprises 3–15 whole cell traces. (*c*) Plot of normalized, peak EOG voltage (V/V_{max}) evoked by odorant versus odorant concentration. (*d*) Plot of normalized, peak negative current (I/I_{max}) evoked by odorant versus odorant concentration. Panel **B**: (*a*) EOG traces induced by odorant/zinc particles mixture at different concentrations of zinc (representative traces of 34 EOG recordings). Odorant concentration was constant for all traces. (*b*) Current traces from whole-cell voltage recordings of olfactory neurons with holding potentials of −70 mV at different concentrations of zinc (typical traces of 23 whole cell recordings). (*c*) Plot of normalized, peak EOG voltage (V/V_{max}) evoked by odorant/zinc particles versus zinc concentration. (*d*) Plot of normalized, peak negative current (I/I_{max}) evoked by odorant/zinc particles versus zinc concentration (Viswaprakash et al. 2009a). By permission from Oxford University Press

Taking logarithm of both sides of Eq. 10 we have

$$\log \frac{Y_m}{1 - Y_m} = n \log[M] - \log k_m \tag{11}$$

The Eq. 11 is an analog of the conventional Hill equation (Segel 1975). A similar expression can be obtained for a case when $[M]$ = constant.

$$\log \frac{Y_o}{1 - Y_o} = m \log[O] - \log k_o \tag{12}$$

The theoretical Eqs. 11 and 12 were used to plot Hill presentations from experimental patch clamp data shown in Fig. 2 (Panel A, c and d; Panel B, c and d). Results are shown in Fig. 4. Hill coefficients derived from these figure are shown in Table 1.

The Hill coefficient, m, defines how many odorant molecules bind a receptor in a single binding event. The value of m determines the cooperativity of odorant binding and the slope of rising phase of the dose–response relation (Segel 1975; Connors 1987; Giraldo 2008). The large the value of m the stepper the rising phase. We found that it takes ~ 2 molecules of odorant to activate one receptor/G_{olf} complex. The value of the Hill coefficient for odorant is consistent with that found by other investigators and is reviewed in (Kleene 2008).

Similarly, the Hill coefficient, n, defines how many zinc nanoparticles bind a receptor in a single binding event, as shown in the kinetic diagram (Fig. 3). We

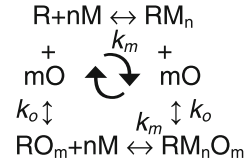

Fig. 3 Schematic diagram of interaction of olfactory receptors with molecules of odorant and metal particles (explanation in text)

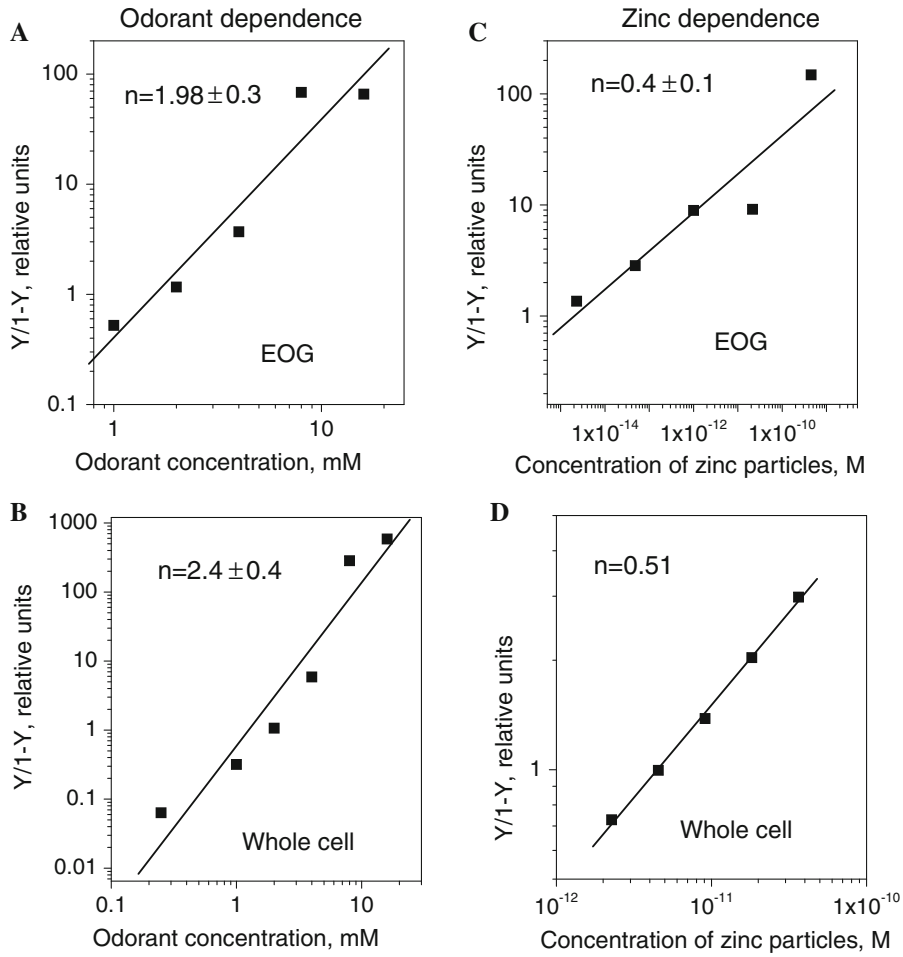

Fig. 4 Hill presentation for odorant and zinc dependence respectively

Table 1 Hill coefficients, *m* and *n*

Methods	Odorant, *m*	Zinc particles, *n*
EOG	2.0 ± 0.3	0.40 ± 0.1
Whole cell	2.4 ± 0.4	0.51 ± 0.01

found that the Hill coefficient for zinc nanoparticles is ~0.5. That means that one metal nanoparticle binds two receptor molecules to creates a dimer (Giraldo 2008). Because, this dimer represents a single activation event transmitted through a single G_{olf}, we speculate that a single receptor/G_{olf} complex is composed of two receptors, one zinc particle and one G_{olf}.

Discussion

There is compelling evidence that many G protein-coupled receptors form dimers or larger oligomers (Milligan 2004; Fredholm et al. 2007; Milligan 2007, 2008; Skrabanek et al. 2007). We found no reports of olfactory receptor homodimerisation, but there is strong evidence, obtained by atomic force microscopy, of rhodopsin homodimerisation in optic disc membranes (Fotiadis et al. 2003a, b).

Previously, we speculated (Viswaprakash et al. 2009a) that zinc particle positioned between the receptor and G_{olf} is consistent with Luca Turin's (1996) suggestion that zinc ion binding sites are present both on the odorant receptor protein and the G-protein and that zinc ions assist the signal transfer from receptor to G-protein. These zinc metal particles can work also as electron donors as predicted by the general theoretical model of Brookes and coworkers (2007). I must admit that we misunderstood Turin's interpretation of zinc ions interactions in olfactory cilia. He has never suggested there was a zinc binding site on the G-protein (Turin L, June 20, 2009,

personal communication). He indicated that the zinc ion-binding motif, CGSHL, positions near the cytoplasmic end of the sixth transmembrane domain of the olfactory receptor protein (Turin 1996). In contrast, Turin suggested that zinc nanoparticles are bound to the mucosal side of membrane. He thinks that zinc nanoparticle works as mini-battery. Some zinc atoms spontaneously go into solution as Zn^{2+}-ions and leave electrons behind. These electrons take part in the tunneling process that he believes holds the transduction (Ball 2009).

The resting potential of olfactory sensory neurons ranges between −30 to −90 mV (Lagostena and Menini 2003). The resting electrochemical gradients at the apical end of the rat olfactory receptor neuron (and probably at the cilia membrane) cannot explain such a relatively large negative resting potential. The highest negative reversal potentials of −24 mV is estimated for potassium ions (Kleene 2008). In any way, at the negative resting potential the mucosal side of cilia membrane is positively charged relative to cytosol. If a zinc nanoparticle is positioned on the cytosol side of membrane it creates Zn/Zn^{2+} zinc galvanic half-cell that can be characterized by reaction of oxidation, $Zn \rightleftarrows Zn^{2+} + 2e^{-}$. A standard reduction potential of this reaction is −0.76 V (Bard et al. 1985). In order to convert this half-cell in the fully functional electrochemical cell, we need to provide a reductant to the mucosal side of membrane. Many metal nanoparticles such as Fe, Cd, Co, Ni, and Cu with reduction potentials of −0.44, −.0.40, −0.28, −0.25, and +0.34, respectively, can serve in this capacity. All these metals, including zinc were found in a few mg/L concentrations in human blood (DIONEX 2009). Zn, Fe, and Cu nanoparticles were found in human and animal blood (Samoylov et al. 2005). In our experiments we found that copper nanoparticles stabilized olfactory responses, so that amplitude of the responses sustains for a long time (Viswaprakash et al. 2009a). I speculate that a copper nanoparticle on the mucosal side of ciliary membrane across the zinc nanoparticle creates another Cu/Cu^{2+} galvanic half-cell. Zinc and copper half-cells create a complete electrochemical cell (mini-battery) when they are galvanically connected. A zinc nanoparticle then becomes negatively charged by donating positive zinc ions into cytosol ($Zn \rightarrow Zn^{2+} + 2e^{-}$) and a copper nanoparticle is charged positively when it provides two electrons to convert copper ions into atoms ($Cu^{2+} + 2e^{-} \rightarrow Cu$). The electro motive force (E) of this mini-battery is equal to the difference between standard reduction potentials of copper and zinc (0.35-(−0.76)) and equal to 1.1 V (Bard et al. 1985). The direction of electric field created by this battery coincides with the natural electric field of resting membrane. The positive pole of the mini-battery is in mucus and the negative one is in cytosol. If zinc nanoparticle is positioned in the mucus, then a large negative potential will be imposed on the positively charged membrane side. More experiments are needed to determine position and full functions of zinc nanoparticles in olfactory cilia.

One of the remarkable properties of small metal nanoparticles is their stability in water in blood plasma (Samoylov et al. 2005; Viswaprakash et al. 2009a). It was discovered that metal nanoparticles with 13, 55, 147, 309, 561, and 923, so called "magic number" of atoms, have added stability. These nanoparticles designated as full-shell clusters that are constructed by successively packed layers of metal atoms around a single metal atom (Aiken and Finke 1999; Khanna et al. 2002).

The important role of zinc nanoparticles in olfactory transduction is a further illustration of the significant role of zinc in neurobiology (Frederickson et al. 2005). Free and bound zinc is found in relatively large concentrations in different parts of the brain, including olfactory bulb and olfactory epithelium (Horning and Trombley 2001; Takeda 2001; Persson et al. 2003; Frederickson et al. 2006). In most cases, the distribution of zinc in the brain was studied by methods that cannot discriminate between zinc ions and zinc metal particles (Takeda et al. 1997; Takeda 2001). Zinc transporters in brain are known (Takeda 2001) but it is not known if the same system of zinc homeostasis supports zinc nanoparticles. Small colloidal metal particles were studied by nineteenth century colloidal science (Kruyt 1952; Thomas 1988), then with more advanced methods of chemistry and physics in twentieth century the small metal particles, called metal nanoclusters, were better understood and used in the rapidly developing field of nanotechnology (Khanna et al. 2002; Aiken and Finke 1999). Very recently, metal nanoparticles, now called superatoms, have been intensively studied (Hakkinen 2008; Walter et al. 2008). Superatoms having special electronic structure possess unusual chemical, physical properties, and may have high

stability against aggregation and oxidation. We found a large number of stable metal nanoparticles in human and animal blood (Samoylov et al. 2005). It would be important to learn if they are physiologically significant. We recently demonstrated that zinc nanoparticles enhanced EOG responses of cultured olfactory neurons. The fact that zinc nanoparticle enhancement was observed in both young and mature cultures as well as in dissected olfactory epithelium indicates the importance of this phenomenon for initial events in olfaction (Viswaprakash et al. 2009b).

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