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Short communication

Olfactory responses to explosives associated odorants are enhanced by zinc nanoparticles

Christopher H. Moore, Oleg Pustovyy, John C. Dennis, Timothy Moore, Edward E. Morrison, Vitaly J. Vodyanoy*

Department of Anatomy, Physiology and Pharmacology, Auburn University, Auburn, AL 36849, USA

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ABSTRACT

Many odorants related to manufactured explosives have low volatilities and are barely detectable as odors. We previously reported that zinc metal nanoparticles increased rat olfactory epithelium responses, measured by electroolfactogram (EOG), to several odorants. Here, we report that nanomolar concentrations of zinc metal nanoparticles strongly enhanced olfactory responses to the explosives related odorants cyclohexanone, methyl benzoate, acetophenone, and eugenol. Rat olfactory epithelium was exposed to metal nanoparticles and odorant responses were quantified by EOG. Zinc nanoparticles added to explosive odorants strongly increased the odorant response in a dose-dependent manner. The enzymatic breakdown of the second messenger cyclic adenosine monophosphate (cAMP) was prevented by adding the membrane-permeable phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX). This caused the olfactory cilia cAMP concentration to increase and generated EOG signals. The EOG responses generated by IBMX were not enhanced by zinc nanoparticles. Based on these observations, we conclude that zinc nanoparticles act at the receptor site and are involved in the initial events of olfaction. Our results suggest that zinc metal nanoparticles can be used to facilitate a canine detection of explosive odorants.

1. Introduction

Detecting explosives buried in soil or otherwise camouflaged is extremely difficult. Physical and chemical explosive detection methods work relatively well in the laboratory but serious challenges exist for detection in the field [1]. Sniffer dogs have been the most effective explosive detectors [2] but even trained dogs are limited because most plastic explosive materials have very low vapor pressures and their detection is therefore difficult. To facilitate detection of plastic explosives, detectable agents, called taggants, with relatively high vapor pressures can be added to explosives during their manufacture [3]. The most common taggant added to plastic explosives is 2,3-dimethyl-2,3-dinitrobutane (DMNB), which can be detected by physical methods in laboratory conditions. Surprisingly, field studies indicated that DMNB is not detectable by trained detector dogs [2,4].

In the search to increase sniffer dogs' capacity for low vapor pressure odorant detection, we found that low concentrations of zinc metal nanoparticles enhanced main olfactory epithelial responses to odorants as measured by electroolfactogram (EOG) and sensory neuron responses using whole cell patch-clamp [5] (discussed in [6]). A small number of zinc nanoparticles added to a butyrate, eugenol, and (+) and (−) carvone odorant mixture increased responses by a factor of ~2.5 but zinc nanoparticles alone produced no odor effects. The effects are dose-dependent and reversible in that the particles are spontaneously cleared from the system. Odorant response enhancement was observed regardless of the delivery method: with odorant vapor, with an extracellular pipette, or intracellular microinjection [5]. Other metal nanoparticles, specifically copper, gold, and silver, did not produce the effects found with zinc. When zinc nanoparticles were replaced by Zn^{2+}-ions at the same concentrations, we observed a reduction in receptor neuron response.

EOG studies on rat olfactory mucosa showed that some compound vapors associated with plastic explosives like benzene, naphthalene, hexachloroethane, styrene, toluene and chlorobenzene, elicited measurable electrical responses. On the other hand, TNT and RDX did not stimulate detectable electrophysiological signals [7].

In this work we asked whether or not other chemical compounds associated with explosives induce measurable EOG

Abbreviations: cAMP, cyclic adenosine monophosphate; CNG, cyclic nucleotide-gated ion channel; DMNB, 2,3-dimethyl-2,3-dinitrobutane; EOG, electroolfactogram; G_{olf}, olfactory neuron specific-G protein; IBMX, 3-isobutyl-1-methylxanthine; RDX, research department explosive; TNT, 2,4,6-trinitrotoluene.

* Corresponding author. Tel.: +1 334 8445405; fax: +1 334 8445388.
E-mail address: vodyavi@auburn.edu (V.J. Vodyanoy).

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responses and if zinc nanoparticles enhanced EOG responses evoked by these odorants. We also attempted to discover the potential site of zinc nanoparticles action in the initial signal transduction events.

2. Experimental

2.1. The epithelial slice

An epithelial sample was prepared by the method described in [5,8]. Rat septal olfactory mucosa was removed from the septum and placed in an RC-8 Warner Instrument patch-clamp recording chamber. The basal side was immersed in physiological buffer solution buffer (137 mM NaCl, 5.3 mM KCl, 4.2 mM $NaHCO_3$, 0.4 mM KH_2PO_4, 3.4 mM Na_2HPO_4, 5.6 mM D-glucose, 0.8 mM $MgSO_4$, and 1.2 mM $CaCl_2$; pH 7.4) and the apical epithelial surface was exposed to the air.

2.2. Electroolfactogram recording

The EOG recording electrode, a Ag/AgCl wire in a glass pipette with a ~24 μm tip opening and filled with physiological buffer, was connected to an electronic amplifier to detect olfactory epithelial responses. Glass pipettes were fabricated from borosilicate capillary pipettes (World Precision Instruments, Sarasota, FL, USA) pulled in a P-87 pipette puller (Sutter Instruments, Novato, CA, USA). Once contact between the electrode and the olfactory epithelial surface was made, responses to odorant puffs applied over a several minute time course were amplified by a MultiClamp Amplifier 700A (Axon Instruments, Union City, CA, USA), filtered at 2–5 kHz, and recorded.

Odorants were purchased from Sigma–Aldrich. We used a mixture of explosive-associated odorants [9] that contained cyclohexanone, methyl benzoate, acetophenone and eugenol in water. As a control, we used an odorant solution containing ethyl butyrate, eugenol, and (+) and (−) carvone in water, which we investigated previously [5,8]. The delivery method of odorants with zinc nanoparticles was described previously [5]. Briefly, odorants were mixed with a vortex and then diluted at concentrations of 1, 2, 4, 8, and 16 mM. Odorant mixtures at each concentration were kept in 100 ml dark-glass bottles and the odorant vapor contained in the bottle headspaces was applied to the isolated olfactory epithelium through a calibrated multibarrel pipette equipped with a small glass nozzle.

For stimulation, a 0.25 s pulse of the odorant mixture at 8 pounds per square inch was formed by a computer controlled Pneumatic PicoPump PV800 (World Precision Instruments). A pulse of positive pressure drove the odorant into a calibrated multibarrel pipette fitted with a glass nozzle directed at the olfactory epithelium. Each pipette barrel could pass a puff of distinct odorant composition and concentration. The residual odorant was cleared by air between each stimulus application. The automatic computer routine was composed of 0.25 s pulses at 20 s intervals. One series of 10 pulses at 20 s intervals constituted one 'EOG recording'. Thus, in the automatic regime, the single 'EOG recording' had a 200 s duration and corresponded to as many as 10 response traces.

A nanoparticle suspension was mixed with odorant solutions to make final nanoparticle concentrations of a few nM. During the puff, the odorant vapor containing metal nanoparticles was delivered to the olfactory epithelium surface.

A special three-pipette system was designed to deliver the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX). Three pipettes were mounted on the Soma MX1100 R High-Precision Micromanipulator so that the pipette tips were simultaneously directed to an area a few hundred microns in diameter on the olfactory epithelium. One pipette contained buffer and served as the EOG electrode. A second contained IBMX dissolved in buffer and the third contained IBMX in buffer with zinc nanoparticles. After the microelectrode formed a stable contact with the olfactory epithelium (OE), a 0.25 s pulse of IBMX or IBMX + zinc was delivered. We used 400 μM IBMX solutions to evoke an EOG response.

3. Results

A typical EOG response to the standard odorant pulses is shown in Fig. 1a. The EOG signal amplitude produced by purified air was much smaller compared with those excited by mixed odorants. When olfactory receptor neurons were excited by odorant mixed with a suspension of zinc nanoparticles, the EOG odorant response amplitude was significantly increased.

The mixture of explosive-associated odorants also elicited an EOG response that was enhanced by zinc nanoparticles in dose dependent manner (Fig. 1b). Zinc nanoparticles at a concentration of 5.6 nM caused an approximate fivefold increase in EOG amplitude compared to that elicited by odorant alone. The EOG kinetic parameters, half-rise and half-decay times, defined in [10] left unaltered at the zinc presence.

Application of 400 μM IBMX in buffer without odorant to the OE surface evoked an EOG response, while delivery of a pulse of buffer alone elicited no response (Fig. 1c). The response to IBMX + zinc nanoparticles was indistinguishable from that of the IBMX alone (Fig. 1d).

4. Discussion

By measuring electrical responses to odorants, we determined that rat olfactory receptors are sensitive to a mixture of four odorants related to explosives: cyclohexanone, methyl benzoate, acetophenone, and eugenol [9]. The EOG signal amplitudes produced by this mixture were much smaller compared with those excited by the standard odorant mixture used as a control [8,10]. The similarities in kinetics between EOG responses evoked by explosives-associated and conventional odorants indicate that those substances related to explosives work as conventional odorants (through olfactory receptors) and they do not noticeably change functional properties of rat olfactory neurons. Finally, our data agree well with rat EOG results obtained with the other explosives-associated compounds [7]. They demonstrated that benzene, naphthalene, hexachloroethane, styrene, toluene and chlorobenzene, elicited measurable electrical responses.

In vitro EOG studies on rat olfactory mucosa showed that vapors of some compounds associated with plastic explosives, specifically benzene, naphthalene, hexachloroethane, styrene, toluene and chlorobenzene, elicited measurable electrical responses but the EOG amplitudes for these compounds are significantly lower than that elicited by the conventional odorant amyl acetate. Furthermore, TNT and RDX in these experiments did not evoke the detectable EOG signals [7]. These low EOG amplitudes evoked by explosive compounds are consistent with facts of very low vapor pressure and difficulties of their detection by sniffer dogs. A taggant for plastic explosive, DMNB, did not facilitate canine detection of plastic mines [1,2,11].

The binding of the odorant to the olfactory receptor leads to an excitation of the receptor neuron, thru a second messenger pathway. In mammals the odorants stimulate adenylyl cyclase to synthesize a second messenger, cAMP, via a G_{olf} protein. cAMP, opens a cyclic nucleotide-gated ion channel (CNG) producing an influx of Ca^{2+} and Na^{+} ions into the cell, slightly depolarizing it. The Ca^{2+} in turn opens a Ca^{2+}-activated chloride channel, leading to

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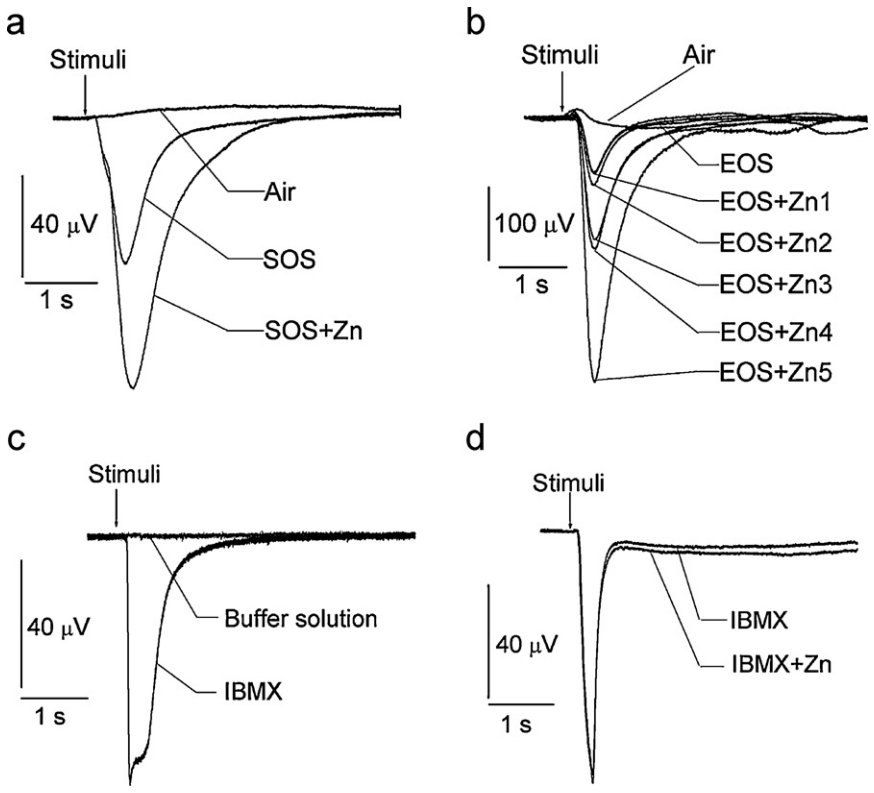

Fig. 1. EOG traces recorded from rat olfactory epithelium. The arrows in a–d indicate the time at which the 0.25 s stimuli were applied. a. EOG recording of odor induced responses. The responses were induced by air, 16 mM standard odorant solution (SOS), and SOS with 1.4 nm zinc nanoparticles (SOS + Zn). The figure shows representative traces of 3 tissues and 23 recordings. b. EOG traces induced by vapors of 8 mM explosive-associated odorant solutions with varying concentrations of zinc nanoparticles from Zn1 to Zn5 = 0.6, 1.4, 2.8, 4.3, 5.6 nM, respectively. The figure represents typical responses obtained from 4 tissues and 35 recordings. c. EOG response induced by a pulse of 400 μM IBMX without odorant. The pulse of buffer solution was applied as a control. The figure shows representative traces from 3 tissues and 21 recordings. d. EOG response induced by a pulse of 400 μM IBMX with and without 4 nM zinc nanoparticles. The figure shows representative traces from 3 tissues and 25 recordings.

efflux of Cl^-, further depolarizing the cell. The termination of the olfactory signal occurs through decomposition of cAMP by phosphodiesterase resulting in cell membrane polarization [12]. The kinetics of membrane depolarization and polarization can be seen in electroolfactogram recording that reflects a consecutive increase and decrease of cAMP level and activation and termination olfactory response to odorant, respectively [13].

To identify the site at which the zinc nanoparticles act in the olfactory transduction pathway, we generated EOG responses without olfactory receptor activation using IBMX, a phosphodiesterase inhibitor that reduces adenylyl cyclase produced cAMP hydrolysis. EOG responses evoked by IBMX without odorant were similar to those observed by other researchers [7,14,15]. We further demonstrated that zinc nanoparticles do not affect the IBMX-induced olfactory response. Therefore, zinc nanoparticles do not act at the ion channel level, which suggests that zinc is involved in earlier transduction steps. Because zinc nanoparticles alone cannot evoke an EOG response [5], they do not modulate cAMP production by adenylyl cyclase and the receptor/G-protein complex is the most likely place of nanoparticle action. Previously, we found that one zinc metal nanoparticle binds two receptor molecules to create a dimer, which is consistent with evidence that many G-protein-coupled receptors form dimers or larger oligomers [16]. The fact that zinc nanoparticle enhancement was observed in both young and mature tissue cultures as well as in olfactory epithelium tissue fragments indicates the importance of this phenomenon for initial events in olfaction [10].

Here, we demonstrated that zinc nanoparticles added to explosive odorants can enhance rat EOG signals in response to those odorants and these results are consistent with previous data obtained with conventional odorants [5]. It remains to be determined whether or not EOG signal enhancement by zinc nanoparticles boosts odor perception at the level of consciousness. Doty and co-workers (1990) [17] demonstrated that perceived odor intensity in humans correlates with an EOG amplitude. There is, therefore, some justification in speculating that if zinc nanoparticles are added to explosive odorants, then perceived odor intensity is increased and, subsequently, detection probability by sniffer dogs is also increased.

5. Conclusions

Sniffer dogs are trusted and, so far, irreplaceable detectors of explosives. Here, we report a new method of smell enhancement using zinc nanoparticles in low concentrations. In particular, we show: (1) explosives-associated compounds induce measurable EOG responses; (2) nanomolar suspensions of zinc nanoparticles enhance EOG responses to these compounds by a factor of 5; (3) the receptor/G-protein complex is the most likely site of nanoparticle action; and (4) our data suggest that zinc nanoparticles added to explosive-associated odorants may enhance the odor intensity and detection probability to sniffer dogs. Future investigations with live animals will show the utility of zinc nanoparticles in explosives detection.

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