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Nucleotide modulates odor response through activation of purinergic receptor in olfactory sensory neuron

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ABSTRACT

Extracellular nucleotides are important neurotransmitters, neuromodulators and paracrine factors in the neural sensory system [16]. Most of purines and pyrimidines act on the associated purinergic cell-surface receptors to mediate sensory transduction and modulation. Previously, we reported a subgroup of heptaldehyde (H)/2-hepatanone (Ho)-responsive olfactory sensory neurons (H/Ho-OSNs) in the ventral endoturbinates [31]. Through the calcium image recording, we characterized that ATP elicited $[Ca^{2+}]_i$ increase in the presence of extracellular calcium, while depletion of intracellular calcium stores blocked UTP-evoked $[Ca^{2+}]_i$ increase. Pharmacological studies indicated that $P2X_3$ was expressed in the H/Ho-OSNs, modulating both heptaldehyde (H) and 2-hepatanone (Ho)-induced responses. These data indicated that activation of purinergic receptor negatively modulated odor response, providing the evidence to support the possible protective effect of purinergic receptor in OSNs.

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1. Introduction

Purinergic nucleotides are important extracellular signaling molecules involved in peripheral visual [25], olfactory [27,8], auditory [20] and gustatory sensory system [5]. They act on purinergic receptors to play vital roles in development, neurotransmission, neuromodulation and neuropathology [2,4,16]. Two types of purinoceptors are identified as P1 and P2 [1]. P1 receptors are subdivided into four types, A_1, A_{2A}, A_{2B}, A_3 [1]. P2 receptors are composed of P2X and P2Y subtypes. P2X receptor family comprises seven subtypes ($P2X_1$–$P2X_7$), all of which are ligand-gated ion channel receptors [22]. P2X receptors form intrinsic Ca^{2+}-permeable nonselective cation channel and allow Ca^{2+} influx from extracellular space [22,19]. Until now, eight P2Y receptor subtypes are recognized. Most of P2Y receptors are G-protein coupled receptors, through which intracellular secondary messenger (cAMP or IP_3) is produced, and then calcium is mobilized and released from intracellular stores [1,7].

In vertebrates, the olfactory epithelium is the primary site to sense the odor. The olfactory epithelium is composed of 6–10 million of olfactory sensory neurons (OSNs). Each OSN possesses an olfactory receptor (OR), which is a G-protein coupled seven -transmembrane receptor [21,28]. OSNs respond to odors with OR-mediated generation of intracellular secondary messengers, and then open cyclic nucleotide-gated (CNG) channels, allowing influx of calcium [6]. Clearly, Ca^{2+} is vital as a third messenger in the ofactory transduction [26].

Recent studies indicated that multiple purinergic receptor subtypes are expressed in the mouse olfactory epithelium, such as $P2X_1$, $P2X_4$, $P2Y_2$ in the OSNs [13], $P2Y_4$ in the sustentacular cells [29], and $P2X_1$, $P2Y_2$ in the basal cells [13]. It has also been suggested that extracellular ATP induced a suppression of activity of OSNs, and activation of P2X and P2Y modulated odor sensitivity [13]. Besides, purinergic receptor antagonists decreased the proliferation rate of basal cells in the tadpole olfactory epithelium [12], and it blocked induction of heat shock protein 25 in mouse olfactory epithelium [15]. Activation of purinergic receptors induced proliferation, neuronal differentiation, and neuropeptide Y release in mouse olfactory epithelium [17,18]. All these researches provided a clue that activation of purinergic receptors may initiate protective signaling pathways when OSNs are overexposed to odors or toxicants.

Nucleotides-induced calcium responses have been characterized in sustentacular cells and basal cells of tadpole olfactory epithelium [11]. Here, we identified the purinergic receptor subtypes and characterized the nucleotides-evoked calcium signaling in heptaldehyde (H)/2-hepatanone (Ho)-responsive OSNs (H/Ho-

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OSNs). We also determined the role of nucleotides in regulating the odor responses in this subgroup of OSNs. Furthermore, we elucidated the possible interaction between odor response and activation of purinergic receptor in OSNs. This may facilitate us better understanding the mechanism involved in modulation of odor sensitivity via neurotransmitters, such as ATP.

2. Materials and methods

2.1. Chemicals and solutions

All odorants used here were purchased from Sigma Aldrich (St Louis, MO). They were freshly made to 100 μM each by directly diluted in Ringer's saline containing 145 mM NaCl, 5 mM KCl, 20 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (Hepes), 1 mM $MgCl_2$, 2 mM $CaCl_2$, 1 mM Na pyruvate, and 5 mM D-glucose. Ca2+-free solution was made by omitting $CaCl_2$ in saline plus 3 mM ethylene glycol tetraacetic acid (EGTA). All solutions were adjusted to pH 7.4 and oxygen-saturated before use.

Uridine 5′-diphosphate (UDP), adenosine 5′-diphosphate (ADP), adenosine 5′-triphosphate (ATP) adenosine and pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) were purchased from Sigma. Uridine 5′-triphosphate (UTP) was purchased from Fermentas (Glen Burnie, MA). α,β-methylene ATP (α,β-meATP), Uridine-5′-(γ-thio)-triphosphate (UTPγS) trisodium salt, 2-thiouridine 5′-triphosphate (2-ThioUTP) tetrasodium salt, suramin, RO-3, NF449, ARL67156 trisodium salt, and BAPTA AM were purchased from TOCTIS bioscience (Ellisville, MI). All nucleotides were dissolved in concentrated solutions to 100 mM. The stock concentration of ARL67156 and BAPTA AM were 50 mM. PPADS and suramin were 20 mM. RO-3, and NF449 were 10 mM. All chemicals were diluted with 1000 times in Ringer's solution just before use.

2.2. Preparation of intact mouse turbinates

All procedures of animal handling were carried out in accordance with the protocols approved by the Institutional Animal Care and Use Committee of the Illinois Institute of Technology. Adult C57BL/6 mice at 2–3 months of age were used in the experiments. The decapitated mouse head was opened along the mid line, and the endoturbinates were exposed by removing the septum. The olfactory bulb and bones around ectoturbinates were removed and the turbinates were loaded with calcium sensitive dye Fura-2 AM (Invitrogen, Carlsbad, CA) similar to described before [24]. In brief, the turbinates were incubated in 10 μM fura-2 AM and 0.02% nonionic dispersing agent Pluronic F-127 at 37 °C for 1 h. The turbinates were mounted to a recording chamber with endoturbinates facing up and were continuously perfused with oxygenated saline throughout the experiments.

2.3. Calcium imaging and data evaluation

Ratiometric calcium imaging recording was performed at excitation of 340 nm (F340) and 380 nm (F380) in an Olympus upright microscope (BX51WI) equipped with a 20x, 0.9 numerical aperture water immersion objective, a filter wheel (Sutter Instruments, Novato, CA), a 175w xenon lamp and a cooled CCD camera (SensiCam qe; Cooke Corporation, Romulus, MI). Images were collected every 4 s using Imaging Workbench 5.2 (Indec Biosystems, Santa Clara, CA). Data were binned every four frames after recording using the Excel program and presented in the ratio of F340/F380 (Fr). The olfactory response magnitude of individual OSN $\Delta F/F$ was calculated as $(Fr - F)/F$, where Fr was the response to a stimulus at any time point and F was baseline activity, obtained by averaging 10 frames before stimulation. Data were presented as mean ± SEM. Paired Student's t-tests were used to determine significant differences. A p value less than 0.05 is considered as different and highly different if p was less than 0.01.

3. Results

3.1. Responsiveness to purinergic agonists in H/Ho-OSNs

Previous studies indicated that several purinergic receptor subtypes were expressed in olfactory sensory neurons [13,8]. To determine which purinergic receptor subtype(s) were expressed in H/Ho-OSNs located in the ventral region of olfactory epithelium, a variety of purinergic agonists activating different purinergic receptor subtypes were applied to this subgroup of neurons. We found that H/Ho-OSNs respond to ATP (a nonselective purinergic agonist), UTP (a nonselective P2Y receptor agonist), and α,β-MeATP (a $P2X_1/P2X_3$ receptor agonist) (Fig. 1A). ATP responsive potency was always greater than that of UTP or α,β-MeATP (Fig. 1A). These data showed that both P2X and P2Y receptors were present in the H/Ho-OSNs. However, adenosine, which is known to activate P1 receptor [1], did not elicit a Ca^{2+} transient (Fig. 1A). Also, ADP or UDP potency was weaker than that of ATP or UTP (Fig. 1A). Furthermore, Both UTPγS (a selective $P2Y_2/P2Y_4$ receptor agonist) and 2-ThioUTP (a selective $P2Y_2$ receptor agonist) can lead to intracellular calcium increase in the H/Ho-OSNs (Fig. 1A). 2-ThioUTP response is generally more potent than that of UTPγS, suggesting the expression of $P2Y_2$. However, this did not exclude the possibility of $P2Y_4$ expression in the H/Ho-OSNs. Collectively, our results indicated that $P2X_1/P2X_3$ and $P2Y_2/P2Y_4$ receptors were expressed in the H/Ho-OSNs.

3.2. ATP and UTP evoked intracellular calcium increases via different Ca^{2+} sources

Transient intracellular calcium increase occurs through calcium influx from extracellular space, or intracellular calcium store release [23]. We investigated the calcium sources to the purinergic stimulus in the H/Ho-OSNs through application of Ringer's salt without calcium and with cytosolic calcium chelator BAPTA AM. ATP-evoked calcium response was significantly inhibited without extracellular calcium in H/Ho-OSNs. Repeated applications of ATP in Ca^{2+}-free Ringer's solution resulted in gradual rundown of the Ca^{2+} responses (First: 72.7 ± 2.5% of control, $p < 0.01$; Second: 52.6 ± 2.5% of controls, $p < 0.0001$; Third: 42.2 ± 2.4% of controls, $p < 0.0001$, $n = 10$, Fig. 1B). Returning to standard Ringer's solution did not fully recover the ATP response (71.9 ± 3.9% of control, Fig. 1B). UTP-evoked calcium response was not blocked in the absence of extracellular calcium (94.9 ± 4.2% of control, $p > 0.1$, $n = 10$, Fig. 1C). By contrast, superfusion of BAPTA AM significantly reduced UTP-induced calcium transient by 55.3 ± 4.7% ($p < 0.0001$, Fig. 1C). To sum up, our data provided evidences to support that ATP-evoked calcium response required extracellular calcium, whereas UTP-induced calcium transient was predominately mediated by calcium release from intracellular stores.

3.3. ATP-induced Ca^{2+} transient was specific and correlated to purinergic receptors

To determine the specificity of ATP-evoked intracellular calcium increase, the dose dependency of ATP-induced $[Ca^{2+}]_i$ increases in the H/Ho-OSNs was shown in Fig. 2A. EC_{50} value of ATP-induced calcium transients was 114.3 μM. Then, we examined the effect of two non-selective P2 receptor antagonists, Suramin and PPADS, on ATP-evoked calcium response. Both Suramin and PPADS superfusion effectively inhibited ATP-induced $[Ca^{2+}]_i$ increase in H/Ho-

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