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Cellular Mechanisms of Olfactory Signal Transduction

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The sense of smell has the exquisite capacity to recognize and discriminate among an immense variety of volatile chemical compounds that are present in the environment. In mammals, olfactory cues are detected by sensory neurons belonging to three different sensory modalities harboured in the nasal cavity. Olfactory and vomeronasal primary sensory neurons (OSNs and VSNs, respectively) detect odorants and pheromones, whereas free nerve endings of the V cranial nerve (*N. trigeminus*) constitute the common chemical sense and convey pain, touch, temperature and chemosensory information. Our interest is to understand the molecular and cellular mechanisms of chemosensation in the diverse subsystems. A first common step in signal transduction is the specific binding of odorant molecules to receptor proteins located in specialized membrane protrusions: the cilia, microvilli or free nerve endings innervating the nasal mucosa. Olfactory and pheromone receptor proteins of OSNs and VSNs, respectively, are identified and functionally characterized to some extent. The intracellular mechanisms that transduce the initial chemical information into an electrical signal in OSNs involve the adenylate cyclase–cyclic nucleotide-gated channel pathway in addition to a recently identified phosphoinositides pathway. In contrast, a phospholipase C–diacylglycerol–transient receptor potential (TRP) channel pathway seems to play the major role in VSNs. However, the molecular mechanisms used by trigeminal neurons to detect odorant molecules are largely unknown. The beginning characterization of thermosensitive TRP channels among other receptors and ion channels expressed in trigeminal ganglion cells revealed their role in detection of chemical compounds and chemosensory signalling in these cells.

The detection and interpretation of chemical cues in an animal's environment is performed by the action and interaction of all chemosensory subsystems. Here, we will focus on the cellular and molecular biology of chemosensory transduction in olfactory sensory neurons (OSNs) of rodents. These cells express a repertoire of ~1000 odorant receptor (OR) genes (Buck and Axel, 1991; Zhang *et al.*, 2004) coding for G protein-coupled receptors with a putative seven transmembrane (7 TM) domain topology. It is generally believed that a mature OSN can express only one OR gene from one allele (monoallelic expression, one OSN–one receptor hypothesis; Chess *et al.*, 1994; Serizawa *et al.*, 2003). The type of OR expressed by an individual OSN determines the ligand specificity, so that individual OSN may recognize a spectrum of odorant molecules and a certain odor might be detected by a combination of different OSN types (combinatorial code; Malnic *et al.*, 1999). The binding of an odorant molecule to an adequate receptor is transduced to G protein-mediated stimulation of adenylyl cyclase, cAMP generation and influx of sodium and calcium ions through cyclic nucleotide-gated (CNG) channels (Brunet *et al.*, 1996). The entry of calcium ions is a central event in olfactory signal transduction and triggers the downstream processes (Schild and Restrepo, 1998; Zufall and Munger, 2001) leading to generation of action potentials and transmission of information to the olfactory bulb. Using calcium-imaging recordings, we have recently shown that a co-application of more than one odorant to individual rat OSNs may lead to inhibition of cells that could be activated by a single odorant (Spehr *et al.*, 2002). Our data suggest that—at least in a subpopulation of OSNs—certain odorants can induce the activation of the phosphatidyl-inositol 3-kinase (PI3K) pathway and the generation of phosphatidyl-inositol-3,4,5-trisphosphate (PIP3) which is acting as a signalling molecule. PIP3 is then able to inhibit primary signal transduction and calcium signalling in rat OSNs most likely by directly targeting the olfactory CNG channel. We found that application of PIP3 to isolated OSN inhibited odor- or forskolin-induced calcium signals, whereas treatment of cells with the PI3K inhibitor wortmannin or LY294002 released the OSN from an odor-induced inhibition. To establish a second line of evidence we now extended our studies to electrophysiological investigation of dissociated mouse OSN by whole-cell voltage-clamp recordings. As a model system, we used transgenic mice expressing the mouse I7 odorant receptor under control of the OMP (olfactory marker protein) promoter (mice were from R.R. Reed, Howard Hughes Medical Institute, Baltimore, MD). Using Northern blot analysis, Zhao and Reed (2001) showed that I7 mRNA was expressed in olfactory epithelium at levels one-tenth of that of OMP. We found the biophysical properties of UbI7 OSNs not different from those of wild type C57Bl/6 mice: The resting membrane potential was –56 ± 17 mV in wild type (n = 22) and –54 ± 12 in UbI7 mice (n = 69). Voltage-activated sodium currents activated at ~–50 mV in wild type (n = 13) and UbI7 mice (n = 23) and were maximal at~–30 mV. The activation kinetics and mean amplitudes of voltage-activated A-type and delayed rectifier potassium currents were not different in both mouse strains. The investigation of odorant-induced membrane currents and voltage signals was performed in calcium-free bathing solution to increase the ion-flux through olfactory CNG-channels (Zufall and Firestein, 1993; Thürauf *et al.*, 1996). We then challenged wild type and UbI7 OSN with the complex odorant mixture Henkel 100 (Wetzel *et al.*, 1999) or with octanal, one of the identified I7 ligands (Krautwurst *et al.*, 1998; Zhao *et al.*, 1998; Araneda *et al.*, 2004). We observed 1 out of 13 (7%) wild type OSNs responding to application of octanal at a concentration of 1:10 000, whereas 11 out of 25 (44%) UbI7 OSNs responded to that stimulus. Application of Henkel 100 (1:1000) stimulated 8 out of 15 UbI7 OSNs (53%), but only 2 out of 8 wild type neurons (25%). Signals reached ~40 mV amplitude when recorded in current-clamp configuration. Using that system, we tested for the effect of the PI3K inhibitor LY294002 (10 μM) on cells that were challenged with the odorant mix Henkel 100 (1:1000) and found 4 out of 13 cells (31%) which responded to Henkel 100 only in simultaneous presence of LY294002, but not to Henkel 100 alone. In current-clamp recordings, membrane potential was depolarized ~40 mV in response to co-application of Henkel 100 and LY294002. Voltage-clamp recordings of those neurons revealed currents of ~100 pA (Figure 1). These data are in good accordance with our results obtained earlier in calcium-imaging experiments (Spehr *et al.*, 2002). We could show that PIP3 is able to inhibit odorant or forskolin-induced calcium signalling, suggesting that PIP3 is acting at the level or downstream of the adenylyl cyclase. Conducting the electrophysiological experiments we could now show that complex odorants can both activate and

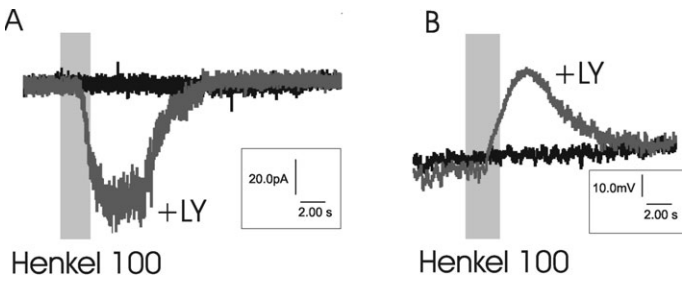

Figure 1 A freshly dissociated UbI7 OSN responding to application of the complex odorant Henkel 100 (1:1000) in presence of the PI3-kinase inhibitor LY294002 only. Voltage-clamp **(a)** and current-clamp recording **(b)** of the same cell. Bars indicate application of Henkel 100.

inhibit the transduction current and that the inhibition could be abolished by the action of the PI3K inhibitor LY294002. From that we can conclude that PIP3 is acting directly at the CNG channel or at upstream targets. Further patch-clamp recordings of native or heterologously expressed olfactory CNG channels should clarify the molecular mechanism of PIP3 action on the channel protein.

These results are in favour of the hypothesis that individual OSNs can detect different odorant molecules which are able to stimulate an excitatory and inhibitory pathway in the same cell. OSNs do integrate the activity of the pathways and the output of the cells depends on the result of the integrative computation.

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