IJBCS ISSN: 2147-1428

Review Article

International Journal of Basic and Clinical Studies (IJBCS)
2016; 5(2): 25-32 Neslihan K. and Sude GE.

# Importance of Olfactory Receptors in Human Testes

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**Abstract**

The sense of smell in vertebrates is mediated by proteins called olfactory receptors (OR). In the case of mammals, these proteins are embedded in the membranes of sensory neurons in the nasal cavity. Each neuron is thought to contain a single type of olfactory receptor responding to a single type of odorant. Humans have around 350 so-called odorant receptor (OR) genes with complete open reading frames, and mice have nearly 1000 OR proteins. These receptors are expressed in the neurons of the olfactory epithelium, where they are concentrated at the plasma membrane of the cilia and at axon terminals. Olfactory receptors also reside in tissues other than the olfactory epithelium as, like spermatogenic cells. In mammals, the perception of smell starts with the activation of odorant receptors (ORs) by volatile molecules in the environment. Members of the odorant receptor (OR) family have been found in various ectopic tissues, including testis and sperm. Olfactory receptors were found to be expressed also in human sperm giving rise to the hypothesis that they might play a role in fertility and sexual behavior. For instance, Bourgeonal was demonstrated to be an agonist of sperm cells olfactory receptor, OR1D2. OR1D2 has been found to be expressed in human olfactory epithelium and to play a important role in human sperm chemotaxis.

**Key worlds:** Olfactory Receptors, Human Testes, Odorant Receptor

## Introduction

Olfactory receptors (ORs), the first given molecules with which odorants physically interact to stimulate an olfactory sensation, form the largest gene family in vertebrates, including around 900 genes in human. In mammalian genomes as many as 1,000 OR-encoding genes are presaged, containing 3-5% of the total gene content Each Olfactory receptor gene consists of a single coding exon of about 1 kilobase (kb). Conserved regions that encode transmembrane domains 3 (TM3) and 7 (TM7) have been used to design degenerate oligonucleotides that are specific for the OR gene superfamily. Using such primers, a number of OR gene sequences have been cloned from several other mammalian species such as human, mouse, dog and pig as well as fishes and amphibians. The consensus

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sequence in Drosophila melanogaster is explicit, and the coding region can be seperate by introns.

An organism's perception of the world is filtered through its sensory systems. In vertebrates, the two principal categories of sensory receptors are ion channels and G-protein-coupled receptors (GPCRs), which mediate olfaction. A recurrent theme is the way in which the biochemical and biophysical properties of sensory receptor molecules, and the neurons in which they exist, have been shaped by evolution to capture those signals that are most evident for the survival and reproduction of the organism.

Both GPCRs and ion channels contribute to sensory transduction pathways by initiating or modulating stimulus-evoked responses. In vertebrates, GPCRs preponderate as stimulus detectors in vertebrate visual and olfactory receptor cells.

A similar biochemical logic governs signaling in vertebrate olfactory sensory neurons, where activation of G-protein-coupled odorant receptors increases the synthesis of cAMP, which binds directly to and thereby opens cyclic-nucleotide-gated ion channels in the plasma membrane of olfactory cilia. The resulting depolarization of the plasma membrane initiates an action potential that is transmitted from the sensory neuron's body in the olfactory epithelium to its presynaptic terminal in the olfactory bulb. In both visual and olfactory sensory neurons, temporal control of signaling comes down to a balance between cyclic nucleotide synthesis and degradation. Interestingly, the detection of tastants and pheromones by GPCR-containing gustatory and vomeronasal sensory neurons, respectively, proceeds through a somewhat different signaling pathway involving G-protein-mediated activation of phospholipase C, which promotes hydrolysis of membrane phospholipids to generate second messengers (such as diacylglycerols, inositol phosphates, and polyunsaturated fatty acids) and thereby triggers calcium release from intracellular stores. These actions promote the opening of excitatory TRP ion channels, leading to depolarization and neurotransmitter release (2).

Odorant receptor genes are expressed not only in the nose but also in testes, where they have been hypothesized to play a role in sperm chemotaxis. In psychophysical and physiological experiments, the authors demonstrate that odorant receptors that may play the same role in both sperm and the nose (6). As a result of the studies , the olfactory receptors found in the sperm and testes of urchin, rat, A. Gambiae and also in humans.

Researchers shown that the expression of a subset of ORs in male germ cells

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of A. Gambiae where they act to modulate activation and perhaps orientation of spermatozoa, which are critical to male reproductive fitness (4). A. gambiae adults revealed that a subset of AgOrs is enhanced in whole male bodies. AgOrs are functional in nonhead tissues in males where they are used in noncanonical chemosensory roles. Detection of AgOr transcripts in testes raised the possibility that some Ors are expressed as functional proteins in spermatozoa. AgOrs perform previously unknown functions in A. gambiae spermatozoa where they may mediate responses to chemical signals. The presence of other AgOr transcripts in testes suggests the presence of heteromeric Or complexes in spermatozoa. .Therefore speculated that a subset of the known AgOr ligands would also activate flagella, mimicking the effect of Orco agonists. To examine this, a panel of odorant ligands was used in the spermatozoa flagella bioassay, revealing that fenchone, which can activate several AgOrs, including the testes-expressed AgOr11, induced significant spermatozoa movements in a concentration-dependent manner. The fenchone responses increased from $10^{-6}$ to a peak activity at $10^{-4}$ molar and then decreased at $10^{-3}$ and $10^{-2}$ molar, becoming insignificant compared with buffer alone. A newly identified AgOr6 ligand, indole-3-carboxyaldehyde, also activated A. gambiae spermatozoa in a concentration-dependent manner with highest activity at $10^{-2}$ molar. Importantly, both the fenchone and indole-3-carboxyaldehyde responses were inhibited by the coapplication of the Orco antagonist VUANT, indicating that flagellar responses to both compounds require a functional Orco subunit. These results support the hypothesis that flagellar beating responses of sperm can be modulated by heteromeric AgOr complexes and constitutes evidence for their function outside of sensory neurons in A. gambiae.

Peptide ligands like those of sea urchin oocytes have not been identified in mammals, but there is evidence that odorant-like molecules function as chemoattractants to mammalian sperm. A human odorant receptor specific to the testis was identified, cloned and functionally expressed in human embryonic kidney cells, where stimulation by specific odorant molecules produced $Ca^{2+}$signals (7).

In humans ; it has been known for some time that olfactory receptors (ORs) reside in spermatozoa.After the studies, researchers identified, cloned, and functionally expressed a previously undescribed human testicular OR, hOR17-4. With the use of ratiofluorometric imaging, $Ca^{2+}$ signals were induced by a small subset of applied chemical stimuli, establishing the molecular receptive fields for the

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recombinantly expressed receptor in human embryonic kidney (HEK) 293 cells and the native receptor in human spermatozoa. Bourgeonal was a powerful agonist for both recombinant and native receptor types, as well as a strong chemoattractant in subsequent behavioral bioassays. In contrast, undecanal was a potent OR antagonist to bourgeonal and related compounds. Taken together, these results indicate that hOR17-4 functions in human sperm chemotaxis and may be a critical component of the fertilization process.

In psychophysical and physiological experiments, the authors demonstrate that odorant receptor hOR 17-4 may play the same role in both sperm and the nose. These two organs have in common a role in chemotaxis – directed movement toward chemical substances in the environment. For species that use external fertilization, such as sea urchins and fish, it is crucial to reproductive success that sperm are able to recognize and swim toward the egg. This is accomplished by a combination of signal transduction components loaded onto mature sperm and chemical ligands secreted by the egg. That sperm in internally fertilizing species, such as mammals, would require active chemotaxis is less obvious. However, shortly after publication of the landmark paper that described a super-family of odorant receptors, another group isolated members of this gene family expressed in mammalian testes, associated with mature sperm. This intriguing finding led to the suggestion that mammalian sperm are indeed capable of 'smelling' their way to the egg. Alternatively, sperm odorant receptors may confer directed chemotaxis toward the egg, either generically for all sperm or more specifically to attract only those sperm possessing the particular odorant receptor tuned to the scent of the egg.

Surveying the range of active and inactive odorants, that the presence of analdehyde group connected to an aromatic ring via a carbon chain of defined length (two to four carbons) is a key determinant for aneffective hOR17-4 ligand. The presence or absence of nonpolar methyl groups is largely tolerated, whereas addition of polar structures and double bonds can effectively decrease the ability of a ligand to activate the receptor (3).
HOR 17-4 responds strongly to floral odors such as bourgeonal, which smells like lily-of-the-valley. In accord with previous studies of conventional odorant receptors, this sperm odorant receptor has a receptive range of a number of structurally related odors that suggest a constrained binding pocket for ligand.

hOR 17-4 has functional properties resembling those of odorant receptors

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expressed in the nose. Further, this suggests that sperm are likely to gain specific and nuanced chemotaxis if they express this particular receptor protein. These studies on the pharmacology of the sperm odorant receptor were then extended to the sperm themselves: human sperm showed functional activation and chemotaxis toward bourgeonal that was blocked by undecanal, although it was not possible to ascribe these behaviors directly to the function of hOR 17-4 itself.

In the new study, researchers showed this sperm receptor is in fact also expressed in the olfactory system. Careful analysis of human tissues revealed bona fide expression of hOR 17-4 in nasal epithelium. This prompted the authors to investigate whether hOR 17-4 shows the same functional properties in the nose and sperm. Remarkably, the human nose showed the same types of pharmacology as human sperm, and the hOR 17-4 mediates both responses. Although direct proof that hOR 17-4 alone mediates these responses is still missing, these findings have several important implications. As hOR 17-4 activity is detected in both tissues, this receptor may have evolved a common function in reproduction that is carried out in the nose and sperm. Secondly, it suggests that the functional properties of odorant receptors are similar in these two tissues and that in both instances they mediate specific responses to odors.

If sperm do undergo chemotaxis toward the egg using odorant receptors, it will be of great importance to characterize the chemoattractants that they are responding to. It is unlikely that bourgeonal itself is released by the egg, but endogenous mimics of this odorant that are within the receptive range of hOR 17-4 may be. Large-scale purification of mouse egg extracts, coupled with new techniques of deorphanizing odorant receptors will likely be a powerful approach to identify such substances. These experiments have obvious practical applications, in that sperm could be activated to swim by synthetic egg odor. Inhibitors such as undecanal could act as potent contraceptives that paralyze or disorient swimming sperm (5).

Intracellular calcium concentration is important regulator of sperm motility in animals. Similar calcium channels have yet to be identified in mosquitoes, although an odorant-gated ion channel, Orco, localizes to the sperm flagella and may play a similar role in the regulation of intracellular osmolarity and motility in the mosquitoes A. aegypti and A. gambiae ;. Orco forms a complex with an accompanying odorant receptor, and has been shown to activate mosquito sperm in the presence of various ligands (4).

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To understand relationship between the Hor17-4 and odorants, the full-length hOR17-4 cloned and tested its sensitivity to bour-geonal and undecanal. Whereas bourgeonal activated full-length hOR17-4, this response was inhibited by undecanal. Bourgeonal induced repeatable Ca2 responses in 36% of examined cells. This compound was the most potent ligand tested, and pro-longed application led to sustained Ca2 sig-nals. ( A ranking of ligands ac-cording to magnitude of Ca2 response wassimilar for spermatozoa and hOR17-4– ex-pressing HEK cells. Moreover, sperma-tozoa detected bourgeonal, and other ligands, at very dilute concentrations, about two orders of magnitude lower t han those required by the recombinant receptor, Coapplication of equimolar unde-
canal and bourgeonal completely block ed Ca transients. Together, these data strongly suggest that hOR17-4 is expressed on the surface of human spermatozoa and that signal transduction is a Ca 2-mediated process (3).

There is evidence that both hyperactivation and chemotactic responses involve a rise in $Ca^{2+}$; however, the $Ca^{2+}$ rise produced by activating CatSper channels to induce hyperactivation in mouse sperm originates in the principal piece of the flagellum, while the $Ca^{2+}$ rise detected in response to the odorant bourgeonal originates in the midpiece of human sperm. Because hyperactivation occurs in the oviduct far from the oocyte and even before ovulation, it is likely that sperm are already hyperactivated when they receive odorant signals. Perhaps chemotactic factors act on hyperactivated sperm to trigger brief releases of $Ca^{2+}$ from the RNE store that modulate the flagellar beating pattern just long enough to re-direct the path of the sperm.

Chemotactic factors could serve to direct sperm toward the ampulla, toward the cumulus mass in the ampulla and/or toward the oocyte within the cumulus mass. In mice, the cumulus mass fills the entire lumen in a substantial region of the oviductal ampulla and makes an easy target for sperm; however, mouse sperm may require guidance to locate oocytes within the mass. In humans, the cumulus mass is a very small target for sperm, because it does not fill the ampullar lumen and the lumen is divided into complex branched channels by mucosal folds. Without guidance by chemotaxis, human sperm could easily pass by the cumulus mass (8).

## Conclusion

As we can understand from the researches, there is a certain mechanism

that takes place when we smell. And at that moment the event sequence is evolving. the cilia of the sensory neurons are located in a layer of mucus. Odorant molecules dissolve in the mucus and bind to receptors on the cilia. Binding of the odorant activates a G protein coupled to the receptor on its cytoplasmic side. This activates adenylyl cyclase, an enzyme embedded in the plasma membrane of the cilia. Adenylyl cyclase catalyzes the conversion of ATP to the "second messenger" cyclic AMP (cAMP) in the cytosol. CAMP opens up ligand-gated sodium channels for the facilitated diffusion of $Na^+$ into the cell. The influx of $Na^+$ reduces the potential across the plasma membrane. If this depolarization reaches threshold, it generates an action potential. The action potential is conducted back along the olfactory nerve to the brain. The brain evaluates this and other olfactory signals reaching it as a particular odor.
We can see the same mechanism in the process of reaching the sperm to the oocyte. Some odor receptors are expressed in human sperm. These enable sperm to swim towards certain chemicals (a positive chemotaxis). Whether similar chemicals are released in the vicinity of the egg and thus increase the chances of fertilization remains. (Human sperm also display a positive chemotaxis to a gradient of progesterone which does propel them toward the egg.
As a result of the researches made on this, When the sperm arrives to the ampulla, cumulus of oocyte release a some chemoattractant odor. By this releasing there is a change in sperm motility as a result of stimulation with a secretion of a chemoattractant in the cumulus oocyte. So this chemoattractant activates the olfactory receptors which in the sperm midpiece and this also activates the calcium channel for sperm motility for swimming faster to oocyte . One of the reasons for the failure of fertilization should be proved by the influence of the chemoattractants in the cumulus.

**References**

(1) Quignon, P., Kirkness, E., Cadieu, E., Touleimat, N., Guyon, R., Renier, C., ... & Galibert, F. (2003). Comparison of the canine and human olfactory receptor gene repertoires. Genome biology, 4(12), 1.

(2) Julius, D.,& Nathans, J. (2012). Signaling by sensory receptors. Cold Spring Harbor perspectives in biology, 4(1), a005991.

(3) Spehr, Marc, et al. "Identification of a testicular odorant receptor mediating human sperm

IJBCS ISSN: 2147-1428

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2016; 5(2): 25-32 Neslihan K. and Sude GE.

chemotaxis." *Science* 299.5615 (2003): 2054-2058.

(4) Pitts, R. Jason, et al. "Odorant receptor-mediated sperm activation in disease vector mosquitoes." *Proceedings of the National Academy of Sciences* 111.7 (2014): 2566-2571.

(5) Degner, Ethan C., and Laura C. Harrington. "A mosquito sperm's journey from male ejaculate to egg: Mechanisms, molecules, and methods for exploration." *Molecular reproduction and development* (2016).

(6) Vosshall, Leslie B. "Olfaction: attracting both sperm and the nose." *Current biology* 14.21 (2004): R918-R920.

(7) Spehr, Marc, et al. "Dual capacity of a human olfactory receptor." *Current Biology* 14.19 (2004): R832-R833.

(8) Suarez, Susan S. "Control of hyperactivation in sperm." *Human reproduction update* 14.6 (2008): 647-657.