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Review Article

Olfactory receptors and the mechanism of odor perception ☆

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ABSTRACT

Introduction: The olfactory system plays one of the key roles in the lives of humans and animals. It can detect thousands of different odor molecules through a large family of olfactory receptors (ORs), of a diverse protein sequence, which are located in olfactory sensory neurons (OSNs) in the olfactory epithelium in the nose of humans, and in the vomeronasal organ in animals. The OR family is comprised of 172 subfamilies, whose members have related protein sequences and are encoded by a single chromosomal locus. The human receptor gene family includes 339 intact receptor genes and 297 receptor pseudogenes, unevenly distributed among 51 different loci on 21 human chromosomes. Different parts of the genome may be involved in the detection of different types of structural odorants.

Odor detection is mediated by odorant receptors. Signals generated in OSNs in response to odorants are transmitted to the olfactory bulb (OB) of the brain, i.e., the first relay station in the control of the olfactory system in mammals. Then, signals are transmitted to the olfactory brain cortex, which has a cortical structure with distinct layers and numerous glomerular modules, and forms a map of olfactory axon terminals. The axonal projection of OSNs is precisely organized with a few topographically fixed glomeruli.

Aim: The purpose of this paper is to present the recent literature in the field of the undertaken subject.

Material and methods: The review of articles is devoted to olfactory receptors and the mechanism of odor perception.

Discussion: In the first part, this review summarizes the mammalian protein ORs that are encoded by genes, as well as the location and structure of these receptors. Extensive studies reveal that mammals can have up to 1000 different OR genes, which constitute approximately 1% of the genomic complement of genes. The analysis of the entire receptor family has shown that it is comprised of 172 subfamilies, whose members are 60% identical in protein amino acid sequence and can recognize odorants with related structures. The second part of this review presents the opinions of many authors concerning the olfactory perception that initiates in the olfactory epithelium. Odor signals are further transmitted to the OB, i.e., the first relay station of the central olfactory system in the mammalian brain. Then such signals ultimately reach higher cortical areas involved

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in the conscious perception of odor. At the review's conclusion some diseases associated with smell disorders are discussed.

Results: The olfactory cortex still remains an unexplored and unexplained area in terms of the processing of odorant information. This subject requires further study.

1. Introduction

The sense of smell is a primal sense of enormous significance for humans and animals. The olfactory system was unexplained until 1985. It was known that odorants were detected through receptors located in the epithelium of the nasal cavity in humans, and through the olfactory sense organ, called the vomeronasal organ, in most animals. In the late 1980s Richard Axel, a geneticist from the Institute of Cancer Research of Columbia University, New York, together with Linda Buck, a biochemist from Harvard Medical School, Boston, Massachusetts, began to consider the following questions: How do mammals begin to recognize the vast diversity of odorant molecules that can vary in size, shape, functional groups, and charge? How does odor perception occur? And how is the chemosensory system presented in the brain? These were some of the basic and most challenging questions concerning the field of olfactory research. The olfactory system posed a fascinating problem for these biologists.[7]

Later, it was discovered that a subtle change in the structure of chemical odors dramatically changes their perceived odor. Olfactory sensory neurons (OSNs) that detect odorants express different receptors, elicit different signals in the brain, and thereby, generate distinct odor perception.

Recent *in vivo* and *in vitro* studies have challenged the existing models of olfactory processing in the vertebrate olfactory bulb (OB) and the insect antennal lobe. Lateral connectivity between olfactory glomeruli was previously thought to form a dense, topographically organized inhibitory surrounding. New evidence suggests that lateral connections may be sparse, nontopographic, and partly excitatory. Other recent studies highlight the role of active sensing (sniffing) in the shaping of odor-evoked neural activity and perception.[37]

2. Aim

The purpose of this paper is to present the recent literature in the field of olfactory receptors and the mechanism of odor perception.

3. Material and methods

A review of articles devoted to olfactory receptors and the mechanism of odor perception was performed. Available medical databases PubMed, Scopus, Embase, and ProQuest were used.

4. Discussion

4.1. *Receptors for odorants and pheromones*

Axel and Buck, pioneers in the research of molecular structure of odor and pheromone sensing in animals, employing the technique of gene cloning and other methods (e.g., PCR-reaction), have isolated genes encoding protein-coupled olfactory receptors (ORs) in the human genome. Then, they have determined the chromosomal location of each OR gene, and analyzed the subfamily structure of the human OR family, the chromosome of genes encoding the members of each family, and the subfamily composition of each chromosomal locus.

This has led to the discovery of three distinct families of ORs, each encoded by a multigene family. One family of 1000 genes encodes ORs in the olfactory epithelium.[6] It comprises approximately 1% of the genomic complement of genes, and this family is the largest unit identified in the genome of any species. The other two OR families are expressed in the vomeronasal organ in mammals. They are called: V_1R family with about 35 members,[9] and the V_2R family with about 150 members.[32] Both receptor families are considered to be candidate receptors for pheromones in most mammals, and recognize different types of chemicals, ligands for these receptors.[5] V_2Rs differ clearly from ORs and V_1Rs in having a very large N-terminal extracellular domain. V_2Rs are related to metabotropic glutamate receptors whose large N-terminal domains bind ligands. Short N-terminal extracellular domains in ORs and V_1Rs bind ligands in a pocket that is formed in the membrane by the combination of the transmembrane domain.[36] A single amino acid in the transmembrane domain has been shown to alter odorant specificity.[20] According to the recent studies of Buck, individual ORs recognize multiple odorants, but V_1Rs and V_2Rs might, instead, be selective for specific pheromones – different types of chemicals, ligands for these receptors.

Studies of Buck have indicated that humans have 636 OR genes, 339 of which are intact and therefore likely to encode functional odorant receptors in the olfactory epithelium of the nasal cavity, and 297 OR pseudogenes. It is assumed that mammals can have up to 1000 different OR genes that encode odorant receptors.[4] This comprises approximately 1% of the genomic complement of genes and this family is the largest unit identified in the genome of any species.

The determination of their genomic locations has demonstrated that OR genes are unevenly distributed among 51 different loci on 21 human chromosomes; 38 chromosomal loci have one or more intact genes and are likely to function in odor perception.

An analysis of the entire OR family has shown that it is comprised of 172 subfamilies, whose members are 60% or

more identical as regards the protein sequence and can recognize odorants with related structures.[17,24] The identification of 172 human OR subfamilies indicates the extreme diversity of the human OR family. This information concerning ORs has facilitated the creation of a model in which each subfamily recognizes a particular class of odorant structures.[23] Members of the same subfamily would recognize partially overlapping sets of odorants with highly related structures. The majority of subfamilies are encoded by a single chromosomal locus. Moreover, many loci encode only one or a few subfamilies, suggesting that different parts of the genome may be involved in the detection of different types of odorant structures. Studies of many authors indicate that some odorants can be recognized by ORs that are less than 60% identical and therefore belong to different subfamilies. It should be noted that further studies are necessary to verify the reliability of the applied method and to estimate the individual ORs that interact with odorants in the definite structure.

Moreover, numerous scientists carry out research pertaining to new channels of olfaction. New evidence demonstrates that a putative new candidate, bestrophin-2 (Best2), cannot be involved in the olfactory channel. Additional studies now identify anoctamin-2 (ANO2, also known as TMEM16B) as a strong candidate.[19]

In 2006 Pifferi et al.[30,31] argued that Best2 might be the olfactory Cl^- channel. Best2 can function as a calcium-activated chloride channel (CaCC) when exogenously expressed, although its role *in vivo* has been controversial. The authors showed that Best2 is expressed in the proximal sections of the olfactory cilia. Furthermore, the olfactory Cl^- channel and expressed Best2 channels have similar biophysical properties (anion selectivities, unitary conductances, voltage dependences, and sensitivities to blockers). The authors also highlighted one difference: Best2 is about 12 times more sensitive to Ca^{2+} than is the olfactory channel. But three years later Pifferi et al. presented new evidence that refuted their former hypothesis. When knockout mice lacking Best2 became available, the authors compared them to wild-type mice and found no significant differences in their olfactory physiologies. In both intact epithelia and isolated OSNs, electrical responses to odors and olfactory second messengers have no requirement for Best2. The sensory endings of OSNs lacking Best2 have CaCCs like those in the wild-type mice.

New evidence suggests that TMEM16B is the olfactory Cl^- channel. In 2005, TMEM16B topped a list of proteins preferentially expressed in mouse OSNs.[35,38] It was reported that anoctamin family proteins, CaCCs and TMEM16B, emerged as a likely olfactory transduction channel. TMEM16B was identified in the proteome of rat olfactory cilia.

In 2004 Linda Buck and Richard Axel were awarded the Nobel Prize in physiology and medicine for the discovery of genes encoding ORs, and for their isolation and research.

4.2. *The mechanism of olfactory perception*

In mammals there are two anatomically independent yet integrated olfactory systems: the main olfactory system, which is concerned primarily with environmental odors and is further compartmentalized into zones and subzones, and the vomeronasal system, which is rather specialized in detecting pheromones, although this functional difference is not absolute.[17,18] The latter system modulates behaviors, e.g., aggression.[12,15]

It should be emphasized that a very important feature of olfactory perception is that a slight change in the structure of the odorant can dramatically alter its perceived odor.[6]

It was assumed that odor perception is initiated in the olfactory epithelium of the nasal cavity, where odorants are detected by the large family of ORs. These receptors are members of the seven-transmembrane domain receptors, also known as G-protein-coupled receptors (GPCRs). They are extremely diverse in amino acid sequences, consistent with the ability to recognize a variety of structurally diverse odorants.[26] ORs induce GTP-dependent adenylyl cyclase III activity and an increase in cyclic adenosine monophosphate (cAMP) production. cAMP opens cyclic nucleotide-gated (CNG) cation channels causing membrane depolarization,[2,3,11] and alters membrane potential.[21,28] Signals from different types of receptors are detected and processed at the level of OSNs and further transmitted to the OB. The OB plays a key role in the perception of odor quality of odorants in the brain. It is the first relay station in the central olfactory system in the mammalian brain and contains a few thousand forms of odorant receptor maps.[39] In the OB, OSNs and their axons make synaptic connections with second-order neurons in neotropic structures termed glomeruli.[25] The pattern of glomerular innervations in the OB is critical for innate behavioral responses.[8] In vertebrates, odor is organized beyond the OB in a spatial map within the medial forebrain bundle.

In the identification of the components of odor mixtures, the olfactory system codes characteristic odors of critical components by adjusting the intensity of many potential odor stimuli.[14] Time intensity factors and prior adaptation to other components play a significant role in the perception of odor.[13]

Sensory neurons in the main olfactory system send their axons to the targets in the main OB in humans, whereas vomeronasal sensory neurons send their axons to a well-demarcated dorsal and posterior region of the OB. In both systems, axons of sensory cells synapse onto the dendrites of second order projection neurons known as mitral cells.[32]

The question of how specific chemical features of odors are represented in the OB is not conclusive. It has been proved that odors are spatially distributed in the OB,[22] and an odor evoked pattern of activity does not correlate directly with odor structure. According to the authors, glomeruli are tuned to odors of multiple types and are hierarchically arranged into clusters by their odor-tuning similarity. The spatial organization of glomeruli conforms to the organizational principle in other sensory systems. At present, other authors confirm the existence of the glomerular response cluster to similar odorants,[10] and indicate a partial hierarchical chemotropic organization. Larger glomerular regions are subdivided into smaller areas which are specific in their responses to particular functional groups of odorants.

It is speculated that inputs from different ORs partially overlap in the olfactory cortex,[18,29] and single cortical neurons receive combinational inputs from multiple different ORs.[40] Via these pathways, odor signals ultimately reach

higher cortical areas involved in the conscious perception of odors, as well as limbic areas, such as the amygdale and hypothalamus, which are involved in emotional and motivational responses. How signals derived from different ORs are organized beyond the bulb, and in the cortex, and how those signals are ultimately decoded to yield the perception of an odorant, or a specific endocrine, or behavioral response, is relatively little known.

Cortical neurons require a combination of receptor input for their activation and the merging of the receptor codes of two odorants, which provides novel combinations of receptor inputs that stimulate neurons beyond those activated by the single odorants. These findings explain the manner in which odorant mixtures can elicit novel odor perception in humans. Another olfactory structure in the nasal septum of animals, the vomeronasal organ, has two additional receptor families which detect pheromones and induce hormonal and behavioral responses, through a different projection in the brain.[7]

Recently it has been believed that neurogenesis in the olfactory activity has a functional significance in the recognition of odorant molecules. It occurs continuously in the adult forebrain in mammals. Neurons can migrate into the OB and into the hippocampal dentate gyrus.[33] It has been proven that mutant male and female mice having a deficit in sex-specific innate activities showed dependence on olfaction in sexual behaviors.[33] These results indicate that continuous neurogenesis in the adult forebrain is required for innate olfactory responses.

Further imaging studies in mammals may provide important insight as to how odors are represented within distinct forebrain areas, and how higher order processes within the forebrain permit the confluence of an odorant stream conveying information about different odorant types serving behavioral functions.

While the primary mechanisms of vertebrate olfactory transduction are largely understood, much remains to be learned about the events that establish, maintain, and modulate the encoding of odor signals. Scientists reporting discoveries important to the olfactory cilia found that a phosphofurin acidic cluster sorting protein 1 (PACS-1) is involved in the trafficking of CNG channels into the mammalian olfactory cilia via an interaction with the CNGB1b subunit of the channel, and that the retinitis pigmentosa GTPase regulator, whose isoforms locate to dendritic knobs or olfactory cilia, is necessary for odorant responses. Others reported that odorants stimulate the production of nitric oxide by OSNs and that the dynamics of electro-olfactograms were altered in mice lacking endothelial nitric oxide synthase (eNOS). Other researchers reported that leptin and insulin increase the excitability of rat OSNs, strengthening links between satiety and the sense of smell. This is in accordance with previous reports that the daily regimen of intranasal insulin now used by some diabetics improves object memory and odor discrimination in mice.

4.3. *Smell disorders and quality of life*

Olfactory sensitivity depends on age and sex. Women are superior to men in virtually all aspects of olfactory function, whereas the exact reasons for this remain unclear. Hormonal effects have been discussed, but remain a matter for debate. Possibly, the higher social awareness of women is also instrumental in so far as women, more than men, show interest in odors as social signals (e.g., body odors, food odors). Accordingly, women on average suffer more than men from the loss of the sense of smell. The reduced ability to smell with increasing age has been long known and is also partly due to the decrease in ORNs. Presbyosmia is, however, not unavoidable, but also represents an expression of overall health – people who have “aged well” and do not take any medication seem to have essentially normal smell thresholds. Anosmia refers to the lack of the ability to smell, and specific anosmia relates to the inability to smell a specific odor, whereas the vast majority of odors are normally perceived. Specific anosmias have been described for a series of different odors and are considered a physiological phenomenon. The occurrence of specific anosmias indicates that specific receptors are necessary for perceiving a specific odor. Specific anosmias are of little clinical importance. The term functional anosmia refers to a significantly reduced ability to smell, although some smell sensations can be present. Hyposmia refers to the reduced ability to smell, and hyperosmia to an enhanced ability to smell. Hyposmic conditions are common, but hyperosmias are very rare; they have been encountered, for example, after exposure to toxic vapors and in association with migraines.[16]

The four main causes of smell disorders are (1) trauma, (2) viral infections, (3) nasal causes such as sinusitis or nasal polyps, and (4) smell disorders associated with aging or neurological illnesses such as Parkinson’s disease or Alzheimer’s disease. Posttraumatic smell disorders are possible due to the severance of the fila olfactoria, and probably also the contusion of secondary olfactory-related areas of the brain such as the orbitofrontal cortex. Viral infections are assumed to cause damage to the ORNs; however, the triggering agent is still unclear. Huntington’s disease is associated with moderate hyposmia. Mild olfactory disorders have also been described for some heredoataxias and motor neuron diseases.[16]

5. Conclusions

The information contained in this review suggests that knowledge concerning the mechanisms of olfactory perception and the encoding of odor signals continues to improve, but is not yet firmly established and, hence, requires further study.

Conflict of interest

None declared.

Financial disclosure

Not applicable.

REFERENCES

[2] Belluscio L, Koentges G, Axel R, Dulac C. A map of pheromone receptor activation in the mammalian brain. *Cell*. 1999;97(2):209–220.

[3] Brunet LJ, Gold GH, Ngai J. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide-gated cation channel. *Neuron*. 1996;17(4):681–693.
[4] Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*. 1991;65(1):175–187.
[5] Buck LB. Information coding in the mammalian olfactory system. *Cold Spring Harb Symp Quant Biol*. 1996;61:147–155.
[6] Buck LB. The molecular architecture of odor and pheromone sensing in mammals. *Cell*. 2000;100(6):611–618.
[7] Buck LB. The search for odorant receptors. *Cell*. 2004;116(suppl 2):S117–119.
[8] Cho JH, Prince JE, Cutforth T, Cloutier JF. The pattern of glomerular map formation defines responsiveness to aversive odorants in mice. *J Neurosci*. 2011;31(21):7920–7926.
[9] Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. *Cell*. 1995;83(2):195–206.
[10] Falasconi M, Gutierrez-Galvez A, Leon M, Johnson BA, Marco S. Cluster analysis of rat olfactory bulb responses to diverse odorants. *Chem Senses*. 2012;37(7):639–653. http://dx.doi.org/10.1093/chemse/bjs045 [Epub 2012 Mar 29].
[11] Firestein S. Electrical signals in olfactory transduction. *Curr Opin Neurobiol*. 1992;2(4):444–448.
[12] Firestein S. How the olfactory system makes sense of scents. *Nature*. 2001;413(6852):211–218.
[13] Frank ME, Goyert HF, Hettinger TP. Time and intensity factors in identification of components of odor mixtures. *Chem Senses*. 2010;35(9):777–787.
[14] Goyert HF, Frank ME, Gent JF, Hettinger TP. Characteristic component odors emerge from mixtures after selective adaptation. *Brain Res Bull*. 2007;72(1):1–9.
[15] Halpern M, Martínez-Marcos A. Structure and function of the vomeronasal system: an update. *Prog Neurobiol*. 2003;70(3):245–318.
[16] Hummel T, Landis BN, Hüttenbrink KB. Smell and taste disorders. *GMS Curr Top Otorhinolaryngol Head Neck Surg*. 2011;10:Doc04. http://dx.doi.org/10.3205/cto000077.
[17] Kajiya K, Inaki K, Tanaka M, Haga T, Kataoka H, Touhara K. Molecular bases of odor discrimination: reconstitution of olfactory receptors that recognize overlapping sets of odorants. *J Neurosci*. 2001;21(16):6018–6025.
[18] Kandel ER, Schwart JH, Jessel TM. *Principles in Neural Sciences*. New York: Mc Graw-Hill; 2010.
[19] Kleene SJ. Identifying olfaction's 'other channels'. *J Physiol*. 2009;587(17):4135–4136. http://dx.doi.org/10.1113/jphysiol.2009.178954.
[20] Krautwurst D, Yau KW, Reed RR. Identification of ligands for olfactory receptors by functional expression of a receptor library. *Cell*. 1998;95(7):917–926.
[21] Labarca P, Simon SA, Anholt RR. Activation by odorants of a multistate cation channel from olfactory cilia. *Proc Natl Acad Sci USA*. 1988;85(3):944–947.
[22] Ma L, Qiu Q, Gradwohl S, Scott A, Yu EQ, Alexander R, et al. Distributed representation of chemical features and tunotopic organization of glomeruli in the mouse olfactory bulb. *Proc Natl Acad Sci USA*. 2012;109(14):5481–5486.
[23] Malnic B, Godfrey PA, Buck LB. The human olfactory receptor gene family. *Proc Natl Acad Sci USA*. 2004;101(8):2584–2589.
[24] Malnic B, Hirono J, Sato T, Buck LB. Combinatorial receptor codes for odors. *Cell*. 1999;96(5):713–723.
[25] Mombaerts P. Axonal wiring in the mouse olfactory system. *Annu Rev Cell Dev Biol*. 2006;22:713–737.
[26] Mombaerts P. Molecular biology of odorant receptors in vertebrates. *Annu Rev Neurosci*. 1999;22:487–509.
[28] Nakamura T, Gold GH. A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature*. 1987;325(6103):442–444.
[29] Neville KR, Haberly LB. Olfactory cortex. In: Shepherd GM, ed. *The Synaptic Organization in the Brain*. New York, NY: Oxford; 2004:415–454.
[30] Pifferi S, Dibattista M, Menini A. TMEM16B induces chloride currents activated by calcium in mammalian cells. *Pflugers Arch*. 2009;458(6):1023–1038. http://dx.doi.org/10.1007/s00424-009-0684-9.
[31] Pifferi S, Dibattista M, Sagheddu C, Boccaccio A, Al Qteishat A, Ghirardi F, et al. Calcium-activated chloride currents in olfactory sensory neurons from mice lacking bestrophin-2 *J Physiol*. 2009;587(Pt 17):4265–4279. http://dx.doi.org/10.1113/jphysiol.2009.176131.
[32] Ryba NJ, Tirindelli R. A new multigene family of putative pheromone receptors. *Neuron*. 1997;19(2):371–379.
[33] Sakamoto M, Imayoshi I, Ohtsuka T, Yamaguchi M, Mori K, Kageyama R. Continuous neurogenesis in the adult forebrain is required for innate olfactory responses. *Proc Natl Acad Sci USA*. 2011;108(20):8479–8484.
[35] Stephan AB, Shum EY, Hirsh S, Cygnar KD, Reisert J, Zhao H. ANO_2 is the cilial calcium-activated chloride channel that may mediate olfactory amplification. *Proc Natl Acad Sci USA*. 2009;106(28):11776–11781.
[36] Strader CD, Fong TM, Graziano MP, Tota MR. The family of G-protein-coupled receptors. *FASEB J*. 1995;9(9):745–754.
[37] Wilson RI. Neural and behavioral mechanisms of olfactory perception. *Curr Opin Neurobiol*. 2008;18(4):408–412.
[38] Yu TT, McIntyre JC, Bose SC, Hardin D, Owen MC, McClintock TS. Differentially expressed transcripts from phenotypically identified olfactory sensory neurons. *J Comp Neurol*. 2005;483(3):251–262.
[39] Zou Z, Buck LB. Combinatorial effects of odorant mixes in olfactory cortex. *Science*. 2006;311(5766):1477–1481.
[40] Zou Z, Horowitz LF, Montmayeur JP, Snapper S, Buck LB. Genetic tracing reveals a stereotyped sensory map in the olfactory cortex. *Nature*. 2001;414(6860):173–179.