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DYSREGULATION OF BRAIN OLFACTORY AND TASTE RECEPTORS IN AD, PSP AND CJD, AND AD-RELATED MODEL

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Abstract—Recently, we have shown the expression of novel chemoreceptors corresponding to the olfactory receptor (OR) and taste receptor (TASR) families in the human brain. We have also shown dysregulation of ORs and TASRs in the cerebral cortex in Parkinson's disease. The present study demonstrates the presence of OR mRNA and mRNA of obligated downstream components of OR signaling adenylyl cyclase 3 (ADYLC3) and olfactory G protein (Gnal) in the cerebral cortex of the mouse. Dysregulation of selected ORs and TASRs has been found in the entorhinal cortex and frontal cortex in Alzheimer's disease (AD) in a gradient compatible with Braak and Braak staging; frontal cortex in terminal stages of Progressive Supranuclear Palsy; and frontal cortex and cerebellum in Creutzfeldt–Jakob disease subtypes methionine/methionine at codón 129 of PRNP (MM1) and valine/valine at codón 129 of PRNP (VV2). Altered OR, ADYLC3 and Gnal mRNA expression with disease progression has also been found in APP/PS1 transgenic mice, used as a model of AD. The function of these orphan receptors is not known, but probably related to cell signaling pathways responding to unidentified ligands. Variability in the drift, either down- or up-regulation, of dysregulated genes, suggests that central ORs and TASRs are vulnerable to variegated neurodegenerative diseases with cortical involvement, and that altered expression of ORs and TASRs is not a mere reflection of neuronal loss but rather a modulated pathological response.

Key words: olfactory receptors, taste receptors, Alzheimer, Progressive Supranuclear Palsy, Creutzfeldt–Jakob disease, APP/PS1 transgenic mice.

INTRODUCTION

Chemoreception plays an essential role in human interaction with the environment. On the one hand, chemical odorants, such as pheromones, are important mediators of communication for bacteria, plants and animals (Tillman et al., 1999), including humans. Therefore, the capacity to produce, release, detect and interpret these molecules is critical for community functioning, including mating, obtaining food, reproduction, and orientation, in a number of species. On the other hand, taste discrimination has facilitated the development of an innate preference for energy-providing nutrients and rejection of harmful substances during the course of evolution.

Olfaction initiates in the primary sensory neurons located in the olfactory epithelium of the nasal cavity, which detect odorant molecules and generate an action potential that is thereafter transmitted to the brain. Odorant recognition is accomplished by G-protein-coupled olfactory receptors (ORs) located in the membrane of the cilia of these cells, which provoke the dissociation of the α subunit of an olfactory-specific excitatory G-protein ($G_{\alpha olf}$) after odorant binding. Subsequently, the $G_{\alpha olf}$ subunit activates the integral membrane protein adenylyl cyclase type III (AC3), leading to the conversion of adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). The activation of these molecular gating cyclic nucleotide-gated (CNG) channels causaes a depolarizing influx of Na^+ and Ca^{2+} that generates the action potential in the olfactory neuron (reviewed in Pifferi et al., 2010; reviewed in Malnic et al., 2010). Mice deficient in G_{olf} or AC3 are anosmic (Belluscio et al., 1998; Wong et al., 2000), which indicates that these molecules are obligate mediators of the olfactory signaling pathway.

ORs have been identified as a large family of proteins sharing conserved transmembrane motifs (Malnic et al., 2010), and approximately 400 different functional ORs have been estimated to be expressed in humans (Niimura and Nei, 2005; Zhang et al., 2007; Malnic et al., 2010). The genes encoding the vast repertoire of ORs are distributed all over the genome except in chromosomes 18 and Y (Glusman et al., 2001; Malnic et al., 2010; Niimura and Nei, 2005; Zhang et al., 2007),

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Abbreviations: AD, Alzheimer's disease; ADYLC3, adenylyl cyclase 3; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CJD, Creutzfeldt–Jakob disease; CNG, cyclic nucleotide-gated; Gnal, olfactory G protein; GUS-β, β-glucuronidase; HSD, Honestly Significant Difference; IDIBAPS, Institute for Biomedical Investigation August Pi i Sunyer; IDIBELL, Institute for Biomedical Investigation of Bellvitge; MM1, methionine/methionine at codón 129 of PRNP; OR, olfactory receptor; PCR, polymerase chain reaction; PD, Parkinson's disease; PS1, presenilin 1; PSP, Progressive Supranuclear Palsy; TASR, taste receptor; VV2, valine/valine at codón 129 of PRNP; XPNPEP1, X-prolyl aminopeptidase P1XPNPEP1.

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and their expression is tightly regulated by still-unknown mechanisms.

Taste detection occurs at the taste buds of the oral cavity, mainly located in the tongue. Each taste bud contains between 50 and 100 sensory epithelial cells, each one able to transduce one of the classic five categories of the basic tastes: bitter, sour, umami, sweet and salty (Iwatsuki and Uneyama, 2012). The most extensively-studied gustatory signaling events are those taking place during the transduction of bitter compounds. Bitter taste perception initiates through bitter tastant binding to a bitter taste receptor in the membrane of the microvilli of the gustatory cells. These receptors belong to the taste 2 receptors family and are coupled to a gustatory-specific G-protein, α-gustducin (Iwatsuki and Uneyama, 2012). Mice null for α-gustducin show a defective detection of sweet and bitter tastes (Wong et al., 1996), which suggests that these mediators are required for sweet and bitter taste transduction. α-Gustducin activation triggers an intracellular cascade involving phospholipase C-beta 2 and inositol 1,4,5-triphosphate receptor type 3, which subsequently opens transient receptor potential channels generating, a Na^+ influx that results in cell depolarization (Ishimaru and Matsunami, 2009; Iwatsuki and Uneyama, 2012). Eventually, the change in the membrane potential provokes the release of ATP toward the first neuron of the gustatory nerve (Iwatsuki and Uneyama, 2012). In human, 25 genes encoding bitter taste receptors (TASRs) have been identified (Behrens et al., 2007).

Expression of ORs in non-olfactory tissues has been reported in testis, kidney, heart and lung (Parmentier et al., 1992; Vanderhaeghen et al., 1997; Branscomb et al., 2000; Feldmesser et al., 2006; Zhang et al., 2007; De la Cruz et al., 2009). Expression of the olfactory functional mediators (G_{olf}) has been found in the kidney (Pluznick et al., 2009). ORs have been identified in the cerebral cortex of mice (Otaki et al., 2003).

In addition to the tongue, TASRs are expressed in a variety of tissues including the respiratory tract, digestive system, pancreas, liver, kidney and testes (Behrens and Meyerhof, 2010; Yamamoto and Ishimaru, 2012; Xu et al., 2013). Bitter TASRs together with downstream functional mediators are also expressed in the brain (Singh et al., 2011; Dehkordi et al., 2012).

We recently demonstrated the presence of ORs, obligate downstream components of OR signaling AC3 and $G_{\alpha olf}$, OR transporters Receptor Transporter Proteins 1 and 2 and Receptor Expression Enhancing Protein 1, and OR xenobiotic remover UDP-glucuronosyltransferase 1 family polypeptide A6 in neurons of the cerebral cortex and other regions in the adult human brain (Garcia-Esparcia et al., 2013). These findings lend weight to the idea that ORs in human cerebrum may support novel physiological functions.

OR mRNA expression is dysregulated (mainly down-regulated) in the frontal cortex in cases of Parkinson's disease (PD) at premotor and parkinsonian stages. Dysregulation of several taste receptors, some of them up-regulated and others down-regulated, also occurs in the frontal cortex in PD (Garcia-Esparcia et al., 2013). Identification of altered OR and TASR regulation in PD suggests a new scenario in the still poorly understood chemical signaling system of the brain vulnerable to neurodegenerative diseases.

Based on these antecedents, the present work was first designed to identify OR and obligate downstream components of OR signaling AC3 and $G_{\alpha olf}$ in the cerebral cortex of the mouse and in APP/PS1 transgenic mice, recreated as a model of cerebral β-amyloidosis or Alzheimer's disease (AD) (Borchelt et al., 1996; Aso et al., 2012), and, second, to check possible modifications in the expression of selected ORs and bitter TASRs receptors in the human cerebral cortex (entorhinal cortex and frontal cortex) at different stages of AD; frontal cortex at terminal stages of Progressive Supranuclear Palsy (PSP); and frontal cortex and cerebellum of Creutzfeldt–Jakob disease (CJD) cases subtypes methionine/methionine at codón 129 of PRNP (MM1) and valine/valine at codón 129 of PRNP (VV2).

EXPERIMENTAL PROCEDURES

APP/PS1 transgenic mice and wild type normal littermates

Double-transgenic APP/PS1 mice express a chimeric mouse/human APP (Mo/HuAPP695swe: APP Swedish mutation) and a mutant human presenilin 1 (PS1-dE9), both directed to the central nervous system neurons (Borchelt et al., 1996). The included Swedish mutation (K595N/M596L) elevates the amount of β-amyloid and the mutant PS1 allele accelerates the β-amyloid deposition rate as well as exacerbating pathological severity. APP/PS1 mice develop β-amyloid deposits throughout the brain and exhibit memory impairment by the end of the sixth month (Savonenko et al., 2005; Aso et al., 2012). Animal procedures were conducted according to ethical guidelines (European Communities Council Directive 86/609/EEC) and approved by the local ethics committee (University of Barcelona-Institute for Biomedical Investigation of Bellvitge (IDIBELL)). Female APP/PS1 transgenic mice and control littermates aged 0 days, and 3, 6, and 12 months; $n = 6$ for every phenotype and time-period were killed under anesthesia, the brains rapidly removed from the skull, dissected, and the left cerebral cortex immediately frozen at −80 °C whereas the right hemisphere was fixed in 4% paraformaldehyde for morphological studies.

Human cases

Brain tissue was obtained from the Institute of Neuropathology Brain Bank (HUB-ICO-IDIBELL Biobank) and the Biobank of Hospital Clinic – Institute for Biomedical Investigation August Pi i Sunyer (IDIBAPS) following the guidelines both of Spanish legislation on this matter and of the local ethics committee. The post-mortem interval between death and tissue processing was between 1 h 45 m and 24 h 30 m in all cases. One hemisphere was immediately cut in coronal sections, 1 cm thick, and selected areas of