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Ligand-selective activation of heterologously-expressed mammalian olfactory receptor

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ABSTRACT

Mammalian olfactory receptors (ORs) appear to have the capacity to couple to multiple G protein-coupled signaling pathways in a ligand-dependent selective manner. To better understand the mechanisms and molecular range of such ligand selectivity, we expressed the mouse eugenol OR (mOR-EG) in HEK293T cells together with Gα15 to monitor activation of the phospholipase-C (PLC) signaling pathway and/or Gαolf to monitor activation of the adenylate cyclase (AC) signaling pathway, resulting in intracellular Ca^{2+} release and/or Ca^{2+} influx through a cyclic nucleotide-gated channel, respectively. PLC-dependent responses differed dynamically from AC-dependent responses, allowing them to be distinguished when Gα15 and Gαolf were co-expressed. The dynamic difference in readout was independent of the receptor, the heterologous expression system, and the ligand concentration. Of 17 reported mOR-EG ligands tested, including eugenol, its analogs, and structurally dissimilar compounds (mousse cristal, nootkatone, orivone), some equally activated both signaling pathways, some differentially activated both signaling pathways, and some had no noticeable effect even at 1–5 mM. Our findings argue that mOR-EG, when heterologously expressed, can couple to two different signaling pathways in a ligand selective manner. The challenge now is to determine the potential of mOR-EG, and perhaps other ORs, to activate multiple signaling pathways in a ligand selective manner in native ORNs.

1. Introduction

G-protein coupled receptors (GPCRs) can show a hierarchy of downstream signaling imposed primarily by the conformational stage of the ligand-GPCR complex, such that different ligands reproducibly shift the balance of the cell's signaling network towards different transduction pathways. This concept, originally introduced in the 1990s [1–3] and most commonly referred to as ligand induced selective signaling or biased agonism, has since become a well-recognized phenomenon of significance to both basic and clinical science [4–6]. Mechanisms mediating ligand selective signaling can be complex, including processes such as activation of multiple G-protein isoforms [7], β-arrestins [8], the heteromerization of GPCRs [6], and direct interaction of GPCR and G-protein subunits with a variety of ion channels, including Ca^{2+} channels [9,10] and G-protein-gated inwardly rectifying potassium channels, GIRKs [11], among others.

Despite the fact that olfactory receptors (ORs) represent the largest family of mammalian GPCRs [12], their potential for ligand-selective signaling has received little attention, notwithstanding indirect evidence that activation of native ORNs can involve adenylate cyclase (AC)- as well as phosphoinositide (PI)-dependent signaling [13] and that activation of the two signaling pathways in native ORNs can be ligand selective [14,15]. A major constraint to implicating ligand-induced selective signaling in ORNs is the identification of odorants that target each signaling pathway for a given OR, given that deorphanizing ORs for even a single ligand is not trivial. Most attempts to deorphanize mammalian ORs involve heterologous expression of ORs with a specific isoform of G-protein. Co-expression of the OR with Gαolf allows coupling the OR to AC signaling, which can be monitored by an increase in intracellular Ca^{2+} through a co-expressed cyclic nucleotide-gated channel [16] or by a variety of biochemical assays [17,18], although Gαs alone can serve the same function as Gαolf [18,19]. Co-expression of the OR with the promiscuous Gα15/16 allows coupling the OR to PI (phospholipase-C, PLC)-dependent signaling, yielding release of Ca^{2+} from intracellular stores [20]. Interestingly, monitoring PLC-dependent signaling appears to bias the ligand ranking from that

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obtained by monitoring AC-dependent signaling [16], potentially reflecting ligand specific bias in the activation of the two different G proteins in the heterologous system.

In order to better understand the potential for ligand selective signaling by mammalian ORs, we used a Ca^{2+} imaging based approach that allows us to monitor activation of both pathways when activated by the mouse eugenol receptor mOR-EG, an OR with numerous known ligands. We expressed mOR-EG in HEK293T cells together with Gα15 to target PLC signaling (mOR-EG/PLC) and/or Gαs(olf) to target AC signaling (mOR-EG/AC) and differentiated the

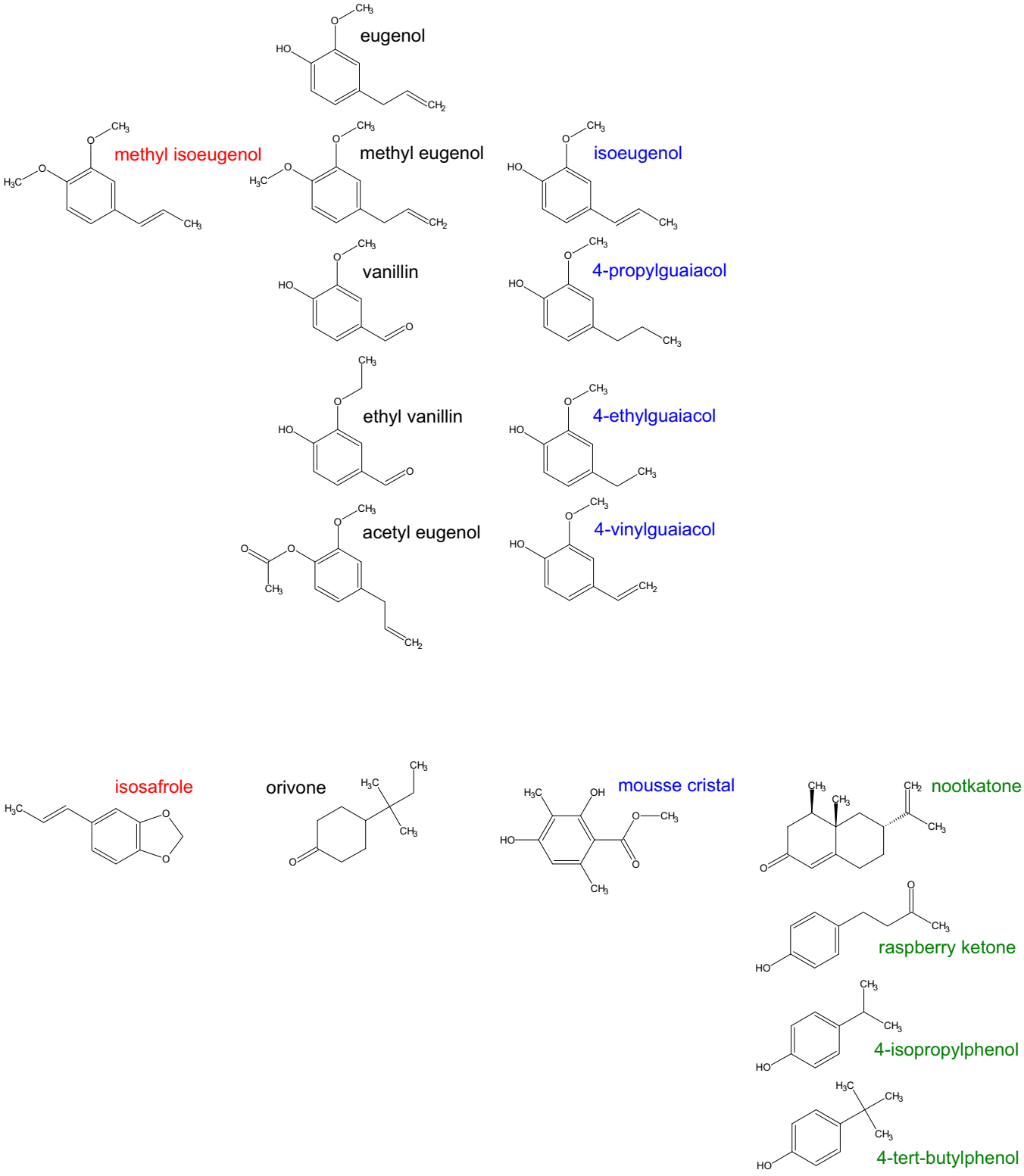

Fig. 1. Chemical structures of mOR-EG ligands used in the study, including eugenol, some eugenol derivatives, and the structurally dissimilar compounds mousse cristal, nootkatone, and orivone. Some of the ligands failed to give a measurable response (green). Some ligands activated both PLC- and AC-mediated outputs, seven of them equally (black) and four preferentially favoring the PLC-dependent output (blue). Methyl isoeugenol and isosafrole are known antagonists (red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)