OR1D2 is a broadly tuned human olfactory receptor

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Introduction.

OR1D2 is one of the first deorphanized human olfactory receptors (ORs). It was initially found to respond to bourgeonal and to a limited series of analogs including canthoxal, lilial and florazone (Spehr et al., Science 2003, **299**, 2056-58). In addition to its putative role in olfactory perception, this receptor was also shown to mediate sperm chemotaxy.

Here, we present the results of different screening campaigns and structure activity relationship studies that were performed on this receptor. We observed that the range of agonists of OR1D2 is larger than initially expected. The receptor responds to a large diversity of chemical structures.

Table 1. Agonists of OR1D2

	Agonist	Log EC50	Main organoleptic note
1	Tetrahydromyrcenol	-6.20	citrus
2	2,6-dimethyl-7-octen-2-ol	-5.63 ± 1.92	citrus
3	Citral dimethyl acetal	-5.47 ± 0	citrus
4	Dihydroisojasmonate	-5.43 ± 0.13	floral
5	(E),(E)-2,4-Decadienal	-5.18 ± 0.66	fatty
6	γ-undecalactone	-5.14 ± 1.17	fruity
7	Myrcenol	-5.12	citrus
8	δ-dodecalactone	-5.09 ± 0.88	tropical
9	Coranol	-4.96 ± 0.22	floral
10	Zinarine	-4.95 ± 0.66	green
11	Rosaphen	-4.87 ± 0.06	floral
12	benzyl acetone	-4.86 ± 0.24	floral
13	Methyl heptenone	-4.84 ± 0.35	citrus
14	Tetrahydrolinalool	-4.81 ± 0.26	citrus
15	Ethyl cinnamate	-4.78 ± 0.14	balsamic
16	Tetrahydrogeraniol	-4.78 ± 0.54	floral
17	1-octyl-2-pyrrolidone	-4.76 ± 0.16	citrus
18	Pivarose	-4.73 ± 0.57	floral
19	Methyl naphthyl ketone	-4.67 ± 0.37	floral
20	Mefrosol	-4.66 ± 0.13	floral
21	Floridile	-4.65 ± 0.74	fruity
22	Violet nitrile	-4.65 ± 0.15	green
23	Cassione	-4.57 ± 0.1	floral
24	Florymoss	-4.54 ± 0.44	floral
25	Citral	-4.52	citrus
26	δ-undecalactone	-4.52 ± 0.67	coconut
27	Dihydromyrcenol	-4.51 ± 0.26	citrus
28	Citronellol	-4.51 ± 0.29	floral
29	Methyl-trans-cinnamate	-4.49 ± 0.59	balsamic
30	Hypo-lem	-4.49 ± 0.1	citrus
31	Milk lactone 2067	-4.49 ± 0.85	creamy
32	cis-5-octen-1-ol	-4.48 ± 0.78	green
33	cis-6-nonen-1-ol	-4.48 ± 0.17	melon
34	Ethyl linalool	-4.47 ± 0.48	floral
35	Petiole	-4.47 ± 0.19	green
36	Citronellyl nitrile	-4.43 ± 0.28	citrus
37	Frutonile	-4.38 ± 0.29	fruity
38	1-octen-3-ol	-4.38 ± 0.38	earthy
39	benzyl isobutyrate	-4.31 ± 0.49	floral
40	Phenyl ethyl isovalerate	-4.3 ± 0.2	floral
41	7-octen1-ol	-4.3 ± 0.02	citrus
42	1-nonanol	-4.26 ± 0.41	floral
43	Iso jasmone	-4.26 ± 0.39	floral
44	Linalool	-4.26 ± 0.34	floral
45	γ-dodecalactone	-4.25 ± 0.77	fruity
46	9-decen-1-ol	-4.24 ± 0.3	floral
47	Ethyl p-anisate	-4.24 ± 0.13	anisic
48	Nerolidyl acetate	-4.21 ± 0.19	floral
49	dimethyl ethyl phenyl carbinol	-4.19 ± 0.1	floral
50	allyl cyclohexylpropionate	-4.19 ± 0.31	fruity
51	Jasmatone	-4.12 ± 0.21	floral
52	3-methyl-3-nonanol	-4.12 ± 0.007	citrus
53	(S)-(-)-Citronellal	-4.12	citrus
54	benzyl butyrate	-4.07 ± 0.41	fruity
55	Ethylene Glycol Monophenoxyacetate	-4.06 ± 0.23	powdery
56	Empetal	-4.02 ± 0.15	aldehydic
57	Geraniol	-4.00	floral
58	Quintone	-3.98 ± 0.42	floral
59	Methyl tuberate	-3.95 ± 0.5	floral
60	Benzyl propionate	-3.95 ± 0.32	fruity
61	Benzyl acetate	-3.94 ± 0.12	floral
62	Phenyl ethyl acetate	-3.91 ± 0.08	floral
63	β-methylphenylethylamine	-3.91	animal
64	δ-tetradecalactone	-3.9 ± 0.63	waxy
65	δ-2-decenolactone	-3.86 ± 0.08	creamy
66	trans-2-nonenal	-3.86 ± 0.87	green
67	undecene-2-nitrile	-3.86 ± 0.27	citrus
68	3-Octyl acetate	-3.86	herbal
69	5-decanol	-3.85 ± 0.5	fruity
70	Heptone	-3.78 ± 0.3	fruity
71	Citronellyl oxyacetaldehyde	-3.77 ± 0.3	aldehydic
72	γ-decalactone	-3.75 ± 1.27	fruity
73	7-Methylindole	-3.75 ± 0.56	animal
74	Nonalactone	-3.64 ± 0.44	coconut
75	Cinnamyl nitrile	-3.63 ± 0.44	spicy
76	Clonal	-3.58 ± 0.15	citrus
77	β-phenoxy-ethyl-isobutyrate	-3.56 ± 0.98	green
78	5-Methylindole	-3.53 ± 0.38	animal
79	Cinnamic alcohol	-3.47 ± 0.23	balsamic
80	Ethyl phenyl glycidate	-3.43 ± 0.21	fruity
81	Anisyl acetate	-3.38 ± 0.2	powdery
82	Geranyl formate	-3.31	floral
83	octanal	-3.29 ± 0.27	aldehydic
84	Neryl acetate	-3.25 ± 0	floral
85	δ-Nonalactone	-3.19 ± 0.78	coconut
86	δ-tridecalactone	-3.14 ± 0.45	creamy
87	Geranyl propionate	-3.10	floral
88	para-methoxyacetophenone	-3.09 ± 1.05	anisic
89	Tetrahydro citral	-3.05 ± 0	citrus
90	δ-decalactone	-2.99 ± 0.59	coconut
91	Geranyl butyrate	-2.96	fruity
92	Geranyl acetate	-2.91	floral
93	1-Methylindole	-2.89	animal
94	heptanal	-2.71 ± 0.02	green
95	Methyl nicotinate	-2.7 ± 0.43	herbal
96	nonanal	-1.71 ± 2.41	aldehydic
97	diethyleneglycol hexyl ether	-1.52 ± 0.88	green

Materials and Methods.

Screening was performed in HEK293T-hRTP1S/hRTP2 cells using the CRE-luciferase reporter assay system. Briefly, cells plated one day before, were transfected with OR and pGL4.29 plasmids using TransIT®-LT1 (Mirus) according to the manufacturer's protocol. Twenty hours after transfection and four hours after incubation with tested compounds, cells were lysed and processed for luminescence measurement using a Spectra Max M5 reader (Molecular Devices).

The compounds of the screening library are distributed in 96 well plates (80 compounds per plate) at a defined concentration. Each molecule of the screening library is tested at three different concentrations : 316 µM, 100 µM and 31.6 µM.
Hits are defined as compounds eliciting a Luciferase response over the plate median value + 2 standard deviations for at least two concentrations.
Hits are further validated by concentration-response analyses using the same functional assay. Before being considered as true agonist of the tested OR, validated hits are tested on mock cells to confirm the specific activation of the receptor.

Structure-activity relationship studies that compare the activity of different activators of OR1D2 were performed using the Luciferase assay. Results of concentration-response analyses are expressed as the percentage of the response induced by 10 µM of Forskolin and were fitted to the Hill's equation.

Direct measurements of cyclic AMP were performed using the HTRF-based assay (CisBio). It corresponds to a competitive immunoassay, where a time-resolved fluorescence energy transfer (FRET) occurs upon binding between the cryptate-labelled anti-cAMP antibody and the d2-labelled cAMP. Introduction of cAMP produced by the cells upon the stimulation of the receptor decreases the FRET signal, proportionally to the concentration of the added unlabelled cAMP. Practically, cells expressing the receptors are incubated in an assay plate, in KRH buffer containing the tested ligand. After 30 min, d2-labelled cAMP and cryptate-labelled anti-cAMP are sequentially added. The FRET signal is read 1 h latter on Synergy II microplate reader (Biotek). are expressed as the percentage of the response induced by 10 µM of Forskolin and were fitted to the Hill's equation .

OR1D2 is a broadly tuned receptor.

OR1D2 has been included in a series of screening campaigns aiming to deorphanize human olfactory receptors. On a total of 1100 molecules screened, 77 were found to activate OR1D2. It represents one of the highest hit rate recorded so far for an olfactory receptor. In addition to these screenings, short structure-activity relationship studies have been performed, leading to the identification of 20 additional agonists (Table 1).

These results were obtained using the Luciferase-based gene rapporter assay. This assay was shown to be very sensitive and suitable to demonstrate the activation of ORs by their agonists. For some of the identified activators of OR1D2, an additional validation was performed using an alternative functional assay that directly measure the cAMP produced upon receptor activation. As illustrated on figure 1, both assays gave similar results, although the estimate potency is higher with the luciferase-based assay (logEC50= -4.98) compared to HTRF-based assay (logEC50= -4.21). This probably reflects the higher sensitivity of the gene reporter assay.

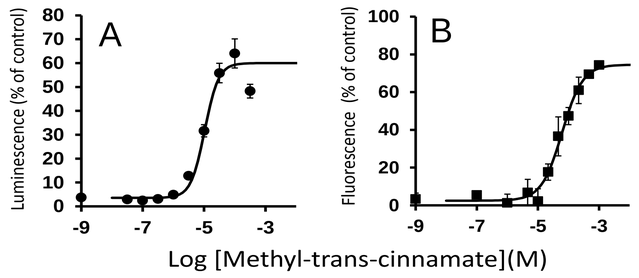

Figure 1. Comparison of OR1D2 activation with 2 different functional assays.
Response of OR1D2 to the methyl-trans-cinnamate has been monitorated using the luciferase-based gene reporter assay (A) or the HTFR-based cAMP immunoassay (B).

Considering the agonist list, no shared structural characteristic emerges from the comparison of the different activators that can be alcohols, aldehydes, esters, lactones,… Likewise, the different agonists are not linked by a common organoleptic characteristic or note. Both pleasant and unpleasant odors are represented, although a majority of ligands are categorized as floral, fruity or citrus.

Notwithstanding the apparent poor selectivity of OR1D2, the comparison of the EC50 of agonists belonging to homogenous molecular series reveals structural features that are correlated with a higher potency. In the examples disclosed on figure 2, a series of γ-lactones and δ-lactones have been compared. The most active compounds correspond to the γ-undecalactone and the δ-dodecalactone. Analogs with a shorter or a longer side chain display a lower potency.

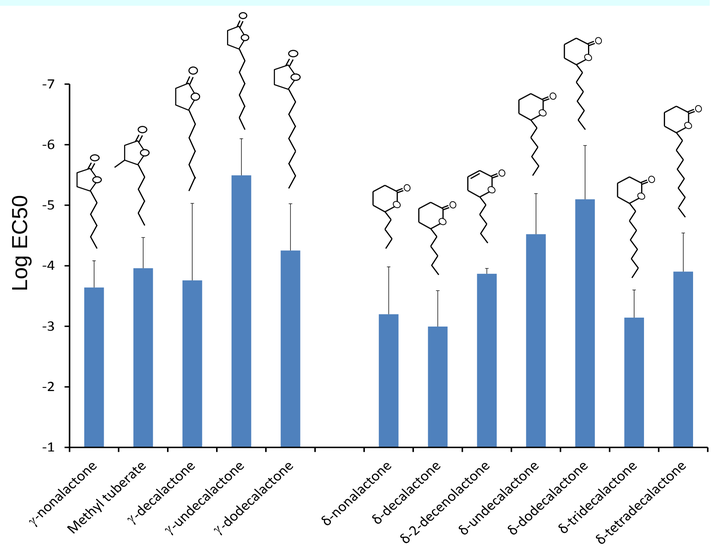

Figure 2. Comparison of potency of different lactones on OR1D2.

Discussion.

With a non-exhaustive list of more than 90 agonists, our study clearly shows that OR1D2 belongs to the intriguing category of broadly tuned receptors. The role of these particular receptors in the discrimination of odors remains to be understood. Nevertheless, a possible function of OR1D2 in odor detection and discrimination can not be excluded, since the receptor has been previously shown to be highly expressed in the human mucosa. In addition, in view of the large differences in agonist potencies, it is can be hypothesized that a stronger sensitivity of OR1D2 to some ligands can play a role in their detection.
Interestingly, OR1D2 is also expressed in spermatozoa. Its ability to respond to a large number of ligands could shed a new light on its function in sperm chemotactism.

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