High-Potency Olfactory Receptor Agonists Discovered by Virtual High-Throughput Screening: Molecular Probes for Receptor Structure and Olfactory Function

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DOI 10.1016/j.neuron.2008.11.014

SUMMARY

The detection of diverse chemical structures by the vertebrate olfactory system is accomplished by the recognition of odorous ligands by their cognate receptors. In the present study, we used computational screening to discover novel high-affinity agonists of an olfactory G protein-coupled receptor that recognizes amino acid ligands. Functional testing of the top candidates validated several agonists with potencies higher than any of the receptor’s known natural ligands. Computational modeling revealed molecular interactions involved in ligand binding and further highlighted interactions that have been conserved in evolutionarily divergent amino acid receptors. Significantly, the top compounds display robust activities as odorants in vivo and include a natural product that may be used to signal the presence of bacteria in the environment. Our virtual screening approach should be applicable to the identification of new bioactive molecules for probing the structure of chemosensory receptors and the function of chemosensory systems in vivo.

INTRODUCTION

The vertebrate olfactory system receives and decodes sensory information from a myriad chemical cues. The first step in this process is the recognition of these cues by receptors expressed by the primary sensory neurons in the olfactory epithelium (Firnstein, 2001). Vertebrate olfactory receptors comprise four different families of G protein-coupled receptors (Mombaerts, 2004) (GPCRs): the OR receptor family, the largest family with ~1000 functional members in some mammalian species (Zhang et al., 2004); the trace amine-associated receptors (Liberles and Buck, 2006) (TAARs; ~20 members); the V1R vomeronasal receptors (Zhang et al., 2004) (~150 members); and the V2R vomeronasal receptors (Yang et al., 2005) (~60 members). The V2R receptors belong to the C family of GPCRs, which includes the calcium-sensing receptor (CaSR), metabotropic glutamate (mGlu) receptors, GABA-B receptors, and T1R taste receptors (Pin et al., 2003).

We previously identified a V2R-like receptor from the goldfish olfactory epithelium that is activated by all 20 natural amino acids, which are potent odorants for fish (Luu et al., 2004; Speca et al., 1999). This receptor, called receptor 5.24, responds preferentially to the long-chain basic amino acids, lysine and arginine, although other amino acids can bind to this receptor with lower affinities. This broad tuning of receptor 5.24 embodies the promiscuous nature of the odorant receptors, a mechanism that allows the olfactory system to recognize a diversity of chemical structures exceeding the actual number of receptors encoded by the genome. It is therefore of great interest to elucidate the molecular determinants of ligand selectivity—using receptor 5.24 as a prototypical receptor—in order to understand how the olfactory/vomeronasal C family GPCRs have evolved to recognize their cognate ligands.

Unlike other GPCRs, members of the C family GPCRs are characterized by a large extracellular N-terminal domain (NTD), the location of the orthosteric ligand binding site. The NTD adopts a conserved clamshell-like fold—also referred to as the Venus fly trap domain (VFTD)—with two lobes connected by a flexible hinge. Analysis of protein crystal structures and molecular modeling have identified ligand interactions with the inner surfaces of lobes 1 and 2 that stabilize a closed conformation of the VFTD, leading to receptor activation (reviewed by Pin et al., 2003). An inspection of the core binding residues reveals numerous potential contacts with ligand that can be sorted into two groups: the “proximal” and “distal” binding pockets. The proximal pocket residues are predicted to bind the amino acid ligand’s glycine moiety (i.e., the α-carboxyl together with the α-amino group and α-proton). Residues residing in the distal pocket interact with the amino acid ligand’s side chain and are responsible for conferring selectivity for distinct side-chain
structures. In the case of goldfish receptor 5.24, through homology modeling we previously identified several distal pocket residues that can account for this receptor’s preference for long-chain basic amino acids (Luu et al., 2004).

In the present study, we sought to utilize novel chemical structures to probe more deeply into the structure and function of the receptor 5.24 binding pocket. We further wished to know whether high potency agonists that were selected based on their interactions with a single receptor would also be active in eliciting olfactory responses in vivo. To this end, we developed and applied a suite of computational techniques to screen for receptor 5.24 agonists. This “virtual high-throughput screening” (vHTS) approach identified numerous active compounds, with several showing significantly higher potency than any of the previously known natural ligands for this receptor. Docking of the most active compounds in three-dimensional models of the receptor confirmed the importance of several binding pocket residues in determining affinity and selectivity. Interestingly, analysis of one series of ligands reveals a conserved ligand-stabilized helix-helix interaction in lobe 1 that is associated with ligand recognition and receptor activation in evolutionarily divergent amino acid receptors. Finally, electrophysiological recordings from goldfish olfactory epithelium indicate that the computationally identified agonists can indeed elicit robust responses by olfactory sensory neurons in vivo. One novel odorant discovered, diaminopimelic acid, is a precursor in the lysine and peptidoglycan biosynthetic pathways of bacteria, suggesting that the fish olfactory system may use the presence of this metabolite to detect bacteria in their environment. Together, these results demonstrate the utility of our vHTS approach in identifying novel compounds for probing C family GPCR structure and function and for interrogating olfactory function in vivo.

RESULTS

Virtual Screening for the Discovery of Novel Receptor Agonists

Elucidation of the principles underlying ligand binding and activation in the metabolotropic glutamate receptors has been greatly facilitated by an array of high affinity agonists and antagonists (Pin et al., 1999). By analogy, we expect that the identification of novel high-affinity ligands for receptor 5.24 will similarly provide new molecules with which to probe the structure and function of this and potentially other chemosensory C family GPCRs. To achieve this goal, we implemented a computational screening paradigm to search for ligands based on two independent criteria: (1) chemicals that “fit” in the predicted ligand binding pocket structure (structure-based vHTS) and (2) chemicals that exhibit features identified by a pharmacophore model of activating ligands (ligand-based vHTS). Since our vHTS approach requires a detailed structural model of the receptor binding pocket as well as an extensive pharmacological profile for the receptor, we implemented this vHTS approach on goldfish receptor 5.24, for which we already have extensive data (Luu et al., 2004).

vHTS Protocol Design and Optimization

As with any screening protocol, a balance must be found to maximize the identification of true positive “hits” while avoiding an inordinate number of false positives. We therefore designed the computational protocols according to the receiver operating characteristic (ROC) curve approach, which provides an objective measure of the ability of a given screening workflow to discriminate known active molecules from known inactive molecules—a critical test of the design of any screening protocol (Triballeau et al., 2005). Thus, the ROC curve approach can be used to facilitate decision making about the selection criteria used for computational screening.

Computational protocols were designed and optimized for both structure- and ligand-based vHTS workflows according to the ROC curve method described previously (Triballeau et al., 2005; see Figure S1 and Supplemental Experimental Procedures available online for details). The structure-based vHTS protocol utilized a molecular model of the receptor 5.24 binding pocket (Luu et al., 2004). For ligand-based vHTS, we used pharmacophore modeling to identify compounds in chemical space that share characteristics similar to known receptor 5.24 ligands (Speca et al., 1999). Our resulting pharmacophore model (Figure S2) comprises features that are consistent with the conformation and interaction of L-arginine docked in a structural model of receptor 5.24 (Luu et al., 2004).

Chemical Library and vHTS Implementation

We used our vHTS protocols to screen a database representing a compilation of 1.6 million commercially available compounds (see Experimental Procedures). A series of computational filters (Triballeau et al., 2005) was applied to the database prior to implementation of the vHTS workflows to reduce the number of candidates to 758 unique chemical structures (see Supplemental Data for details). Screening the 758 compounds using our vHTS protocols for receptor 5.24 resulted in 468 compounds that satisfied our selection criterion in the structure-based protocol and 50 compounds that met our criterion in the ligand-based protocol. Forty-six compounds are common to both lists (“common hits”), and include L-amino acids known to activate the receptor.

Table 1. vHTS Summary: Number of Compounds Retained after Each Selection Stage

<table>
<thead>
<tr>
<th>vHTS Selection Method</th>
<th>Number of Virtual Hits</th>
<th>Number of Hits Tested in Functional Assays</th>
<th>Number of Active Compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Docking only</td>
<td>422</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Common virtual hits</td>
<td>46</td>
<td>36</td>
<td>16</td>
</tr>
<tr>
<td>Pharmacophore only</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>472</td>
<td>45</td>
<td>19</td>
</tr>
</tbody>
</table>

*A compound was operationally defined as active if it elicited ≥40% maximum receptor activity at 20 μM.

Functional Validation of Compounds Identified by vHTS

We reasoned that chemical structures identified by two independent approaches should comprise the most promising candidates for subsequent validation by functional assays. Thus, we focused mainly on compounds that were retained in common
by both screening protocols. Compounds were functionally validated by screening for activation of receptor 5.24 expressed in HEK293 cells, using a calcium mobilization assay in 96-well plates. Chemicals were screened at 20 µM, a concentration ~10 times higher than the EC50 for the receptor’s most potent natural agonists, lysine and arginine (EC 50 = 2 µM). We reasoned that only those compounds showing significant activity at this concentration would be viable candidates as high affinity agonists. Thirty-six compounds were initially tested from the common hit list. After examining the structures of the most active compounds identified in this preliminary functional screen (Table 2), we selected additional compounds from the structure-based (seven compounds) or ligand-based (two compounds) vHTS hit lists, as well as a few structural analogs of the top hits. Altogether 54 compounds were tested, with 45 from the three vHTS hit lists (Figure S4; Tables 1, 2, and S1). Forty-two percent (19/45) of the compounds tested from the vHTS hit lists showed significant activity at 20 µM (operationally defined as ≥40% maximum activity), which is remarkable given the more typical validation rate of 10% or less in such screening protocols (Augen, 2002; Tribal-leau et al., 2005, 2006). In addition, of the nine compounds selected based on their structural similarities to the active compounds, five exhibited significant activity as receptor 5.24 agonists. We next generated dose-response curves to calculate EC50 values for the 24 validated compounds (Tables 2 and S1).

**Table 2. Activities of the Top Five vHTS Hits and Known Natural Agonists**

<table>
<thead>
<tr>
<th>Structure of Hits</th>
<th>Hit Number and Name</th>
<th>Hit Class</th>
<th>EC50 (µM)</th>
<th>SEM</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>HO2C NH2 O NO2</td>
<td>L-glutamic acid-γ-p-nitroanilide</td>
<td>Q</td>
<td>0.72</td>
<td>0.09</td>
<td>6</td>
</tr>
<tr>
<td>HO2C NH2 O NH2</td>
<td>(S)-4-oxalysine</td>
<td>K/R</td>
<td>0.89</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>HO2C NH2 O NH2</td>
<td>L-canavanine</td>
<td>K/R</td>
<td>0.96</td>
<td>0.09</td>
<td>4</td>
</tr>
<tr>
<td>HO2C NH2 O NH2</td>
<td>L-lysine</td>
<td>K/R</td>
<td>1.58</td>
<td>0.26</td>
<td>3</td>
</tr>
<tr>
<td>HO2C NH2 CO2H</td>
<td>L-cystathionine</td>
<td>diAA</td>
<td>1.7</td>
<td>0.13</td>
<td>3</td>
</tr>
<tr>
<td>HO2C NH2 NH2</td>
<td>L-arginine</td>
<td>K/R</td>
<td>2.36</td>
<td>0.1</td>
<td>39</td>
</tr>
<tr>
<td>HO2C NH2 CO2H</td>
<td>LL- and DL-α,ε-diaminopimelic acid</td>
<td>diAA</td>
<td>2.37b</td>
<td>0.07</td>
<td>3</td>
</tr>
<tr>
<td>HO2C NH2 O NH2</td>
<td>L-glutamine</td>
<td>Q</td>
<td>9.72</td>
<td>1.21</td>
<td>3</td>
</tr>
</tbody>
</table>

*aQ = glutamine derivative; K/R = lysine/arginine derivative; diAA = di-amino acid.
*bReceptor activation assay was performed with a mixture of LL, DL, and DD isomers.

**Structure-Activity Analysis of vHTS Hits Reveals a Strong Preference for Amino Acid Ligands with Unbranched Side Chains**

Our previous molecular modeling study of receptor 5.24 predicted interactions between specific residues and contact sites on the bound ligand’s glycine core (the so-called proximal binding pocket) as well as on the ligand’s side chain (the “distal” binding pocket) (Luu et al., 2004). It is striking that all vHTS hits that were validated as active compounds are linear amino acids with no substitutions at the β and γ positions (Table S1). This characteristic probably reflects the restricted steric tolerance of the residues interacting with this portion of the side chain, serine-151 (S151) on lobe 1 and asparagine-310 (N310) on lobe 2. From our three-dimensional model of receptor 5.24, the bound
ligand’s side chain’s β and γ methylenes are in Van der Waals contact with S151 and N310 (Luu et al., 2004) (Figure S5). These contacts would preclude substitutions on the ligand’s side chain and explain why, for example, phenylalanine, tyrosine, tryptophan, and their derivatives are poor agonists of receptor 5.24.

Molecular Determinants of Ligand Binding and Receptor Activation Revealed by Top vHTS Hits

The identification of novel high-potency agonists provides an opportunity to probe the molecular interactions underlying ligand recognition and receptor activation. A close look at the side chains of the most active molecules among the hits identified by both the docking and pharmacophore models allowed us to classify them into three groups: lysine/arginine-like (hit #’s 244, 48, 677), glutamine-like (hit #’s 338, 506, 586, 137, 583), and di-amino acids (hit #’s 380, 299, 565, 612, 652, 479) (Tables 2 and S1). Most of the other validated hits (identified by the docking model alone or selected based on structural similarity to some of the top hits) belong to one of these three categories (Table S1). To gain insight into the interactions underlying the properties of these novel receptor agonists, we generated molecular models of receptor 5.24 bound to selected compounds from the three groups of chemicals.

Lysine/Arginine Analogs

In the lysine- and arginine-like series, substitution of a methylene group with an oxygen atom in the alkyl chain of lysine (L-oxalysine, hit # 244) or arginine (L-canavanine, hit # 677) results in an ~2-fold increase in ligand potency (Table 2). The interactions with the ligands’ terminal amino (oxalysine) or guanidinium (canavanine) groups (Figures 1A and 1B) are similar to those predicted for lysine and arginine, respectively (Luu et al., 2004). Carbon atoms are depicted in gray, oxygens in red, nitrogens in blue, sulfurs in yellow, and hydrogens in white (hydrogens shown only on ligands). Predicted hydrogen bonds are displayed as dashed green lines. Novel receptor-ligand interactions with the oxygen heteroatoms of oxalysine and canavanine as compared to their natural amino acid counterparts (lysine and arginine, respectively) are noted with green arrowheads (A and B). Note the different binding of each di-amino acid’s distal carbonyl to distal pocket residues (far right of [C] and [D]). Comparison of the dockings in (E) and (F) reveals the participation of L-glutamic acid-γ-p-nitroanilide’s nitro substituent in a distal hydrogen bond network, which may explain this ligand’s enhanced potency relative to glutamine.

Di-amino Acids

The strongest activation in this class was observed with hit # 380 (L-cystathionine; EC50 = 1.7 μM) and hit # 299 (LL- and DL-α,β-diaminopimelic acid; EC50 = 2.4 μM for a mixture of LL, DL, and DD enantiomers) (Table 2). Docking of L-cystathionine in a structural model of receptor 5.24 suggests ligand-receptor interactions with the distal carboxylate in addition to interactions with the distal amino group similar to those predicted for L-lysine or L-oxalysine (compare Figures 1A and 1C). In contrast, docking...
Interaction Conserved in Divergent Amino Acid Receptors

Among the residues predicted to contact the distal nitrophenyl moiety of L-glutamic acid-γ-p-nitroanilide (Figure 1F), K74, Q78, D388, and M389 are located within helices αl and αIX. These two helices comprise part of a conserved lobe 1 core structure in all proteins belonging to the structural periplasmic binding protein-like I superfamily, which includes the mGlu receptors and bacterial amino acid binding proteins such as LIVBP, as well as receptor 5.24 (Luu et al., 2004; Figure 2A). From the crystal structures of mGlu1 (PDB code 1ewk) and LIVBP (PDB code 1216), coordination of ligand by these two helices relies upon interactions with residues at positions corresponding to Q78 and M389 in receptor 5.24 (highlighted in Figure 2A). In mGlu1, bound glutamate forms a bridge between the two helices via ionic interactions with R78 on helix αl (via a bridging water molecule) and K409 on helix αIX (Figure 2B). Similarly, in LIVBP, the hydropobic side chain of bound leucine interacts with Y18 (helix αl) and F276 (helix αIX), drawing the two helices in close apposition (Figure 2C).

Whereas bound L-arginine is not predicted to interact directly with residues on helix αl (although hydrogen bonding may occur between Q78 on helix αl and D388 on helix αIX; Figure 2D), our model predicts that bound L-glutamic acid-γ-p-nitroanilide forms a bridge between these two helices via an novel interaction with Q78 in helix αl and hydrophobic interactions with M389 (as well as polar interactions with D388) in helix αIX (Figure 2E). It is remarkable that, although the amino acid residues in mGlu1, LIVBP and receptor 5.24 are divergent at positions 78 and 389 (receptor 5.24 coordinates: Figure 2A), the function of these sites in conferring both affinity and selectivity appears to have been conserved in these amino acid binding proteins.

Identification of a Ligand-Mediated Helix-Helix Interaction Conserved in Divergent Amino Acid Binding Proteins

The docking model also predicts hydrogen bonds (as well as polar interactions with D388) in helix αl and M389 (as well as polar interactions with D388) in helix αIX). A hydrophobic interaction is also predicted between the ligand’s phenyl ring and M389 (as well as polar interactions with D388) in helix αIX. In addition, an orthogonal interaction (Paulini et al., 2005) may occur between the distal nitro group and D388. Polar interactions are depicted by dashed green lines (small red sphere in [B] represents a bridging water molecule); hydrophobic contacts are shown as transparent gray spheres.

of LL- and DL-α,β-diaminopimelic acid suggests a reorganization of the distal binding pocket interactions in order to accommodate the distal acidic group of this di-amino acid in either diastereoisomeric configuration (the LL isomer is shown in Figure 1D). The docking model also predicts hydrogen bonds with the sulfur heteroatom of L-cystathionine (Figure 1C), as seen with the oxygen heteroatoms in the oxalysine and canavanine side chains (Figures 1A and 1B). However, when the adjacent δ-CH2 group of L-cystathionine is replaced by a sulfur atom (hit # 479) or selenium atom (hit # 443), the potency is markedly decreased (Table S1).

Glutamine Analogs

While glutamine displays modest potency (EC50 = 9.7 μM), we discovered that its N-4-nitrophenyl derivative, L-glutamic acid-γ-p-nitroanilide (hit # 338; EC50 = 0.72 μM) is over 10 times more potent than L-glutamine and ~2- to 3-fold more potent than lysine or arginine, the two most potent natural amino acid ligands of receptor 5.24 (Luu et al., 2004; Specia et al., 1999). This enhanced potency derives mainly from the nitro moiety, as the N-phenyl glutamine analog (compound Q2, EC50 = 7.9 μM for the racemic mixture) is only slightly more potent than L-glutamine. None of the other substitutions of the phenyl ring or polar N substituents such as -NH2 or -OH, induce a similar increase in potency (e.g., hit #s 506, 583, 588, and compounds Q1 and Q3; Table S1). Several factors may be responsible for the enhanced potency of L-glutamic acid-γ-p-nitroanilide. From molecular modeling, we predict that in addition to making similar contacts as bound glutamine (Figure 1E), the distal nitro group participates in a set of novel polar interactions with Q78 and D388 in the distal pocket (Figure 1F). The distal nitro moiety may also constrain the conformation of L-glutamic acid-γ-p-nitroanilide’s phenyl ring to allow optimal interaction with M389 (and possibly other hydrophobic residues in the vicinity), which we previously demonstrated to be critical for activation by high-affinity ligands (Luu et al., 2004).
Figure 3. Compounds Identified by vHTS Function as Odorants In Vivo
Electro-olfactogram (EOG) recordings were performed on goldfish olfactory epithelium to measure olfactory responses to L-arginine and selected top vHTS hits. Representative traces are shown in which each compound was applied at 10^{-5} M concentration. Each compound elicited a negative potential characteristic of an excitatory olfactory neuronal response. H_2O, water control; L-Arg, L-arginine; 338, L-glutamic acid-γ-p-nitroanilide; 244, L-oxalysine; 299, diaminopimelic acid; 677, L-canavanine.

Functional Validation of Top vHTS Hits as Odorants In Vivo
The data presented thus far demonstrate the utility of our vHTS approach in identifying novel molecules that can be used to probe the structure and function of the receptor 5.24 ligand binding pocket. We next wished to determine whether agonists identified in this way—i.e., based on their interactions with a specific receptor—could also function as odorants by eliciting responses in olfactory sensory neurons. Candidate compounds were tested for activity in vivo by measuring evoked changes in voltage across the goldfish olfactory epithelium. This measurement, known as the electro-olfactogram (EOG; Ottoson, 1956), is an extracellular field potential that locally summates the activity of a population of cells in the sensory epithelium and is widely used as a reliable assay for olfactory neuron responses in a variety of vertebrates, including fish (Scott and Scott-Johnson, 2002).

We selected four of the top vHTS hits (# 338, L-glutamic acid γ-p-nitroanilide; # 244, oxalysine; # 299, diaminopimelic acid; and # 677, L-canavanine) for testing by EOG in the goldfish olfactory epithelium. As shown by the representative traces in Figure 3, all compounds elicited robust EOG responses at 10^{-5} M, showing peak EOG amplitudes comparable to the response generated by 10^{-5} M L-arginine. Testing of L-glutamic acid γ-p-nitroanilide, L-oxalysine, diaminopimelic acid, and L-arginine over a wide concentration range revealed detectable responses at 10^{-5} M (Table S2). The responses did not show saturation at 10^{-4} M, which is typical of amino acid-evoked EOGs in fish (Byrd and Caprio, 1982; Rolen et al., 2003) and probably reflects the recruitment of low-affinity receptor interactions at higher ligand concentrations.

Interestingly, whereas L-glutamic acid γ-p-nitroanilide and L-oxalysine exhibit higher apparent affinities to receptor 5.24 than L-arginine (Table 2), in vivo they elicit somewhat lower peak responses across all concentrations tested (Table S2). A possible explanation for this observation is that L-glutamic acid γ-p-nitroanilide and L-oxalysine interact with a smaller number of receptors than L-arginine (i.e., they are more specific). On the other hand, diaminopimelic acid and L-canavanine are slightly more potent than L-arginine in vivo, suggesting that their interactions with the population of receptors expressed in the olfactory epithelium may be more promiscuous than those of the other compounds (including L-arginine). Whatever the case, EOG recordings clearly demonstrate that the top hits identified by our receptor 5.24-based computational screening paradigm can indeed function as odorants in vivo.

DISCUSSION

In the present study, we applied a computational high-throughput screening approach to discover new agonists of receptor 5.24, an olfactory C family GPCR. Our strategy utilized ligand-based and structure-based models that together identified several agonists showing higher potency than any of the receptor’s previously identified natural ligands. Interestingly, compounds that bind to lobe 1 of an mGlu receptor but prevent closure of the VFTD are antagonists (Bessis et al., 2002). It therefore may be possible to identify receptor 5.24 antagonists by screening for compounds that fit a structural model of the receptor’s ligand binding domain in the open, but not closed conformation. More broadly, our vHTS approach should be applicable to the identification of ligands for other chemosensory C family GPCRs (the V2R/V2R-like vomeronasal/olfactory receptors and T1R taste receptors) (Alioto and Ngai, 2006; Shi and Zhang, 2007) as well as the OR family olfactory receptors, whose transmembrane ligand binding domains have been characterized using molecular modeling techniques (Abaffy et al., 2007; Katada et al., 2005; Schmiedeberg et al., 2007).

Conservation of Molecular Determinants Underlying Ligand Recognition in the Amino Acid Receptors: Functional Implications
L-glutamic acid-γ-p-nitroanilide is the most potent agonist identified in this screen. We attribute the enhanced potency of this compound to novel interactions between its distal nitrophenyl moiety and sites on helices αI and αX of the ligand binding domain. Significantly, the positions of these determinants on helices αI and αX—and their role in dictating affinity and selectivity—appear to be a conserved feature of highly divergent amino acid receptors, including bacterial LIVBP and a mammalian mGlu receptor (Figure 2). In this regard it is interesting that, among the top 5 vHTS hits, L-glutamic acid-γ-p-nitroanilide—a nonnatural amino acid—is the only compound predicted to form direct bridging interactions with helices αI and αX. The conserved role of these contact sites in receptor 5.24 suggests the existence of naturally occurring high potency ligands that participate in similar ligand-receptor interactions.

Receptor-Based Computational Screening as a Tool for the Discovery of Novel Odorants
Electrophysiological recordings from goldfish olfactory epithelium confirmed that at least four of the compounds identified...
by computational screening demonstrate robust activities as odorants in vivo. These results validate the overall approach of using a receptor-specific model as a starting point in the search for novel odorants. Interestingly, one compound identified in our screen, diaminopimelic acid, is a component of Gram-negative bacterial cell walls and an intermediate in the bacterial biosynthetic pathways for lysine and peptidoglycans (Born and Blanchard, 1999; Work, 1950). Moreover, the two natural stereoisomers of diaminopimelic acid (L and D) can be found in estuaries at concentrations ranging from ~10 to ~100 μM (Jorgensen et al., 2003), well within the sensitivity of the goldfish olfactory system for this chemical. We speculate that, as fish are thought to use metabolites such as amino acids and nucleotides as feeding cues (Hara, 1994), diaminopimelic acid may signal through the olfactory system the presence of bacteria, which presumably would be localized at or near potential food sources. Application of the high-throughput computational screening described here to other olfactory and taste receptors may reveal additional natural products that function as chemosensory cues.

EXPERIMENTAL PROCEDURES

vHTS

Computational protocols were designed for both ligand-based and structure-based screening workflows using the ROC curve method (Triballeau et al., 2005). These protocols were used to screen the 1.6 million compound CAP database (version 2004, Accelrys Inc.) following a computational prefILTERing of the database. Details of the vHTS workflows are described in the Supplemental Data.

Molecular Modeling with Active Compounds

Selected compounds from the vHTS hit lists, as well as structural analogs, were subjected to computational docking in a molecular model of the receptor 5.24 ligand binding domain, as described previously (Bertrand et al., 2002; Luu et al., 2004).

Receptor Activation Assays

For cell-based receptor activation assays, receptor 5.24 was expressed in HEK293 cells using a CMV expression vector (Luu et al., 2004; Specia et al., 1999). Receptor activation was assayed by measuring changes in intracellular calcium using a Flexstation II fluorescence plate reader (Molecular Devices). Details of these procedures can be found in the Supplemental Data.

Electro-olfactogram Recordings

EOG recordings on goldfish olfactory epithelium were carried out essentially as described previously (Irvine and Sorensen, 1993). Odors were applied for 5 s with a minimum interstimulus interval of 3 min to allow the olfactory epithelium to recover full responsiveness. For each fish, a compound was tested 2–3 times and an average response was computed. The per-fish averaged responses were then normalized to the response elicited by a reference standard odorant, 10−5 M L-arginine. Responses to the reference standard were measured at least every 45 min and bracketed with all trials with each test odorant. Normalized responses from 3–4 fish were then averaged, resulting in a mean normalized response for each compound/concentration tested. All stimuli were tested on every fish in this study.

SUPPLEMENTAL DATA

The Supplemental Data include five figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://www.neuron.org/supplemental/S0896-6273(08)00968-9.

ACKNOWLEDGMENTS

This study was supported by grants from the National Institute on Deafness and Other Communication Disorders (J.N.), and the Fédération pour la Recherche sur le Cerveau, the Agence Nationale pour la Recherche, and the Fondation de France Comité Parkinson (F.C.A.). N.T. was the recipient of a CIFRE grant (ref. 738/2002) provided by the Association Nationale pour la Recherche Technique and Accelrys. D.C. was an LSSURP participant supported by an IGERT grant from the NSF (NSF DGE-0653827). We thank Maria Waldhoer and Jennifer Whistler for their invaluable and generous assistance with the Flexstation assays, Haude Levesque for assistance with the EOG recordings, and Delphine Rigault for technical support with the commercial hits.

Accepted: November 10, 2008

Published: December 10, 2008

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