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SCIENCE DIRECT
Pharmacology & Therapeutics 110 (2006) 525 – 532
Pharmacology & Therapeutics
www.elsevier.com/locate/pharmthera

Associate editor: J. Wess

Orphan GPCRs and their ligands

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Abstract

Due to their diversity, G-protein-coupled receptors (GPCRs) are major regulators of intercellular interactions. They exert their actions by being activated by a vast array of natural ligands, referred to in this article as "transmitters". Yet each GPCR is highly selective in its ligand recognition. Traditionally, the transmitters were found first and served to characterize the receptors pharmacologically. Since the end of the 1980s, however, it is the GPCRs that are first to be found because they are identified molecularly by homology screening approaches. But the GPCRs found this way suffer of one drawback, they lack their natural transmitters, they are "orphan" GPCRs. Searching for transmitters of orphan GPCRs has given birth to the reverse pharmacology approach that uses orphan GPCRs as targets to identify their transmitters. The most salient successes of the reverse pharmacology approach were the discoveries of 9 novel neuropeptide families. These have enriched our understanding of several important behavioral responses. But the application of reverse pharmacology has also led to some surprising results that question some basic pharmacological concepts. This review aims at describing the history of the orphan GPCRs and their impact on our understanding of biology.

Keywords: Orphan GPCR; Reverse pharmacology; Transmitters; Neuropeptides

Contents

1. Introduction

The seminal discovery that the β_2-adrenergic receptor and the opsins share a 7-transmembrane domains (TMs) topology (Dixon et al., 1986), created the concept that receptors that

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0163-7258/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2005.10.001

couple to G proteins form a homologous supergene family, thereafter called the G-protein-coupled receptors (GPCRs) (Hall, 1987). The 7 TMs topology has since evolved to include receptors that do not couple to G proteins, and our understanding of receptor activation has been shown to include proteins other than the G proteins (Bockaert & Pin, 1999; Angers et al., 2002; Pierce et al., 2002; Kristiansen, 2004). Yet, one tenet of the basic concept has remained constant over the last 20 years: all the small molecules that have evolved to direct intercellular interactions interact with GPCRs (7TMRs). This positions the GPCRs at the center of signal transmission and endows them with an extraordinary importance in the organism's life and survival.

1.1. The transmitters

The GPCRs are activated by a plethora of "transmitters" (Civelli et al., 2001), the first messengers that are either present in the environment or released from a cell to carry a message to a second one. These act in an endocrine, paracrine, or exocrine fashion to allow the organism to react to particular physiological challenges. Transmitters are mostly small molecules although few of them are larger polypeptides. They include biogenic amines, neuropeptides, chemokines, lipid mediators, nucleotides, amino acid and derivatives, polypeptide hormones, pheromones, olfactory and gustatory molecules, and some other naturally occurring chemicals such as calcium ions and protons.

Traditionally, the GPCRs are expected to exhibit specificity for the transmitters (Goldstein, 1974). This specificity results from evolutionary processes that aim at diversifying the intercellular interactions. A transmitter may be the natural ligand of more than one GPCR, but those then share a higher degree of homology that groups them into a subfamily. Also a GPCR may bind more than one transmitter, but then these transmitters share structural similarities and are often part of the same synthesis pathway, as in the case of the neuropeptides synthesized from the same precursor (Douglass et al., 1984). A classic example for structurally similar ligands binding structurally related receptors are the opioid receptors and the natural opioid peptides. The concept of GPCR specificity has recently been revised and will be discussed in this review.

1.2. The physiological importance of the G-protein-coupled receptors

Because they direct such a broad spectrum of interactions, the GPCRs participate in about every physiological response. Each cell expresses a few dozen different GPCRs, which implies that its homeostasis can be influenced by numerous transmitters. GPCR activation leads to intracellular chemical changes that may affect directly the state of the cells but can also lead to transcriptional regulation, thus making the role of the GPCRs long lasting. Consequently, every function in the organism can be affected by the activities of particular GPCRs.

For example, the cardiovascular, respiratory, muscular, and endocrine functions are directly dependent on the activities of the adrenergic, histaminergic, muscarinic, and hormone GPCRs, respectively. The organ that is possibly most dependent on GPCR activities is the brain, where practically all the GPCRs are expressed and where their activities add a slower but long lasting dimension to the synaptic transmission mechanisms that direct all brain-related responses. In addition, one will remember that most of our senses such as olfaction, taste, and vision depend directly on the activation of specific GPCRs.

A particular GPCR is often expressed in several tissues. It can be found in the periphery and in the CNS. Its roles in these tissues may ultimately be very different although the second messengers that result from its initial activation are probably the same. We only begin now to dissect the role that the activation of a particular GPCR has on the organism. In some cases, we are helped by the studies of some pathological cases (Schöneberg et al., 2004). Furthermore, and increasingly during the past few years, we expect that genome sequence analyses will point at GPCR structural differences (Balasubramanian et al., 2005) that may help explain pathological differences and consequently unravel the roles of particular GPCRs in particular tissues. But ultimately, the discovery of the predominant role(s) of a GPCR awaits the development of corresponding surrogate agonist or antagonist molecules. Only administration of such drugs to human can answer the question of the GPCR function. Fortunately, developing specific surrogate ligands for GPCRs is in the realm of medicinal chemistry. Indeed about half of the drugs presently on the market are targeting GPCRs (Drews, 2000) and studies of their effects has been a driving force in our understanding of the functions of the GPCRs. Yet these are far from covering the entire supergene family, which amount to some 800 receptors in the human genome (Vassilatis et al., 2003).

This review will describe how the GPCRs became such an imposing supergene family and how they continue to spearhead our understanding of particular physiological responses. It will focus on the search for the ligands of the transmitter GPCRs and will proceed from specificity, to diversity, to finally complexity.

2. The orphan G-protein-coupled receptors

Most of the GPCRs started as orphans. Their discoveries stemmed from the concept that GPCRs would belong to a supergene family and thus would share sequence similarities. Homology screening techniques, low stringency hybridization (Bunzow et al., 1988, 1992) soon followed by PCR-derived approaches (Libert et al., 1989), paved the way for the discoveries of new GPCRs, thus that, by the end of the 1980s, it became clear that the number of GPCRs would be large. This was confirmed, at the turn of the century, with the sequence of the genome. Today, one estimates that the number of GPCRs is about 800, of which more than half are olfactory GPCRs (Vassilatis et al., 2003).

The discovery of new GPCRs found by homology screening suffers from one obvious problem, the receptors found lack their pharmacological identities, their natural ligands. They are

all "orphan" receptors (Libert et al., 1991a, 1991b). The pursuit to unravel their identities was thought to be formidable and to many an unresolvable task that would lead to unglamorous fishing expeditions.

2.1. Reverse pharmacology

In the second part of the 1980s, there existed about 50 transmitters, potential GPCR ligands, that had no cloned cognate receptors. Testing all of these for their binding to new GPCRs was seen as an impossible aim in an academic environment. Yet serendipity and ingenious insights, such as these that could spring from the analysis of the new GPCR tissue expression profile, proved to be successful in matching the first orphan GPCRs to known transmitters. The first deorphanized GPCRs, the 5HT-1A and the D2 dopamine receptors, were already reported in 1988 (Fargin et al., 1988; Bunzow et al., 1988). The strategies used were the same, that is, the orphan GPCR was expressed by DNA transfection in eukaryotic cells, membranes of these cells were then used as targets to determine the binding of potential transmitters. This strategy has become to be known as reverse pharmacology (Libert et al., 1991a, 1991b; Mills & Duggan, 1994).

During the first part of the 1990s, application of the reverse pharmacology strategy led to the pharmacological characterization of many GPCRs. These endeavors were mostly carried out in an "artisanal" fashion; the focus was on one particular receptor tested for few potential known ligands (Table 1). At the same time, however, random searches for new GPCRs, using PCR-based homology screening approaches, were also in full swing, thus the overall number of orphan GPCRs was steadily increasing (Marchese et al., 1994, 1998). This led us to conclude that the GPCRs outnumbered the known potential ligands and more importantly that these receptors must bind ligands that have not been thus far characterized because inactive receptors should be evolutionarily discarded (Civelli, 1998). This recognition inspired enough confidence in a few researchers to utilize orphan GPCRs as baits to isolate their natural ligands, which meant to identify novel transmitters.

2.2. The search for novel transmitters

The concept that an orphan receptor could be used as bait to identify a novel transmitter requested the application of two technologies (Civelli, 1998). First, it necessitated the search for novel transmitters in tissue extracts, therefore in complex molecular mixtures. Then it necessitated the monitoring of receptor activation instead of binding. Both requirements implied the application of countless repetitive assays. Fortunately, such assays were existing or being developed for the pharmaceutical industry (Wess et al., 2001).

The cloning of receptors, enzymes, and other targets of pharmaceutical interest had revolutionized drug screening (Drews, 2000). From that time on, drugs could be searched for by the random screening of large libraries of synthetic compounds (Schreiber, 2000). These are tested for the desired biological and in vivo activity and, if active, chemically modified to fulfill the pharmaceutical constraints of expected drugs. The screening of libraries of compounds required high-throughput assays and such assays were developed with the aim of monitoring GPCR reactivity.

The first orphan GPCR that was used for discovering a novel transmitter was ORL-1, cloned through its homology to the opioid receptors (Henderson & McKnight, 1997). Its

Table 1
Dates in the history of GPCR deorphanization

1986	The β2-adrenergic and the rhodopsin receptors share a 7-transmembrane topology. Birth of the GPCR family concept and recognition that homology screening approaches could lead to new GPCRs.
1987	Identification of G-21, the first orphan GPCR using low stringency hybridization.
1988	The first deorphanizations: G-21 is recognized as the 5-HT1A receptor, RGB-2 as the dopamine D2 receptor. Introduction of the reverse pharmacology approach.
1989	Development of the PCR-based homology screening approach.
1990–1995	Orphan GPCRs are identified by homology screening. The "artisanal" era of GPCR deorphanization. The following GPCRs are characterized: Adenosine A1, A2a, A2b, A3; adrenergic a1A, a1d, a2b, a2c, b1, b3; anaphylatoxin C3a, C5a; angiotensin AT1b; bombesin BB1, BB3; cannabinoid CB1, CB2; chemokine CCR1, CCR2, CCR3, CCr4, CCR5, CXCR2, CXCR3, CXCR4; cholecystokinin CCKa; dopamine D1, D2, D3, D4, D5; follicle-stimulating hormone; formyl-peptide FPR2; galanin type 2; gonadotropin-releasing hormone; histamine H2; lysophosphatidic acid; melanocortin MC1, MC2, MC3, MC4, MC5; melatonin ML1a, ML1B; muscarinic acetylcholine M3, M4, M5; neurokinin NK1, NK3; neuropeptide YY1, YY1-like, YY4; neurotensin NTR2; opioid and μ; prostanoid EP1, EP2, EP3, EP4, DP, FP, IP; protease-activated 2,3; purinoceptor P2Y1, P2Y3, P2Y4, P2Y6, P2Y8; serotonin 5-HT1a, 5-HT1b, 5-HT1d, 5-HT1e, 5-HT1f, 5-HT2a, 5-HT2b, 5-HT4, 5-HT5a, 5-HT5b, 5-HT6, 5-HT7; somatostatin SST1, SST2, SST3, SST4, SST5; thyrotropin-stimulating hormone; vasopressin V1b, V2.
1995	Discovery of the first novel natural ligand of an orphan GPCR: nociceptin/orphanin FQ. Demonstration that orphan GPCRs can be used to identify new transmitters.
1996–2005	The "industrial" era of GPCR deorphanization. The following GPCRs are identified: leukotriene B4, C4, D4; latrotoxin; sphingosine 1-phosphate; lysophosphatidic acid; melanin-concentrating hormone; urotensin II; motilin; neuromedin U; UDP-glucose; sphingosylphosphorylcholine; histamine 3; prostaglandin D2; neuropeptide FF, AF; RFamide-related protein 1,3; lysophosphatidylcholine; adenosine diphosphate; psychosine; trace amines; 5-oxo-ETE; bile acids; bovine adrenal medulla peptide 22; relaxin; relaxin-3; bradykinin; pyroglutamylated arginine-phenylalanine-amide peptide; cortistatin; medium and long fatty acids; nicotinic acid; proton; β-alanine; α-ketoglutarate.
1998–2004	Discovery of 8 novel neuropeptide/receptor system: Orexins/hypocretins, prolactin-releasing peptide, apelin, ghrelin, metastatin, neuropeptides B/W, prokineticins 1/2, neuropeptide S.

The dates presented in this table refer to these mentioned in text. The GPCR lists were derived from Marchese et al. (1998) and Saito and Civelli (2005).

activation was monitored by quantifying intracellular decreases in cAMP levels, which could be measured in newly developed scintillation proximity assays. Because phylogenic analyses classified ORL-1 as a peptidergic GPCR and because ORL-1 is expressed in the CNS, peptidergic brain tissue extracts were prepared, purified, and fractionated. Fractions were tested for their abilities to inhibit adenylyl cyclase activity in cells that were stably transfected with ORL-1. A 17-residue long peptide was ultimately isolated, named orphanin FQ or nociceptin (OFQ/N) (Meunier et al., 1995; Reinscheid et al., 1995). Its structural similarities to the opioid peptides immediately attracted considerable attention, yet it has been proven not to bind the opioid receptors (Reinscheid et al., 1998).

The second successful attempt at discovering novel transmitter through orphan GPCRs screened over 50 different orphan GPCRs by measuring their abilities to induce intracellular calcium release when subjected to peptidic extracts. One receptor did respond and led to the characterization of two peptides, the orexins (Oxs) (Sakurai et al., 1998), also identified through an RNA subtraction approach as hypocretins (Hcrts) (de Lecea et al., 1998). This was immediately followed by the discovery of two novel peptides, prolactin-releasing peptide, and apelin as the natural ligands of the orphan GPCRs GPR10 and APJ, respectively (Hinuma et al., 1998; Tatemoto et al., 1998).

These successes proved the validity of high-throughput screening of orphan GPCRs. It is therefore not surprising that the pharmaceutical industry became its major proponent (Hinuma et al., 1999). Consequently, orphan GPCRs began to be screened randomly against large libraries of ligands, setting the stage for the "industrial" period of deorphanization (Wise et al., 2004). These libraries contained all the ligands that had not been matched to any receptor molecules but also many molecules that are known to exist in cells. This led in a few years to the deorphanization of some 40 GPCRs (Saito & Civelli, 2005).

2.3. Physiological roles of the orphan G-protein-coupled receptors

The impact that the deorphanization of GPCRs had and continues to have on our understanding of the organism's function is fundamental. Deorphanizing a GPCR opens the opportunity to combine anatomical studies on the site of synthesis with that on the sites of action of the system and thus to gain a full understanding of the localization of the system. Most often, the sites of the orphan GPCR expression serve as primary indication of the role of the system. The matching of orphan GPCRs to previously known transmitters has permitted such precise anatomical analyses. Genetic ablations of the orphan GPCRs can also help to understand these new receptor systems (Morita et al., 2004; Piao et al., 2004). But the most striking results came from the discoveries of novel peptides. Our understanding of several physiological responses has greatly gained from these discoveries.

Sleep is 1 physiological response that has been impacted by the orphan GPCR research (Civelli, 2005, in press). While GABA, noradrenalin, and histamine were known to regulate sleep, the discoveries of Hcrts/Oxs have proven that neuropeptides have a prominent role. Inactivation of one of the Hcrts/Oxs receptor induces narcolepsy (Chemelli et al., 1999; Lin et al., 1999). More recently, 3 other orphan GPCR systems, the PrRP (Lin et al., 2002), neuropeptide S (Xu et al., 2004a, 2004b), and urotensin II (Huitron-Resendiz et al., 2005) systems have also been shown to regulate some aspects of sleep. Finally, the prokineticin system has been shown to be a major regulator of the circadian rhythm (Cheng et al., 2002).

Another physiological response that has been shown to depend on the activity of orphan GPCRs is feeding (Xu et al., 2004a, 2004b). While this response was understood as relying on the release of several peptides, in particular leptin (Zhang et al., 1994), melanocyte-stimulating hormone (Marks & Cone, 2001), and neuropeptide Y (Gehlert, 1999), it has been shown that the deorphanized GPCR systems of ghrelin (Kojima et al., 1999; Tschop et al., 2000), melanin-concentrating hormone (Shimada et al., 1998; Marsh et al., 2002), and the Oxs/Hcrts (Willie et al., 2001) play important roles in the central regulation of food intake.

Other physiological responses that have been found to be regulated by orphan GPCR systems include anxiety as it relates to stress, which is impacted by the activity of OFQ/N (Jenck et al., 1997; Koster et al., 1999) or NPS (Xu et al., 2004a, 2004b).

It should be emphasized that, due their novelty, our understanding of the function of the novel neuropeptide systems is still at its infancy. Novel systems such as these are initially tested on the basis of receptor localization. Most of these orphan GPCRs are expressed in several parts of the CNS, their activation should affect the behavioral responses related to these CNS centers. Consequently, the effects that administration of the novel neuropeptide has on behavior depend on the assays used. A novel neuropeptide may modulate a behavioral response in a totally new fashion and that may lead to the discovery of a different function for that neuropeptide. The Hcrt/Ox effect on sleep is an example of such discovery (Chemelli et al., 1999; Lin et al., 1999). It is therefore likely that the deorphanized GPCRs may point at new behavioral or physiological responses that will enlarge our understanding of the function of the organism.

3. The surprises of the reverse pharmacology approach

GPCRs have been deorphanized at a rate of 7–8 per year from 1999 until 2004 (Civelli, 2005). This was mostly the result of large-scale random screenings of practically all molecules known to exist in cells. The primary pharmacological constraint of these screening endeavors was that active compounds exhibit affinities to the orphan GPCRs that are defined by the investigators. But there is no definite rule for predicting the affinity constant of a natural ligand at a particular receptor. Biogenic amines, for example, activate their cognate receptors with potencies that are mostly in the micromolar range while most peptides do so in the nanomolar range. Moreover, the level of receptor expression in a transfected cell can affect ligand potency. Consequently, one has to remember

that the reverse pharmacology approach is based on the investigator's set standards and therefore subject to artefacts (i.e., "made by the art").

These screening endeavors took place mainly in pharmaceutical companies. From a pharmaceutical standpoint, finding a compound that can activate a GPCR is the key to opening the door for the drug discovery process (Robas et al., 2003a, 2003b). It is not a prerequisite that this compound is the genuine transmitter. In doing so, many orphan GPCRs were matched to undoubtedly genuine transmitters, but some were matched to only surrogate ligands. For example, the orphan GPCR PUMA-G/HM74 was matched to nicotinic acid (Soga et al., 2003; Tunaru et al., 2003), a success with therapeutic implications, but one that leaves open the door for the search of the natural ligand. What grew from the intense search for natural and surrogate ligands by reverse pharmacology is that the number of potential genuine transmitters wound down. At that point, the technology began to be pushed to its limits and transmitters began to be found that are unexpected. This is further compounded by some recent discoveries showing that some GPCRs are activated by several transmitters that are chemically unrelated (Civelli, 2005, in press).

3.1. The unexpected transmitters

An "unexpected" transmitter could best be defined as a naturally occurring molecule that was not expected to exert its action through a specific receptor. For example, UDP-glucose was found to activate the orphan GPCR KIAA0001 with affinities in the 100-nM range (Chambers et al., 2000). UDP-glucose was known to be a glucosyl donor in the biosynthesis of carbohydrates. Whether it acts as a transmitter has still to be shown. Other surprising examples are succinate and α-ketoglutarate, which are known as citric acid cycle intermediates but were shown to activate the orphan GPCRs, GPR 91 and 99, respectively, with affinities in the 25- to 70-μM range (He et al., 2004). Succinic acid was identified not by random screening of defined ligands but through the purification of kidney extracts. Succinate was known to have a role on the reabsorption of phosphate and glucose in the proximal tubule and to stimulate gluconeogenesis. In GPR91-deficient mice, succinate was unable to induce hypertension, thus demonstrating that its activity relies on GPR91 activation (He et al., 2004). So there is no doubt that succinate is a genuine activator of GPR91, although the question remains whether it serves as a transmitter. Citric acid intermediates are not expected to be secreted in a regulated manner. They may be released upon mechanical stress or cell death, which would infer that some GPCRs are used as monitors of metabolic breakdown or global injury.

3.2. The promiscuous G-protein-coupled receptors

Another issue regarding pharmacological selectivity has arisen from some of the results of the application of reverse pharmacology. Subfamilies of GPCRs usually bind one or several closely related ligands. Three opioid receptors all bind opioid peptides and have evolved their structure to insure that they do not bind OFQ/N (Reinscheid et al., 1998; Meng et al., 1998). Catecholamine receptors are structurally related as are their ligands. Yet the adrenergic and dopaminergic systems are viewed as separate, although it has been shown that adrenaline and noradrenalin can efficiently activate the dopamine D4 receptor (Lanau et al., 1997). But this does not hold for a recently discovered GPCR subfamily, the Mas-related GPCRs (Mrgs or sensory neuron-specific receptors; SNSRs). This is a family of orphan GPCRs that is predominantly expressed in dorsal root ganglions and thus might have a role in nociception. Variable numbers of Mrgs exist in human, rat, and mouse making any attempt at orthologous classification difficult (Zylka et al., 2003). Being part of a subfamily, one could have expected that the Mrgs would bind similar transmitters. Instead, the Mrgs have been paired to a variety of structurally diverse transmitters: RFamide peptides for some mouse Mrgs (Dong et al., 2001); BAM22 (Lembo et al., 2002) and cortistatin (Robas et al., 2003a, 2003b) for 2 human Mrgs; adenine (Bender et al., 2002) for a rat Mrg; and β-alanine (Shinohara et al., 2004) for an Mrg found in human, rat, and mouse. The matched transmitters are specific to particular Mrgs and activate them efficiently. For example, the RFamides or BAM22 peptides have affinities in the low nanomolar range (Lembo et al., 2002; Han et al., 2002), which is the range that is expected for peptides binding to GPCRs. So can it be that there are GPCR subfamilies that have a broad spectrum of transmitters and if so what does it imply for their function? From a physicochemical standpoint, if a molecule contains a motif that permits its interaction with a receptor, this interaction will take place. The issue is whether the receptor and that surrogate ligand will be in a position to interact in vivo. But this cannot be answered by reverse pharmacology.

3.3. The non-selective G-protein-coupled receptors

Finally, when considering the outcomes of reverse pharmacology that go against one of the tenets of pharmacology, one has also to consider the case of a receptor that is not selective in its ligand recognition. Such a receptor is the orphan GPCR GPRC6A. One has come to accept that glutamate, glycine, or GABA receptors do not bind other amino acids. GPRC6A, on the other hand, can be activated by a series of basic L-α-amino acids with only a preference for basic amino acids (Wellendorph et al., 2005). For example, it is activated by L-Arg, with an affinity of about 50 μM. The concentration of L-Arg in the plasma is in the 100-μM range. So, if Arg acts in an endocrine fashion, the receptor would be constantly and strongly activated. In addition, it would be surprising that any metabolic change would result in a high enough increase in the concentration of L-Arg to account for a significant difference in signaling. This suggests that L-Arg and the other GPRC6A ligands act in a paracrine fashion. Furthermore, because GPRC6A belongs to the receptor family that include the calcium sensing receptor (Brown, 1999), it may sense free amino acid concentrations. Such a notion would help explain the promiscuity of GPRC6A in ligand recognition.

4. The impact of the orphan G-protein-coupled receptor research

The search for the ligands of orphan GPCRs has impacted the basic and therapeutic fields. Although matching known transmitters to their respective GPCRs had the most success, it is the discovery of novel transmitters from tissue extracts that has had perhaps the most impact. By the mid-1990s, ~90 transmitters were known; since then, a dozen new transmitters have been found and one expects that the remaining 120 orphan GPCRs will lead to the discovery of at least 50 more transmitters (Civelli et al., 2001). The deorphanization of GPCRs has revolutionized the discovery of novel transmitters and in turn these have revolutionized many fields of biomedical research in which they have been implicated. For example, the novel neuropeptides found as ligands of orphan GPCRs have changed our understanding of the mechanisms that regulate sleep or food intake.

Orphan GPCR research has also dramatically impacted drug discovery. The GPCRs are de facto preferential drug targets. Although, as mentioned above, most of the GPCR deorphanizations that took place until 1995 were achieved in academia, many of those deorphanized GPCRs were already targets of drug development programs. They had been sought using traditional pharmaceutical techniques, often membrane binding assays. The deorphanization successes allowed the pharmaceutical industry to develop defined assays based on cloned receptors. The discovery of OFQ/N showed that orphan GPCRs could open the door to untapped drug targets. Furthermore, this line of research required high-throughput techniques that were in use in the pharmaceutical industry. It is therefore not surprising that the pharmaceutical industry was enthusiastic to espouse the reverse pharmacology approach (Wise et al., 2004). From 1995, the majority of the GPCR deorphanizations have been carried out in pharmaceutical companies, and in this respect, Takeda Chemical Industries should be recognized as the most successful entity at discovering novel transmitters.

So there is no doubt that orphan GPCRs are used as potential drug targets. That there is no marketed drug directed at any of the ones that have been deorphanized since 1995 is of no surprise knowing the length of time required to bring a drug on the market. But are there drugs directed at any of these new deorphanized GPCRs in the pipeline? This is an answer that would, of course, require knowledge of proprietary drug discovery programs. Yet, one can foresee that several orphan GPCRs are being used as targets in drug discovery programs (Table 2). The orphan GPCRs that bind novel neuropeptides represent pharmaceutical targets that may approach therapeutic needs from a totally novel standpoint. They have the potential to not only treat known indications but also to, possibly, define new ones. If pursued, they can lead to drugs with unmatched competitive advantages. They can also lead surprises that could prove of great benefits as, for example, OFQ/N that has been shown to be a potent antitussic agent (McLeod et al., 2001). Deorphanized GPCRs represent also assay targets. The targets listed in Table 1B, for example, were recognized of therapeutic interest but could not be developed for drug screening. It is the deorphanization research that made this aim possible. If one considers that the entire number of biochemical targets that have led to marketed drugs are fewer than 500, one has to recognize that the orphan GPCRs are an outstanding source of novel pharmaceutical targets. Whether drugs will ultimately reach patients can only be hoped.

Table 2
Foreseen impact of orphan GPCR research on drug discovery since 1995

A. Novel transmitter systems with therapeutic implications

OFQ/N	Anxiety
Hcrt/Ox	Narcolepsy/obesity
Ghrelin	Obesity
Prokineticin 2	Sleep
NPS	Arousal/anxiety

B. Assay systems of therapeutic interest

Receptor	Ligand	Indication
SCL-1/GPR24	MCH	Obesity/CNS
GPR14	Urotensin II	High blood pressure
P2Y12	ADP	Thrombosis
CysLT1	LTD4	Asthma
CysLT2	LTC4/LTD4	Asthma
NPGPR	NPFF and NPAF	Pain
TA1	Trace amines	Depression
GPR40	M and L chain fatty acids	Diabetes
HM74	Nicotinic acid	Dyslipidemia

The therapeutic indications are derived from the authors' knowledge of the biology of the different systems. Deorphanized GPCR systems for which the authors do not foresee therapeutic indications of significant market size are not mentioned.

Acknowledgments

This work was supported by NIH grants MH60231, DK63001, DK70619, and the Stanley Medical Research Institute.

References

Angers, S., Salahpour, A., & Bouvier, M. (2002). Dimerization: an emerging concept for G protein-coupled receptor ontogeny and function. *Annu Rev Pharmacol Toxicol 42*, 409–435.

Balasubramanian, S., Xia, Y., Freinkman, E., & Gerstein, M. (2005). Sequence variation in G-protein-coupled receptors: analysis of single nucleotide polymorphisms. *Nucleic Acids Res 33*, 1710–1721.

Bender, E., Buist, A., Jurzak, M., Langlois, X., Baggerman, G., Verhasselt, P., et al. (2002). Characterization of an orphan G protein-coupled receptor localized in the dorsal root ganglia reveals adenine as a signaling molecule. *Proc Natl Acad Sci U S A 99*, 8573–8578.

Bockaert, J., & Pin, J. P. (1999). Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J 18*, 1723–1729.

Brown, E. M. (1999). Physiology and pathophysiology of the extracellular calcium-sensing receptor. *Am J Med 106*, 238–253.

Bunzow, J. R., Van Tol, H. H., Grandy, D. K., Albert, P., Salon, J., Christie, M., et al. (1988). Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature 336*, 783–787.

Bunzow, J. R., Zhou, Q. Y., & Civelli, O. (1992). Cloning of the dopamine receptors: homology approach. *Methods Neurosci vol. 9* (pp. 441–453).

Chambers, J. K., Macdonald, L. E., Sarau, H. M., Ames, R. S., Freeman, K., Foley, J. J., et al. (2000). A G protein-coupled receptor for UDP-glucose. *J Biol Chem 275*, 10767–10771.

Chemelli, R. M., Willie, J. T., Sinton, C. M., Elmquist, J. K., Scammell, T., Lee, C., et al. (1999). Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. *Cell 98*, 437–451.

Cheng, M. Y., Bullock, C. M., Li, C., Lee, A. G., Bermak, J. C., Belluzzi, J., et al. (2002). Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature 417*, 405–410.

Civelli, O. (1998). Functional genomics: the search for novel neurotransmitters and neuropeptides. *FEBS Lett 430*, 55–58.

Civelli, O. (2005). GPCR deorphanizations: the novel, the known and the unexpected transmitters. *Trends Pharmacol Sci 26*, 15–19.

Civelli, O. (in press). Orphan GPCRs in the regulation of sleep and circadian rhythm. *FEBS J*.

Civelli, O., Nothacker, H. P., Saito, Y., Wang, Z., Lin, S. H., & Reinscheid, R. K. (2001). Novel neurotransmitters as natural ligands of orphan G-protein-coupled receptors. *Trends Neurosci 24*, 230–237.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., et al. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc Natl Acad Sci U S A 95*, 322–327.

Dixon, R. A., Kobilka, B. K., Strader, D. J., Benovic, J. L., Dohlman, H. G., Frielle, T., et al. (1986). Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature 321*, 75–79.

Dong, X., Han, S., Zylka, M. J., Simon, M. I., & Anderson, D. J. (2001). A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. *Cell 106*, 619–632.

Douglass, J., Civelli, O., & Herbert, E. (1984). Polyprotein gene expression: generation of diversity of neuroendocrine peptides. *Ann Rev Biochem 53*, 665–715.

Drews, J. (2000). Drug discovery: a historical perspective. *Science 287*, 1960–1964.

Fargin, A., Raymond, J. R., Lohse, M. J., Kobilka, B. K., Caron, M. J., & Lefkowitz, R. J. (1988). The genomic clone G-21 which resembles a beta-adrenergic receptor sequence encodes the 5-HT1A receptor. *Nature 335*, 358–360.

Gehlert, D. R. (1999). Role of hypothalamic neuropeptide Y in feeding and obesity. *Neuropeptides 33*, 329–338.

Goldstein, A. (1974). *Principles of Drug Action: The Basis of Pharmacology*. (2nd ed.). West Sussex & New York: Wiley.

Hall, Z. A. (1987). Three of a kind: the β-adrenergic receptor, the muscarinic acetylcholine receptor, and rhodopsin. *Trends Neurosci 10*, 99–100.

Han, S. K., Dong, X., Hwang, J. I., Zylka, M. J., Anderson, D. J., & Simon, M. I. (2002). Orphan G protein-coupled receptors MrgA1 and MrgC11 are distinctly activated by RF-amide-related peptides through the Gaq/11 pathway. *Proc Natl Acad Sci U S A 99*, 14740–14745.

He, W., Miao, F. J., Lin, D. C., Schwandner, R. T., Wang, Z., Gao, J., et al. (2004). Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. *Nature 429*, 188–193.

Henderson, G., & McKnight, A. T. (1997). The orphan opioid receptor and its endogenous ligand nociceptin/orphanin FQ. *Trends Pharmacol Sci 18*, 293–300.

Hinuma, S., Habata, Y., Fujii, R., Kawamata, Y., Hosoya, M., Fukusumi, S., et al. (1998). A prolactin-releasing peptide in the brain. *Nature 393*, 272–276.

Hinuma, S., Onda, H., & Fujino, M. (1999). The quest for novel bioactive peptides utilizing orphan seven-transmembrane-domain receptors. *J Mol Med 77*, 495–504.

Huitron-Resendiz, S., Kristensen, M. P., Sanchez-Alavez, M., Clark, S. D., Grupke, S. L., Tyler, C., et al. (2005). Urotensin II modulates rapid eye movement sleep through activation of brainstem cholinergic neurons. *J Neurosci 25*, 5465–5474.

Jenck, F., Moreau, J. L., Martin, J. R., Kilpatrick, G. J., Reinscheid, R. K., Monsma, F. J., et al. (1997). Orphanin FQ acts as an anxiolytic to attenuate behavioral responses to stress. *Proc Natl Acad Sci U S A 94*, 14854–14858.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., & Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature 402*, 656–660.

Koster, A., Montkowski, A., Schulz, S., Stube, E. M., Knaudt, K., Jenck, F., et al. (1999). Targeted disruption of the orphanin FQ/nociceptin gene increases stress susceptibility and impairs stress adaptation in mice. *Proc Natl Acad Sci U S A 96*, 10444–10449.

Kristiansen, K. (2004). Molecular mechanisms of ligand binding, signaling, and regulation within the superfamily of G-protein-coupled receptors: molecular modeling and mutagenesis approaches to receptor structure and function. *Pharmacol Ther 103*, 21–80.

Lanau, F., Zenner, M. T., Civelli, O., & Hartman, D. S. (1997). Epinephrine and norepinephrine act as potent agonists at the recombinant human dopamine D_4 receptor. *J Neurochem 68*, 804–812.

Lembo, P. M., Grazzini, E., Groblewski, T., O'Donnell, D., Roy, M. O., Zhang, J., et al. (2002). Proenkephalin A gene products activate a new family of sensory neuron-specific GPCRs. *Nat Neurosci 5*, 201–209.

Libert, F., Parmentier, M., Lefort, A., Dinsart, C., Van Sand, J., Maenhaut, C., et al. (1989). Selective amplification and cloning of four new members of the G protein-coupled receptor family. *Science 244*, 569–572.

Libert, F., Vassart, G., & Parmentier, M. (1991a). Current development in G protein-coupled receptors. *Curr Opin Cell Biol 8*, 218–223.

Libert, F., Schiffmann, S. N., Lefort, A., Parmentier, M., Gerard, C., Dumont, J. E., et al. (1991b). The orphan receptor cDNA RDC7 encodes an A1 adenosine receptor. *EMBO J 10*, 1677–1682.

Lin, L., Faraco, J., Li, R., Kadotani, H., Rogers, W., Lin, X., et al. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell 98*, 365–376.

Lin, S. H., Arai, A. C., Espana, R. A., Berridge, C. W., Leslie, F. M., Huguenard, J. R., et al. (2002). Prolactin-releasing peptide (PrRP) promotes awakening and suppresses absence seizures. *Neuroscience 114*, 229–238.

McLeod, R. L., Parra, L. E., Mutter, J. C., Erickson, C. H., Carey, G. J., Tulshian, D. B., et al. (2001). Nociceptin inhibits cough in the guinea-pig by activation of ORL(1) receptors. *Br J Pharmacol 132*, 1175–1178.

Marchese, A., Docherty, J. M., Nguyen, T., Heiber, M., Cheng, R., Heng, H. H. Q., et al. (1994). Cloning of human genes encoding novel G protein-coupled receptors. *Genomics 23*, 609–618.

Marchese, A., George, S. R., & O'Dowd, B. (1998). Cloning of G protein-coupled receptor genes. In K. R. Lynch (Ed.), *Identification and Expression of G Protein-Coupled Receptors1* (pp. 1–26).

Marks, D. L., & Cone, R. D. (2001). *Central melanocortins and the regulation of weight during acute and chronic disease*. *Recent Prog Horm Res 56*, 359–375.

Marsh, D. J., Weingarth, D. T., Novi, D. E., Chen, H. Y., Trumbauer, M. E., Chen, A. S., et al. (2002). Melanin-concentrating hormone 1 receptor-deficient mice are lean, hyperactive, and hyperphagic and have altered metabolism. *Proc Natl Acad Sci U S A 99*, 3240–3245.

Meng, F., Ueda, Y., Hoversten, M. T., Taylor, L. P., Reinscheid, R. K., Monsma, F. J., et al. (1998). Creating a functional opioid alkaloid binding site in the orphanin FQ receptor through site-directed mutagenesis. *Mol Pharm 53*, 772–777.

Meunier, J. C., Mollereau, C., Toll, L., Suaudeau, C., Moisand, C., Alvinerie, P., et al. (1995). Isolation and structure of the endogenous agonist of opioid receptor-like ORL_1 receptor. *Nature 377*, 532–535.

Mills, A., & Duggan, M. J. (1994). Orphan seven transmembrane domain receptors: reversing pharmacology. *Trends Pharmacol Sci 14*, 394–397.

Morita, H., Mazerbourg, S., Bouley, D. M., Luo, C. W., Kawamura, K., Kuwabara, Y., et al. (2004). Neonatal lethality of LGR5 null mice is associated with ankyloglossia and gastrointestinal distension. *Mol Cell Biol 24*, 9736–9743.

Piao, X., Hill, R. S., Bodell, A., Chang, B. S., Basel-Vanagaite, L., Straussberg, R., et al. (2004). G protein-coupled receptor-dependent development of human frontal cortex. *Science 303*, 2033–2036.

Pierce, K. L., Premont, R. T., & Lefkowitz, R. J. (2002). Seven-transmembrane receptors. *Nat Rev Mol Cell Biol 3*, 639–650.

Reinscheid, R. K., Nothacker, H. P., Bourson, A., Ardati, A., Henningsen, R. A., Bunzow, J. R., et al. (1995). Orphanin FQ: a neuropeptide that activates an opioidlike G protein-coupled receptor. *Science 270*, 792–794.

Reinscheid, R. K., Higelin, J., Henningsten, R. A., Monsma, F. J., & Civelli, O. (1998). Structures that delineate orphanin FQ and dynorphin A pharmacological selectivities. *J Biol Chem 273*, 1490–1495.

Robas, N., Mead, E., & Fidock, M. (2003a). MrgX2 is a high potency cortistatin receptor expressed in dorsal root ganglion. *J Biol Chem 278*, 44400–44404.

Robas, N., O'Reilly, M., Katugampola, S., & Fidock, M. (2003b). Maximizing serendipity: strategies for identifying ligands for orphan G-protein-coupled receptors. *Curr Opin Pharmacol 3*, 121–126.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., et al. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell 92*, 573–585.

Saito, Y., & Civelli, O. (2004). GPCR deorphanizations. R. J. Bradley, R. A. Harris, & P. Jenner *Int Rev Neurol vol. 65* (pp. 180–211).

Schöneberg, T., Schultz, A., Biebermann, H., Hermsdorf, T., Rompler, H., & Sangkuhl, K. (2004). Mutant G protein-coupled receptors as a cause of human diseases. *Pharmacol Ther 104*, 173–206.

Schreiber, S. L. (2000). Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science 287*, 1964–1969.

Shimada, M., Tritos, N. A., Lowell, B. B., Flier, J. S., & Maratos-Flier, E. (1998). Mice lacking melanin-concentrating hormone are hypophagic and lean. *Nature 396*, 670–674.

Shinohara, T., Harada, M., Ogi, K., Maruyama, M., Fujii, R., Tanaka, H., et al. (2004). Identification of a G protein-coupled receptor specifically responsive to beta-alanine. *J Biol Chem 279*, 23559–23564.

Soga, T., Kamohara, M., Takasaki, J., Matsumoto, S., Saito, T., Ohishi, T., et al. (2003). Molecular identification of nicotinic acid receptor. *Biochem Biophys Res Commun 303*, 364–369.

Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M. X., et al. (1998). Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem Biophys Res Commun 251*, 471–476.

Tschop, M., Smiley, D. L., & Heiman, M. L. (2000). Ghrelin induces adiposity in rodents. *Nature 407*, 908–913.

Tunaru, S., Kero, J., Schaub, A., Wufka, C., Blaukat, A., Pfeffer, K., et al. (2003). PUMA-G and HM74 are receptors for nicotinic acid and mediate its anti-lipolytic effect. *Nat Med 9*, 352–355.

Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., et al. (2003). The G protein-coupled receptor repertoires of human and mouse. *Proc Natl Acad Sci U S A 100*, 4903–4908.

Wellendorph, P., Hansen, K. B., Balsgaard, A., Greenwood, J. R., Egebjerg, J., & Brauner-Osborne, H. (2005). Deorphanization of GPRC6A: a promiscuous L-α-amino acid receptor with preference for basic amino acids. *Mol Pharm 67*, 589–597.

Wess, G., Urmann, M., & Sickenberger, B. (2001). Medicinal chemistry: challenges and opportunities. *Angew Chem Int Ed 40*, 3341–3350.

Willie, J. T., Chemelli, R. M., Sinton, C. M., & Yanagisawa, M. (2001). To eat or to sleep? Orexin in the regulation of feeding and wakefulness. *Annu Rev Neurosci 24*, 429–444.

Wise, A., Jupe, S. C., & Rees, S. (2004). The identification of ligands at orphan G-protein coupled receptors. *Annu Rev Pharmacol Toxicol 44*, 43–66.

Xu, Y. L., Jackson, V., & Civelli, O. (2004a). Orphan G protein-coupled receptors in obesity. *Eur J Pharmacol 500*, 243–253.

Xu, Y. L., Reinscheid, R. K., Huitron-Resendiz, S., Clark, S. D., Wang, Z., Lin, S. H., et al. (2004b). Neuropeptide S: a novel neuropeptide promoting arousal and anxiolytic-like effects. *Neuron 43*, 487–497.

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., & Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homolog. *Nature 372*, 425–432.

Zylka, M. J., et al. (2003). Atypical expansion in mice of the sensory neuron-specific Mrg protein-coupled receptor family. *Proc Natl Acad Sci U S A 100*, 10043–10048.