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The current status and prospects of research on orphan G protein-coupled receptor

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Abstract. This review provides an overview of the current status and prospects of research on orphan G protein-coupled receptors (oGPCRs). It begins by discussing the classification and structure of GPCRs and then delves into the methods used to identify and characterize ligands for oGPCRs, including ligand screening strategies, correlation analysis, functional analysis techniques, and interaction evaluations. The review further examines the functionality and de-orphanization research of specific oGPCRs, such as GPR35, GPR55, GPR75, and GPR88. It discusses their basic information, signalling pathways, research on endogenous agonists, and their associations with diseases and physiological activities. Finally, the review concludes with a discussion of future directions and prospects for research on oGPCRs, emphasizing the importance of understanding receptor functionality and signalling pathways for drug development

Keywords: Orphan G Protein-Coupled Receptor, Signal pathway, Ligand.

1. Introduction

G protein-coupled receptors (GPCRs) constitute a broad classification encompassing a substantial cohort of membrane receptors, orchestrating their physiological impacts via G proteins [1]. The native ligands for nearly 210 of these GPCRs have been characterized, while approximately 140 others retain undefined endogenous ligands, thereby denoted as orphan G protein-coupled receptors (oGPCRs). A large number of oGPCRs have not been functionally clarified and so remain of great research value. The discovery of endogenous ligands of oGPCRs can significantly advance the exploration of their physiological roles and pathological implications, thereby offering fresh theoretical underpinnings for investigating the mechanisms underlying associated diseases and fostering drug development efforts targeting these receptors. For examples Doxycycline, a potential drug for cancer therapy, is potentially active against several oGPCRs associated with tumour growth and metastasis [2]. Studies conducted in this domain have garnered considerable scholarly interest.

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2. Orphan G protein-coupled receptors

2.1. Structure of G protein-coupled receptor

Based on the structural attributes of GPCR protein molecules, they can be broadly partitioned into three domains: intracellular region, transmembrane region, and extracellular region. The intracellular domain engages in recognizing G protein signaling molecules and binding with pertinent signaling moieties. The transmembrane domain constitutes the foundational framework of GPCR protein molecules, facilitating signal transduction and ligand binding throughout the reaction cascade. The extracellular domain predominantly assumes the role of recognizing signaling molecules [3].

The extracellular domain of GPCR protein molecules comprises three extracellular loop segments and the N-terminal region. The extracellular component of GPCRs exerts a stabilizing influence on the receptor's spatial conformation, facilitated by the presence of two highly conserved cysteine residues capable of forming a disulfide bond. Additionally, glycosylation frequently occurs on the extracellular loops of GPCRs.

The transmembrane domain of GPCR protein molecules is composed of seven transmembrane helices, categorized into two segments: helices I-IV and helices V-VII. Helices II, III, VI, and VII principally compose the substrate-binding pocket of GPCRs, serving the primary purpose of substrate recognition. Conversely, helix V predominantly engages in substrate molecule binding and the conformational adaptations required for distinct substrates, thereby exerting additional modulation over the receptor's diverse functionalities [4].

The intracellular portion of the GPCR protein molecule encompasses the helical conformation of the eighth segment positioned within the cell, the three-segment intracellular loop domain, and the intracellular segment of the GPCR's carboxy-terminal region [5]. The initial two out of the three intracellular loops are comparatively brief and lack distinct biological functionalities, in contrast to the third intracellular loop, which exhibits greater dimensions. Its principal role involves binding with downstream effector proteins to facilitate signal transduction [4].

2.2. Regulating Pathways

The G protein pathway represents a frequently employed signaling cascade in GPCR-mediated signal transduction. The G protein associated with GPCRs is composed of α, β, and γ subunits. In the absence of ligand stimulation, the α subunit of the G protein remains bound to GDP, maintaining an inactive state. Upon activation of the receptor by a ligand, the G protein undergoes a conformational alteration. GTP replaces GDP on the α subunit, leading to its dissociation from the β and γ subunits. The α subunit transitions into an active state, while the unbound β and γ subunits gain the capacity to activate their respective effectors [6]. Upon activation, the α-subunit engages with the respective enzyme or ion channel, initiating a sequence of cascading reactions that modulate downstream signal transduction pathways, thereby regulating associated physiological processes.

Based on the downstream signals and the similarity of the α-subunits, the α-subunits can be classified into four groups: Gs, Gi/o, Gq/11, and G12/13 [7].

Upon activation of GPCRs coupled to GS, Gs proteins activate intracellular adenylate cyclase (AC), which catalyzes the cyclization of ATP into cyclic AMP (cAMP), thereby regulating cellular metabolism as well as the expression of specific genes. cAMP can be degraded by intracellular phosphodiesterase to 5'-AMP, which terminates the signalling transduction. In contrast, GPCR coupled to Gi/o inhibits cAMP synthesis by inhibiting AC activity upon activation [6].

2.3. Related Diseases

Several orphan GPCRs have undergone investigation and have been linked to particular diseases. Notably, certain oGPCRs have exhibited potential associations with cancer metastasis and progression; for instance, GPR35 has demonstrated a robust association with conditions like lung cancer, while heightened GPR55 expression levels have shown a positive correlation with the malignancy grade of glioblastoma.

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Moreover, it has been demonstrated that orphan GPCRs are implicated in specific neurological disorders. As an illustration, GPR85, which researchers have indicated via animal experiments to play a role in brain size determination, the modulation of animal behavior, and a potential association with schizophrenia, exhibits overexpression, resulting in various cognitive and behavioral abnormalities in individuals [8]. These abnormalities encompass diminished social aptitude and disruptions in motor-sensory coordination. In a separate investigation, GPR85 is identified as an etiological factor in schizophrenia and exerts a negative regulatory influence on hippocampal adult neurogenesis as well as neurogenesis-related processes tied to learning and memory [9].

Certain immune system disorders are also subject to modulation by orphan GPCRs. Evidence suggests that GPR18 is linked to immune cell migration and inflammatory responses, implying a potential role in immune-related diseases [10]. Furthermore, the de-orphanized receptor EDG1 actively participates in immune system regulation, with particular significance in lymphocyte migration and activation processes. EDG1 is expressed on immune cell types like T cells and B cells, where it exerts control over their activation, migration, and circulation by binding to S1P.

3. Ligands of oGPCRs

The identification of endogenous ligands for orphan receptors remains incomplete, constituting a central research challenge. The primary objective in the quest for these ligands is to elucidate the functions and biological roles of these receptors, a pursuit imbued with significant biological and medical implications. oGPCRs are typically pivotal in orchestrating a diverse array of intracellular physiological processes, including but not limited to cellular growth, differentiation, and immune responses. The discovery of their respective ligands holds the potential to shed light on the receptor's role in cellular signaling and physiological regulation. Moreover, the identification of ligands for orphan receptors presents promising avenues for the development of novel drug targets, thereby offering prospects for the creation of innovative therapeutics spanning a broad spectrum of ailments, including cancer, autoimmune disorders, and neurological conditions.

3.1. Workflow

Presently, the most widely employed approach for the discovery of ligands for orphan receptors involves the utilization of an orphan receptor strategy. In this strategy, the orphan receptor serves as a 'bait' to isolate novel ligands and elucidate their functional roles. The fundamental procedure entails the transfection of the cloned orphan receptor gene into vector cells, which subsequently express the corresponding receptor. These transfected cells are then exposed to tissue extracts containing potential natural ligands for the receptor. The interaction between the ligand and the receptor triggers the generation of a second messenger, and alterations in the second messenger within the vector cells are measured as a parameter to monitor the purification of ligands specific to the orphan receptor [11]. High-throughput screening methodologies are employed to evaluate a large number of candidate ligands in the quest for ligands suitable for orphan receptors.

3.2. Several Common Detection Techniques

The primary assay technique presently employed for the identification of endogenous ligands for oGPCRs involves the analysis of variations in intracellular Ca^{2+} concentrations subsequent to phospholipase C (PLC) activation. This method is favored due to its stability, amenability to automated detection, and high throughput capacity. PLC, an enzyme that typically interacts with receptor proteins on cell membranes, becomes activated when the target receptor binds to its ligand, thereby initiating dynamic changes in intracellular Ca^{2+} concentrations. Receptor-ligand interactions are evaluated by monitoring fluctuations in intracellular Ca^{2+} levels to unveil ligands that correspond to oGPCRs. Several commonly employed assays for this purpose are delineated below.

Fluorescence imaging reader method (FLIPR) It is an experimental technique commonly used in bioscience research to monitor and measure the fluorescence emission of cells or biomolecules, is commonly used to study biomolecules within cells and can provide information about their localization;

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Cell visualization It is an experimental technique employed to observe and investigate various aspects of living cells, including their structure, function, behavior, interactions, and more. This method offers an intuitive visual comprehension of specific biological processes occurring within cells;

Jellyfish luminescence is a scientific method that leverages bioluminescent reactions to investigate cellular activity and molecular interactions. This approach relies on the luminescent properties of calcium-sensitive receptor proteins, allowing for the real-time monitoring and visualization of cellular processes;

Using activation of the phospholipase A2 (PLA2)-Ca^{2+} pathway to identify ligand The radioactivity associated with the radioligand bound to orphan G protein-coupled receptors (oGPCRs) was quantified using the arachidonic acid release assay.

4. Functionality and De-Orphanization Research of several oGPCRs

4.1. GPR35

4.1.1. Basic information. G protein-coupled receptor 35 (GPR35) is a class A, rhodopsin-like orphan GPCR that has been implicated in a variety of diseases and physiological activities, but for which no endogenous ligand has been identified.

4.1.2. Signaling pathway. Its signaling pathway is widely distributed, and it can couple with Gαi/o and Gα13 while recruiting β-arrestin [12]. Among these, GPR35/Gαi/o promotes the phosphorylation of c-Jun amino-terminal kinase and extracellular signal-regulated kinase 1/2, thereby participating extensively in physiological activities such as cell migration and immune regulation [13]. It also activates peroxisome proliferators, receptor γ coactivator-1α, and promotes lipid metabolism, thermogenesis, and the expression of anti-inflammatory genes [14]. GPR35/Gα13 impacts actin regulation, cytoskeletal rearrangement, and vascular remodeling through Ras homolog A/Rho kinase signaling. GPR35 can also activate Gαi/o and Gα13 signaling, and it translocates to the outer mitochondrial membrane where it binds to ATPase inhibitory factor 1, thus preventing ATP loss during ischemia [15].

4.1.3. Research related to endogenous agonists. Agonists are molecules that induce receptor activation by binding to the receptor, such as KA(Kynurenic Acid), lysophosphatidic acids (LPA), chemokine 17 (CXCL17), 5-hydroxyindole acetic acid (5-HIAA) and the ethyl acetate extract of thyme, which are prominent among the endogenous substances [12, 16]. These endogenous substances are currently being investigated as potential agonists for GPR35. Nonetheless, these candidates are not without issues that have prompted debates, thereby complicating the precise identification of the authentic endogenous agonist.

The debates regarding KA as the primary endogenous agonist of GPR35 stem from several factors: (1) To elicit GPR35 activation in vitro, KA concentrations often need to be elevated to the millimolar range, whereas human plasma levels of KA typically remain within the nanomolar range. Nevertheless, during inflammatory or pathological conditions, local KA concentrations can rise to millimolar levels. (2) KA also triggers the activation of other receptors such as NMDAR, α7nAChR, and AhR. Its in vivo effects involve multiple targets and intricate regulatory networks, posing challenges in dissecting its specific association with GPR35. (3) KA exhibits notable selectivity for rodents, with its potency for mouse and rat GPR35 being 40-100 times higher than for human GPR35. This rodent selectivity complicates the confirmation of its precise mechanism [12].

Lysophosphatidic aid (LPA) and chemokine 17 are also frequently investigated as potential endogenous agonists of GPR35. However, it is noteworthy that LPA functions as a biased ligand for GPR35. Specifically, LPA activates Gαq signaling pathways downstream of GPR35, while not affecting Gαi/o, Gα13, or β-arrestin signaling. Consequently, LPA does not induce GPR35 activation in standard GPR35 ligand screening assays [17].

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In vitro GPR35 ligand screening assays have indicated agonist activity for the second messenger cGMP and certain tyrosine metabolites. However, these ligand screening assays did not encompass GPR35-related signaling mechanisms or functionalities, and their findings have not received validation from other investigations [18].

4.1.4. GPR35 is associated with disease. GPR35 is inflammatory bowel disease (inflammatory bowel disease, IBD) risk of genes, and ulcerative colitis (ulcerativecolitis, UC) and Crohn's disease (Crohns disease, CD) and so on. GPR35 plays a protective role in the intestinal tract through various signaling mechanisms: Tsukahara et al. found that PA/GPR35 promotes fibronectin, integrin-5 expression and ERK1/2 phosphorylation, thus promoting colonic mucosal repair [12]. Some research found CX3CR + macrophages of LPA/GPR35 promoting TNF alpha, aldosterone hydroxylase 11 members of the family of the cytochrome P450 b1 (cytochromeP4501b1 Cyp11b1) expression, thus maintaining the steady state [19]. Sun et al. found that KA/GPR35 inhibits the over-activation of NLRP3 inflammasome and reduces the secretion of IL-1β when stimulated by lipopolysaccharides (LPS) [20].

GPR35 is associated with colorectal cancer (CRC), gastric cancer, breast cancer and other cancers, and the data from The Cancer Genome Atlas (TCGA) and The Genote-Tissue Expression (GTEx) databases were found to be positive Compared with normal tissues,GPR35 expression was upregulated in colorectal, gastric, and pancreatic cancers, but unchanged in ovarian and breast cancers. More importantly, clinical data show that GPR35 overexpression is associated with poor prognosis in patients with colorectal cancer, non-small cell lung cancer, and gastric cancer, while GPR35 expression level is associated with tumor differentiation, stage, and metastasis in breast cancer and gastric cancer [12]. Meanwhile, some research shows that the Neuroglobin plays as tumor suppressor by disrupting the stability of GPR35 in colorectal cancer [21].

The components of GPR35 related signaling pathways and their functional roles in various organs and tissues have been obtained. The gradual identification and identification of agonists meet the need for further exploration of the function of GPR35. Further studies are needed to further understand the mechanism of action between agonists such as KA and lysophosphatidic acid and GPR35, which will lay a foundation for the development of its targeted drugs.

4.2. GPR55

4.2.1. Basic information. GPR55 is classified as a member of the class A retinoid-like receptors. Lysophosphatidylinositol (LPI) serves as a potent agonist for GPR55. Furthermore, GPR55 exhibits specific binding affinity for certain cannabinoid ligands, leading some researchers to propose its categorization as a cannabinoid type 3 receptor. However, numerous unresolved issues exist.

4.2.2. Signaling pathway. The physiological roles of GPR55 are currently not well understood. However, it is evident that GPR55 exhibits widespread distribution and is implicated in various physiological activities through its associated signaling pathways. Notably, GPR55 has been shown to couple with G12/13 and Gq proteins, thereby initiating a sequence of downstream signaling cascades [22].

4.2.3. Function. Its functionalities are multifaceted, and existing research has demonstrated its potential as a novel anti-diabetic target that could have favorable impacts on β-cell function and quality. Furthermore, empirical evidence indicates that the LPI/GPR55 axis contributes to pro-carcinogenic processes encompassing cell proliferation, differentiation, migration, invasion, and metastasis. This involvement is facilitated through G12/13 and Gq signaling pathways and is altered in various cancer cell types. Additionally, both GPR55 itself and its bioactive lipids are being explored as potential biomarkers for cancer diagnosis. Notably, an association has been established between elevated or heightened GPR55 expression and the aggressiveness of specific tumors, including acute myeloid leukemia, uveal melanoma, low-grade gliomas, and renal cancer. In the context of studies related to depression, investigations have revealed that the down-regulation of GPR55 expression in the

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hippocampus might mediate depression and anxiety-like phenotypes induced by chronic social defeat stress (CSDS). Conversely, the activation and up-regulation of GPR55 could potentially yield anti-inflammatory and subsequent neuroprotective effects, offering a prospective avenue for depression treatment. Remarkably, the GPR55 agonist O-1602 has shown significant efficacy in mitigating CSDS-induced depression and anxiety-like behaviors, along with the amelioration of neuroinflammatory and neurogenic deficits in the hippocampus [23].

4.2.4. Research related to endogenous agonists. Lysophosphatidylinositol (LPI) exerts potent agonist activity on GPR55. It also exhibits specific binding affinity to certain cannabinoid ligands, prompting some researchers to consider it as a potential ligand for the cannabinoid type 3 receptor. However, this viewpoint is accompanied by unresolved issues. Notably, GPR55 lacks homology to the pro-CB1 and CB2 (cannabinoid receptor 1 and 2) receptors, and traditional cannabis binding sites are not identified [24]. The question of whether cannabis acts as an antagonist or agonist remains complex. Research has indicated that endogenous cannabinoids can function as both antagonists and activators of GPR55. Among these, analogs of cannabidiol have been identified as potent agonists of the GPR55 cannabinoid receptor, demonstrating EC50 values of 13, >30,000, and >30,000 nM for GPR55, CB1, and CB2 receptors, respectively. In vitro studies have revealed that this compound inhibits mouse osteoclast formation, stimulates osteoclast polarization and resorption, triggers Rho and ERK1/2 activation, and impedes the migration of human neutrophils.

Furthermore, a relevant investigation published in the journal “Nature” demonstrated that curcumin activates GPR55, leading to the initiation of serum response element and serum response factor-mediated transcription. This activation is subsequently restrained by Rho kinase and GPR55 antagonists. Both the methoxy and heptadienone functionalities of curcumin are essential for GPR55 activation, with residue F190^5.47 within the GPR55 structure playing a significant role in curcumin interaction. Notably, GPR55 antagonists hinder the curcumin-induced secretion of glucagon-like peptide-1 in GLUTag cells [23].

4.2.5. GPR55 and cancer. Many GPCRS have been shown to be associated with the pathogenesis of various cancers, including promoting cancer cell proliferation, evading immunity, inducing angiogenesis, and promoting invasion and metastasis. Studies have confirmed that the endogenous ligand LPI of GPR55 can promote cell mitosis [25]. Proto-oncogene Ras induces malignant transformation of epithelial thyroid cells and fibroblasts, resulting in the synthesis and release of LPI, and LPI, as a mitogen of these cells, can promote the proliferation of malignant cells, suggesting that LPI is involved in the occurrence and development of RAS-dependent tumors [26].

4.2.6. Summary of GPR55 features. Current studies have confirmed that LPI is the endogenous natural ligand of GPR55, and due to GPR55’s sensitivity to cannabinoids, GPR55 can explain some mechanisms that are not mediated by traditional cannabinoid receptors CB1 and CB2. Therefore, the physiological and pathological states related to LPI, including cell proliferation and differentiation, cancer, cardiovascular diseases, etc., as well as the physiological and pathological states related to cannabinoids, including inflammation and gastrointestinal diseases, are of great significance for further study of GPR55.

4.3. GPR75

4.3.1. Basic Information and Functions. GPR75 is categorized as a class A retinoid-like Gαq protein-coupled receptor. Recent investigations have unveiled the interactions between GPR75 and ligands such as CCL5 and 20-HETE [27]. On the a GPR75 is categorized as a class A retinoid-like Gαq protein-coupled receptor. Recent investigations have unveiled the interactions between GPR75 and ligands such as CCL5 and 20-HETE . On the anatomical scale, GPR75 exhibits a widespread expression across various tissue systems. Within the central nervous system, GPR75 is detectable in distinct brain regions,

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including the retina, cerebral cortex, thalamus, and hippocampus. Within the cardiovascular system, GPR75 manifests its presence in tissues such as vascular smooth muscle cells and the heart. Moreover, GPR75 expression has been identified in tissues like pancreatic islets and the prostate.natomical scale, GPR75 exhibits a widespread expression across various tissue systems. Within the central nervous system, GPR75 is detectable in distinct brain regions, including the retina, cerebral cortex, thalamus, and hippocampus. Within the cardiovascular system, GPR75 manifests its presence in tissues such as vascular smooth muscle cells and the heart. Moreover, GPR75 expression has been identified in tissues like pancreatic islets and the prostate.

4.3.2. Research related to endogenous agonists. CCL5 is classified as an inflammatory chemokine belonging to the CC class subfamily. It binds to CC class chemokine receptors such as CCR1, CCR3, and CCR5. It has been suggested that CCL5 may potentially interact with the orphan receptor GPR75. Studies conducted on cells overexpressing GPR75 and those endogenously expressing GPR75 have indicated that CCL5 elicits intracellular IP3 accumulation, raises Ca2+ levels, and triggers the activation of protein kinase B (PKB/AKT) and mitogen-activated protein kinase (MAPK) signalling pathways [27]. Interestingly, some investigations have indicated lower expression levels of CCR1, CCR3, and CCR5—receptors for CCL5—while GPR75 exhibited significant expression. Silencing GPR75 expression effectively obstructed the Ca2+ increase and pro-insulin secretion induced by CCL5. Furthermore, knockdown of GPR75 expression impeded the CCL5-induced phosphorylation of AKT, GSK3β, and ERK. However, there exists some controversy regarding the potential interaction between CCL5 and GPR75, and no direct binding or interaction between CCL5 and GPR75 has been definitively established.

20-HETE, a vasoactive metabolite derived from the metabolism of arachidonic acid catalyzed by the CYP4A/4F enzymes within the cytochrome P450 enzyme family, has been identified as having significant physiological impact. A study conducted in 2017 unveiled the expression of GPR75 within the vascular system and disclosed the activation and binding of 20-HETE to this receptor. The investigation further confirmed that, in vascular smooth muscle cells, 20-HETE engenders vascular endothelial dysfunction and stimulates smooth muscle cell contraction via a GPR75-dependent mechanism. More recent inquiries have illuminated the modulation of the interaction between 20-HETE and GPR75 by CCL5. Notably, treatment with CCL5 substantially suppressed the 20-HETE-triggered escalation of intracellular Ca2+ concentration and IP-1 accumulation, concurrently thwarting the recruitment of β-arrestin initiated by 20-HETE. These findings strongly suggest distinct binding sites for GPR75 in 20-HETE and CCL5, whereby CCL5 appears to act as a competitive inhibitor by binding GPR75 as a low-affinity ligand, thereby impeding the activation of GPR75 by 20-HETE.

4.3.3. GPR75 and Cancer. Studies have shown that 20-HETE can activate GPR75 and affect signaling pathways in prostate cancer cells [28]. GPR75 can enhance the phosphorylation of EGFR, NFκB, AKT, and p38 through treatment with 20-HETE. On the other hand, CCL5 may act as a competitive inhibitor of 20-HETE by binding to GPR75. These findings indicate that GPR75 and the 20-HETE/GPR75 signaling pathway could have implications in cancer development. Further research is needed to explore the precise involvement of GPR75 in cancer and its potential as a therapeutic target.

4.3.4. Summary of GPR75. In summary, GPR75 is a class A retinoid-like Gαq protein-coupled receptor that exhibits widespread expression in various tissue systems, including the central nervous system, cardiovascular system, pancreatic islets, and prostate. Studies have identified potential interactions between GPR75 and ligands such as CCL5 and 20-HETE. CCL5, an inflammatory chemokine, has been suggested to bind to GPR75, although the evidence for direct binding is inconclusive. 20-HETE, a vasoactive metabolite, has been found to activate GPR75 and impact signaling pathways. These findings indicate potential involvement of GPR75 in various diseases, including cancer, where 20-HETE/GPR75 signaling may play a role. Further research is needed to better understand the exact functions of GPR75 and its potential as a therapeutic target in cancer and other conditions.

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4.4. GPR88

4.4.1. Basic Information and Functions. GPR88 is categorized as a class A G protein-coupled receptor. Its protein expression is detectable in the striatum during early embryonic development and gradually increases as development progresses in the striatum, olfactory bulb, nucleus ambiguous, amygdala, and neocortex, maintaining this pattern into adulthood. However, GPR88 expression is notably discrete in the spinal cord, pons, and medulla. Moreover, GPR88 exhibits transiently elevated expression in certain peripheral tissues, such as the adrenal cortex and cochlear ganglia, while being relatively less expressed in tissues like the retina and spleen.

4.4.2. Research related to endogenous agonists. 2-PCCA represents the first potent small molecule agonist for GPR88 [29]. In cells expressing GPR88, it exerts a GPR88-mediated concentration-dependent inhibition of isoprenaline-stimulated cAMP accumulation. It operates through Gi coupling and does not trigger calcium mobilisation. Investigations have demonstrated the adaptability of the aniline portion of 2-PCCA to various modified sites. In experimental settings, 2-PCCA diminishes locomotor activity in rats and exhibits the potential to mitigate methamphetamine-induced hyperactivity. However, it does not demonstrate the ability to counteract methamphetamine's behavioral effects, encompassing alterations in methamphetamine-induced hyperactivity and discriminative stimulus effects. In summary, 2-PCCA establishes a foundational platform for further investigations and the refinement of GPR88's in vivo functionality.

2-AMPP has also demonstrated agonistic properties towards GPR88 [29]. Through the design of a series of derivative compounds, researchers have identified that structural modifications to 2-AMPP can lead to enhanced activity. Specifically, replacement of the hydroxyl group at position A results in a twofold increase in activity, and the presence of the phenyl group at position B is pivotal for its activity. These discoveries hold significance for refining the model and facilitating deeper exploration into the structural characteristics and specificity of the GPR88 receptor.

4.4.3. GPR88 and related disorders. GPR88 has been implicated in various physiological and pathological processes, including hypertension, alcohol use disorder, and anxiety. In the context of hypertension, GPR88 expression is lower in the aorta but higher in the heart. This differential expression suggests that GPR88 may play a role in the occurrence and development of hypertension, potentially through its influence on abnormal heart contraction and renin secretion, which can increase blood pressure.

In alcohol use disorder, studies with GPR88 knockout mice have demonstrated enhanced seeking and consumption behavior for alcohol, indicating that GPR88 may regulate alcohol consumption [30]. The functional changes of GPR88 in the brain reward system and decision-making processes may affect the reward effect of alcohol and consumption control in individuals with alcohol use disorder.

Regarding anxiety, GPR88 is expressed in brain regions closely linked to anxiety, such as the central amygdala, ventral striatum, and terminal nucleus of the striatum. GPR88 knockout mice have shown decreased anxiety levels, suggesting that GPR88 may play a role in regulating anxiety-like behavior. The mechanism of action for GPR88 in anxiety may involve the modulation of neurotransmitters like dopamine and GABA.

4.4.4. Summary of GPR88. GPR88 is a class C G protein-coupled receptor expressed in various brain regions and transiently in certain peripheral tissues. It has agonists called 2-PCCA and 2-AMPP. GPR88 has been implicated in hypertension, alcohol use disorder, and anxiety. It may play a role in abnormal heart contraction and renin secretion in hypertension. In alcohol use disorder, GPR88 knockout mice show increased alcohol consumption, while its knockout decreases anxiety levels. Further research is needed to explore the mechanisms and develop targeted therapies.

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5. Conclusion

The oGPCRs possess a broad spectrum of clinical and medicinal implications. Investigating their native agonists and delineating their signaling pathways across diverse systems holds the potential to revolutionize drug discovery and advancement. Leveraging recent advancements in biotechnology, structural biology, and receptor pharmacology has enabled us to elucidate the ligand-receptor interactions and signaling cascades of certain receptors. However, the current process of de-orphaning is relatively slow, and higher throughput de-orphaning methods need to be developed. In addition, it is difficult to establish a systematic theory of the signaling pathway. In the forthcoming research on oGPCRs, a focal point should be the interplay between receptor functionality and signaling pathways, thus furnishing a theoretical foundation for drug development.

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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