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SUPER-CONSERVED RECEPTORS EXPRESSED IN THE BRAIN: BIOLOGY AND MEDICINAL CHEMISTRY EFFORTS

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Keywords: autism; GPR173; GPR27; GPR85; G protein-coupled receptor; schizophrenia; SREB family; super-conserved receptors

Abstract

The super-conserved receptors expressed in the brain (SREB) constitute a family of orphan G protein-coupled receptors that include GPR27 (SREB1), GPR85 (SREB2) and GPR173 (SREB3). Their sequences are highly conserved in vertebrates, and they are almost exclusively expressed in the central nervous system. This family of receptors has attracted much attention due to their putative physiological functions and their potential as novel drug targets. The SREB family has been postulated to play important roles in a wide range of different diseases, including pancreatic β-cell insulin secretion and regulation, schizophrenia, autism and atherosclerosis. This review intends to provide a comprehensive overview of the SREB family and its recent advances in biology and medicinal chemistry.

Plain language summary : In recent years, the super-conserved receptors expressed in the brain called GPR27, GPR85 and GPR173 have attracted much interest in the field of medicinal science. They have one important feature in common: they are all almost entirely found in the brain. Researchers have investigated their functions in the body in various animal models, as well as their utility in future drug development. GPR27 has been found to be involved in insulin and blood sugar processes in the body and therefore may be important for diabetes treatment. GPR85 is thought to be linked to brain diseases such as schizophrenia and autism. GPR173 is linked to many different illnesses, including atherosclerosis (the buildup of fats, cholesterol and other substances in arteries) and Type 2 diabetes.

G protein-coupled receptors (GPCRs) are a large family of cell-surface proteins that bind to a range of ligands, including neurotransmitters, peptides and lipids, to activate a wide range of essential physiological signaling processes. Due to their varied structure, ligands and expression, they often serve a vital function in coordinating biochemical processes. They are expressed in a range of different tissues, with approximately 90% of their nonsensory family members being expressed in the brain [1–3]. Their signaling is generally coupled with the activation of G proteins and subsequent induction of

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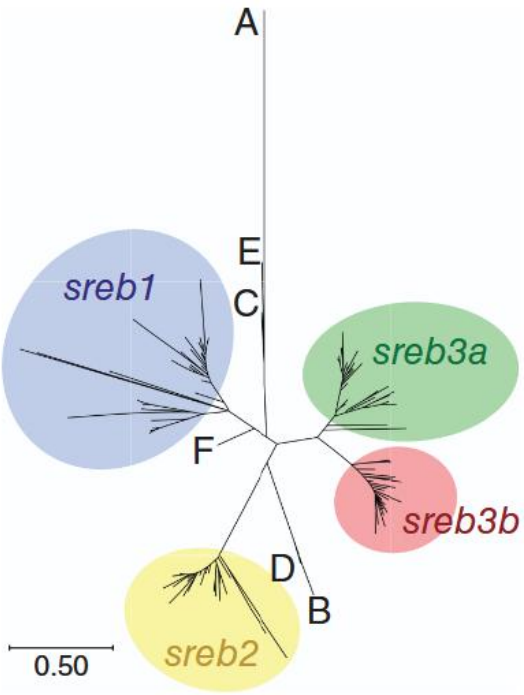
intracellular responses [4,5]. These characteristics, as well as their importance in physiology, made them one of the most heavily studied drug targets [4,6]. Of the drug therapies currently approved by the US FDA, 34% target 103 different GPCRs [6,7]. With 826 members, including 350 druggable non-olfactory receptors, they form the largest group of cell-membrane receptors [8,9]. However, only 165 of them are validated drug targets, leaving many still classified as "orphan receptors," meaning that they don't have known endogenous ligands and have a poorly understood function [4,8–11]. Therefore, they might be the solution to certain well-known medication side effects or drug responses with unknown mechanisms of action. Thus, the need for further research is a matter of importance.

A similarity search using the dopamine D4 receptor sequence recently revealed a unique orphan GPCR subfamily called super-conserved receptors expressed in the brain (SREB). This family consists of three members: SREB1(GPR27), SREB2 (GPR85) and SREB3 (GPR173) [4]. Studies from Breton et al. have also identified a novel fish-specific SREB family member, SREB3b [4]. Therefore, SREB sequences can be clustered into four groups using phylogenetic analysis [4]: SREB1, SREB2, SREB3A and SREB3B (Figure 1).

Figure 1. Phylogenetic tree of SREB gene sequences from verified fish species using Ensembl genome browser 99. Major SREB genes are grouped into four colored clusters (blue, yellow, green, red) and "SREB-like" sequences, which could not be grouped (A–F). Figure adapted from [4] with permission.



The SREB family's extraordinary sequence conservation throughout vertebrate evolution and its broad expression in brain areas with high levels of plasticity, such as the hippocampal dentate gyrus, are noteworthy features [4,12]. The high evolutionary conservation of SREBs in the vertebrate CNS shows their importance [8,13,14], even though their functions are unclear. Interestingly, gonadal SREB genes, specifically SREB1 and SREB2, have been found in non-mammalian vertebrates [4,12,15]. A large similarity in gonad-specific patterns across teleosts for all SREBrelated genes could suggest an important role for SREB genes in testicular development in mammals [4]. Despite their high conservation, SREB orthologs are not encoded in the genomes of Caenorhabditis elegans and

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Drosophila melanogaster [12]. This shows that the SREB family developed after vertebrates diverged from the invertebrate lineage [12].

This review discusses the SREB family, its pharmacological importance and medicinal chemistry approaches.

GPR27 (SREB1)

Among the SREB family, GPR27 (SREB1) has shown special importance, as it exhibits the broadest tissue distribution in mammals and, as recent studies suggest, in different important physiological processes [4,7,8,12,16– 19]. These include modulation of pancreatic β-cell insulin gene transcription and insulin secretion identified by investigating the depletion of GPR27 in the murine cell line MIN-6 and in GPR27 whole-body knockout (KO) mice [7,8,17]. The observed decrease in insulin mRNA level in GPR27 KO mice suggests that GPR27 may play a role in glucose homeostasis. However, the KO mice showed just minor differences in glucose tolerance in comparison with their wild-type littermates [7,8,17]. The function of GPR27 has also been studied in nonmammalian vertebrates, such as in the zebrafish (Danio rerio), which confirmed the involvement of GPR27 in glucose homeostasis [4,20]. Insulin signaling and lipid metabolism seem to be influenced by GPR27 as well [8,20]. However, the endogenous ligand of GPR27 is still unknown. In addition, GPR27 agonists are still scarce and therefore the (patho-)physiological function of GPR27 remains to be explored [4,7,8,21–23].

Research on the fish species *D. rerio* and *D. nigroviridis* enabled a deeper insight into the protein structure of the SREB family [4]. It was found that SREB proteins share the typical characteristics of the GPCRs: an N-terminal extracellular region, a C-terminal intracellular region with an α-helix and seven more α-helical transmembrane (TM) domains. Also, three highly conserved motifs could be identified [4].

GPR27 & GLUCOSE HOMEOSTASIS

The analysis of whole-body GPR27 KO zebrafish in 2020 by Nath *et al.* showed that the loss of GPR27 potentiated the effects of pharmacological and nutritional perturbations that increase glucose levels [20]. Basal glucose levels in GPR27 KO zebrafish remained unchanged [20]. On the contrary, the loss of GPR27 resulted in an increase in medium-chain acylcarnitines, which are known to be linked to insulin resistance in humans [20]. Expression of the CPT1 gene was also found to be elevated, suggesting a connection between GPR27 and the carnitine– shuttle complex, which controls the rate of beta-oxidation of long-chain fatty acids [20]. The GPR27 KO zebrafish also showed resistance to the glucose-lowering effect of exogenous insulin, while metformin, an anti-diabetic drug, showed effects on the KO larvae [20]. Akt-dependent insulin signaling was likewise affected by the loss of GPR27 [20]. The insulin-dependent Akt phosphorylation was abrogated in the KO zebrafish, leading to the suggestion that insulin insensitivity is linked to the loss of GPR27 [20]. Subsequently, Chopra *et al.* investigated the role of GPR27 in glucose homeostasis. They investigated the connection between GPR27 and diabetes *in vivo* by studying wholebody GPR27 KO mice [7]. While the islets of GPR27 KO mice had a lower insulin mRNA expression, the insulin at the protein level remained unchanged [7]. The GPR27

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KO resulted in a reduction of insulin mRNA by ~30% in the islets. These data confirmed previous *in vivo* findings, by the same team, that identified reduced insulin mRNA levels as a consequence of GPR27 depletion in MIN-6 [17]. Those authors hypothesized that this phenotype in GPR27 KO mice is caused by unidentified endogenous ligands, which would remain inactive in the KO mice due to the loss of GPR27. GPR27's basal activity was postulated but then disproven by Lu *et al.*; therefore, it may be ruled out [24]. Also, while the levels of GIIS3, PAX6 and HNF4A remained unchanged, the levels of PDX1 were reduced by ~30%, similar to insulin mRNA [7]. PDX1 (insulin promoter factor 1) is a transcription factor responsible for islet β-cell development, the formation of all pancreatic cell types and adult islet β-cell function [25]. Therefore, it is presumed that the reduction of insulin transcript could be linked to the reduction of mRNA levels of PDX1.

Additionally, the expression of other protein family members of the SREB family, GPR85 and GPR173, was measured. GPR173 was not detectable above background and GPR85 expression remained unaffected in islets of GPR27 KO mice [7].

Notably, the KO of GPR27 also resulted in the mice having reduced body mass, modest worsening of glucose intolerance and decreased plasma insulin levels [7]. In comparison with their wild-type littermates, GPR27 KO mice showed a reduced body mass of 10% at 12 weeks of age [7]. On the other hand, isolated islets from GPR27 KO mice did not display a reduction of total insulin protein concentration, despite insulin mRNA being reduced [7].

Analysis of blood glucose levels confirmed a decline of glucose tolerance in the GPR27 KO mice [7]. This was also proven by measurements of plasma insulin levels after glucose challenge [7]. The reduction of insulin secretion in GPR27 KO mice implies that GPR27 might signal to enhance insulin secretion. This would suggest either Gαs or Gαq coupling [7,26]. Interestingly, the distribution of islet sizes, islet morphology and β-cell mass remained largely unchanged [7].

To observe and verify glucose intolerance, GPR27 KO mice and wild-type littermate controls from 3 to 12 weeks of age were put on a high-fat diet to challenge the mice with increased insulin resistance. The body weights of KO mice and controls were normal, but the glucose tolerance was significantly reduced in GPR27 KO mice [7].

The expression of neighboring genes such as EIF4E3 likewise showed a reduction of mRNA levels in GPR27 KO islets but not in GPR27 KO cerebral cortex [7]. This could be linked to observed splicing events between the antisense strand of the GRP27 exon and EIF4E3 gene, which led to the suggestion that the deletion of the GPR27 exon unintentionally simultaneously caused the removal of the first exon of a subset of EIF4E3 transcripts [7].

To explain the modest worsening of glucose tolerance in GPR27 KO mice, data on EIF4E3 KO mice from the International Mouse Phenotyping Consortium were examined. EIF4E3 KO mice did not show a change in glucose tolerance but, rather, showed a reduction of body mass, which could explain this effect on GPR27 KO mice [7,27]. In islets, but not in the cerebral cortex, EIF4E3 mRNA transcripts were reduced [7]. One explanation could be the existence of EIF4E3 transcriptional start sites on the antisense strand of the GPR27 coding exon [7]. It was found that in the human Refseq annotation there are similar GPR27/EIF4E3 overlapping transcripts, which may be more specific to the islets in mice [7]. As a result, future studies on the GPR27 exon should be approached with caution, as it may have an

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impact on EIF4E3 [7]. Still, GPR27 silencing by RNA interference showed that the mentioned insulin reduction was not dependent on EIF4E3 transcription, since it was not affected and KO mice still showed reduced insulin levels and reduced PDX1 mRNA. Also, EIF4E3 KO mice showed no alteration of their glucose tolerance [7,28].

The signaling of GPR27 remains elusive. Different studies based on GPR27 constitutive activity suggested it could be coupled to $G_{\alpha s}$, $G_{\alpha q}$ or $G_{\alpha i}$ [7,21]. Using a pharmacological approach based on surrogate ligands for GPR27 (see below), the authors of the present study were not able to confirm the ability of GPR27 to couple to G proteins [8]. Other investigations aiming at measuring the spontaneous formation of the GPR27–G protein complex failed to identify any basal activity of the receptor [24]. GPR27 could also signal with plasmalogens, but this hypothesis has not been confirmed yet [7,29].

GPR27 ADVANCES IN MEDICINAL CHEMISTRY

In the search for small molecule agonists for GPR27, Hanson's group conducted a screening campaign using an in-house assay based on the recruitment of β-arrestin-2 by a chimeric version of GPR27 called GPR27V2 [23]. The vasopressin V2 receptor C-terminal tail is included in this design to improve the sensitivity of the test, which is a common method for detecting GPCR ligands. This resulted in a series of molecules with GPR27 agonistic activity (see Figure 2) [23].

Molecules **I** (5128535) and **II** (5217941) with sulfonamide core structure were identified as the most potent GPR27 agonists among all.

When compared with similar constructions for the other SREBs, GPR85V2 and GPR173V2, the agonists showed selectivity for GPR27V2 [7,23]. Having these two lead GPR27 agonists, the authors recently reported an extensive structure–activity relationship [8]. Additionally, a recent report revealed that the activation of GPR27 by compound **I** in 3T3 wild-type embryonic cells resulted in an elevation of cytosolic levels of L-lactate, thus linking GPR27 with L-lactate homeostasis [30]. Moreover, agonists with a wide variety of efficacies, including partial and complete agonists, were discovered with efficacies that were greater than those of lead compound **I** (see Figure 3) [8]. 2-Fluoro-N-(4-[N-phenylsulfamoyl]phenyl)-4-(trifluoromethyl)benzamide (**7x**; pEC50: 6.44; Emax: 50%), and 4-chloro-2,5-difluoro-N-(4-[N-phenylsulfamoyl]phenyl)benzamide (**7y**; pEC50: 6.85; Emax: 37%) were the most potent agonists, followed by 4-chloro-2-methyl-N-(4-[N-phenylsulfamoyl]phenyl)benzamide (**7p**; pEC50: 6.04; Emax: 123%) and 2-bromo-4-chloro-N-(4-[N-phenylsulfamoyl]phenyl)benzamide (**7r**; pEC50: 5.99; Emax: 123%). The solubility and cellular toxicity of these compounds were assessed in parallel and proved to have limited influence at pharmacologically relevant concentrations [8].

Furthermore, the binding mode of the best compound, **7p**, was predicted with GPR27 against the homology model structures of the three most relevant GPCR structures from the same family (https://gpcrdb.org). As shown in Figure 4, ring A of compound **7p** was fully occupied in the high-energy hydration sites of GPR27 [8]. This property may play a role in the compound's selectivity for the GPR27 over the GPR85 and GPR173 receptors. This hypothesis needs to be confirmed experimentally by comparing the activity of various compounds on the three SREBs.

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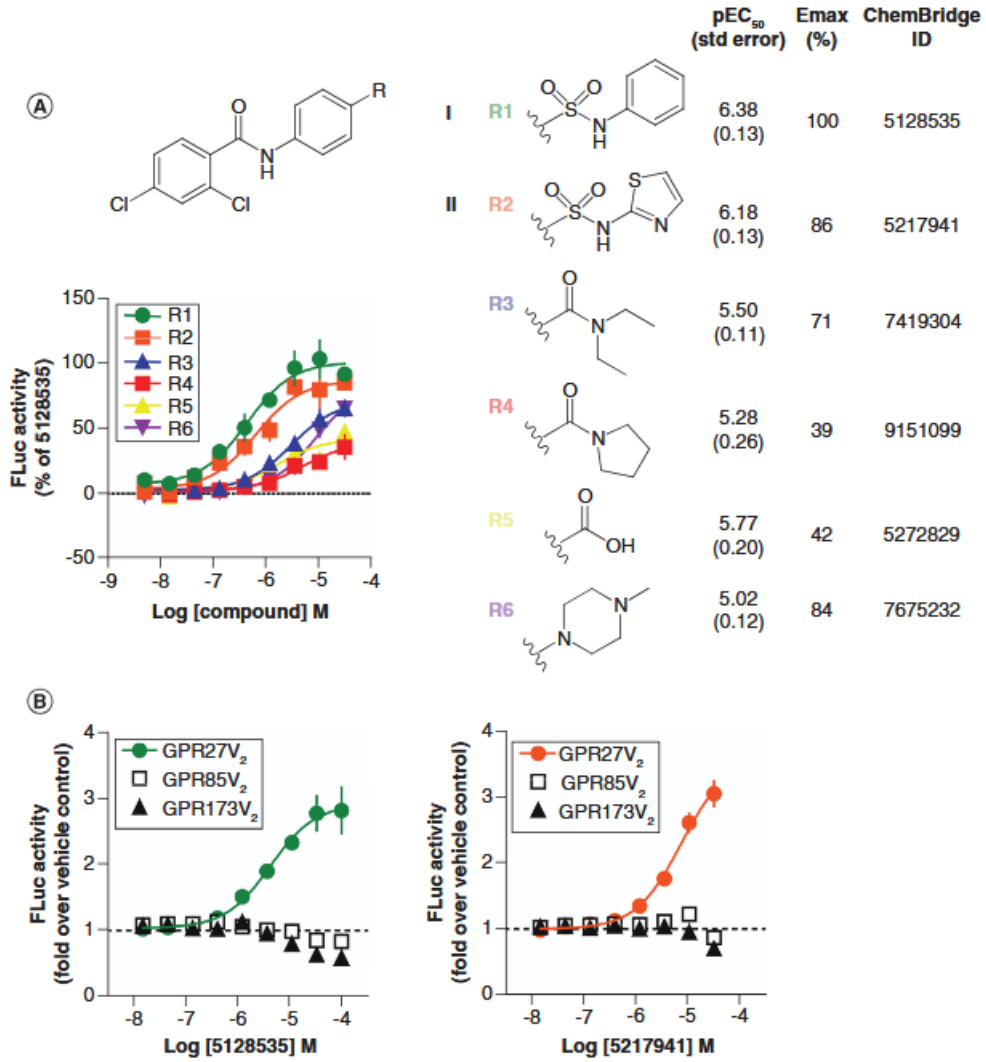
Figure 2. Structure–activity relationships and selectivity of the GPR27 agonists versus GPR85 and GPR173. **(A)** A common *N*-phenyl-2,4-dichlorobenzamide scaffold and the effect of various substituents (R) on compound activity in a firefly luciferase complementation assay based on β-arrestin-2. E_{max} is expressed as the percentage of 5128535 maximal activities. **(B)** Selectivity of GPR27 agonists I and II in the firefly luciferase complementation assay versus GPR85 and GPR173.



Yanai *et al.* found numerous inverse agonists for SREBs by looking at their constitutive activity [22]. They used a GPR27–Gsα/GPR85–Gsα/GPR173–Gsα fusion protein and a [35S]GTPγS-binding assay to screen chemical compounds. This led to the discovery of new non-selective inverse agonists for the SREB family with individual maximal EC_{50}s of over 30 µm incorporating pyrazole and coumarin scaffolds (see Table 1). The chemical with the highest potency has an IC_{50} of around 8 µm. In the present study's test setup, however, the authors were not able to detect GPR27-specific signals with these compounds [23]. The reasons for these discrepancies are not clear but may reflect different assay technologies and cell systems.

Based on the previous lead compounds, NPD6061 and NPD6145, the same group recently published a preprint about the development of new ligands for the SREB family with improved inverse agonistic activity compared with the previous study [29]. All final compounds were characterized using a GPR85–Gsα fusion protein and a [^{35}S]GTPγS-binding assay [29]. It was discovered that compounds **2g**, **3h** and **3i** were effective inverse agonists of the SREB family (Table 2) [31]. On the other hand, it should be

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noted that the compounds were not evaluated for their specificity against an unrelated control receptor. Thus, future investigations should investigate these aspects and confirm the mechanism of action of this family of molecules.

Figure 3. Structures and activities of GPR27 full and partial agonists.

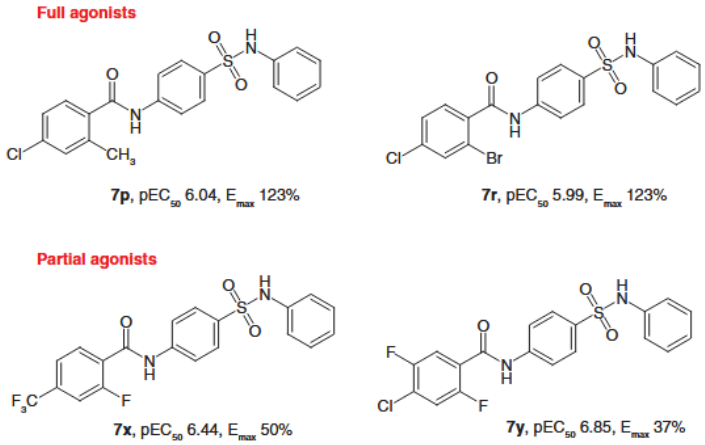
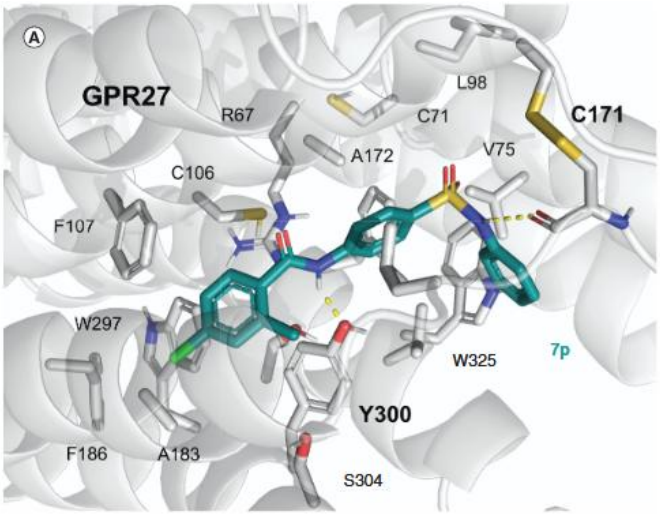


Figure 4. Docking studies of 7p in the binding pocket of the homology model of GPR27.



They also studied the neurochemical nature of compound **3i** and the physiological relevance of GPR85 using cerebellar Purkinje cells expressing GPR85 and an electrophysiological technique. The GPR85 inverse agonist chemical regulated potassium channel opening, according to the findings [31].

GPR85 (SREB2)

GPR85 is the most evolutionarily conserved SREB of the three, with the primary amino acid sequence of GPR85 being 100% identical in humans, rats and mice [12,32,33]. In addition, when compared with other GPCRs, human GPR85 and its zebrafish counterpart have 94% amino acid similarity [12].

GPR85 is highly expressed in the dentate gyrus of the hippocampus, which has been associated with psychiatric illnesses and cognition [12]. It is also found in Purkinje cells [22].

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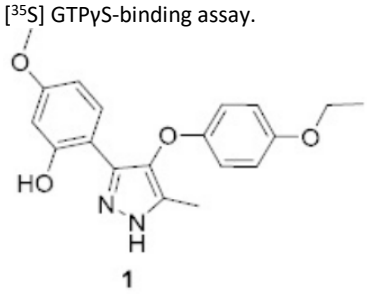
Table 1. Proposed inverse agonists for super-conserved receptors expressed in the brain– Gsα fusion proteins measured by [^{35}S] GTPγS-binding assay.



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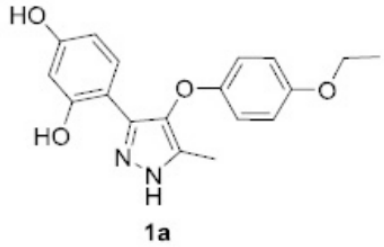

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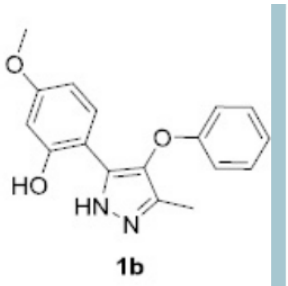

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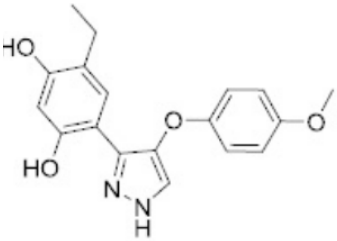

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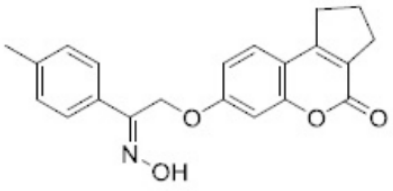

2

Compound	GPR27–Gsα IC_{50} (μM)	GPR85–Gsα IC_{50} (μM)	GPR173–Gsα IC_{50} (μM)
1	23 ± 1.3	32 ± 3.0	8.1 ± 0.6
1a	10 ± 4.1	14 ± 4.3	12 ± 1.1
1b	26 ± 8.1	29 ± 5.6	27 ± 5.5
1c	58 ± 1.2	52 ± 1.5	60 ± 1.4
2	29 ± 1.8	53 ± 2.7	23 ± 1.3

Data taken from [22].

The hippocampal dentate gyrus is a portion of the brain structure that may grow new neurons as it matures, making the hippocampus morphologically and functionally adaptable [34–36]. An increasing body of data shows that shrinking of the hippocampus formation is linked to mental diseases, resulting in functional deficits including learning and memory loss, as well as mood dysregulation [37,38].

Matsumoto *et al.* showed that the expression profile of GPR85 mRNA in adult human, monkey and rat forebrains was largely conserved [18]. SREB2 mRNA was identified in neurons across the brain in all of the species investigated, with the hippocampal dentate gyrus showing the greatest levels of expression.

The creation of the hippocampus, the olfactory system and the supraoptic and paraventricular nuclei, all of which are highly malleable, is associated with high levels of SREB2 mRNA expression [18]. SREB1 and SREB3 anatomical expressions overlapped with that of SREB2 in the adult monkey brain [18]. These findings suggest a link between the SREB family and brain plasticity [18].

GPR85 transcripts was found in a variety of human brain locations, as well as at lower levels in the spleen and placenta, according to Hellebrand et al., but GPR85 is solely found in the brains of mice [32]. Following that, the same group investigated the distribution of the GPR85-containing outermost layer of the cerebral cortex during mouse development and in the adult brain in great detail. GPR85 was identified in both the embryonic and adult brains of mice [14]. The majority of the GPR85 transcripts were discovered in neuroectodermal tissues. During the early stages of neuron formation, GPR85 is located in the CNS. The greatest amounts of transcripts were identified in the developing

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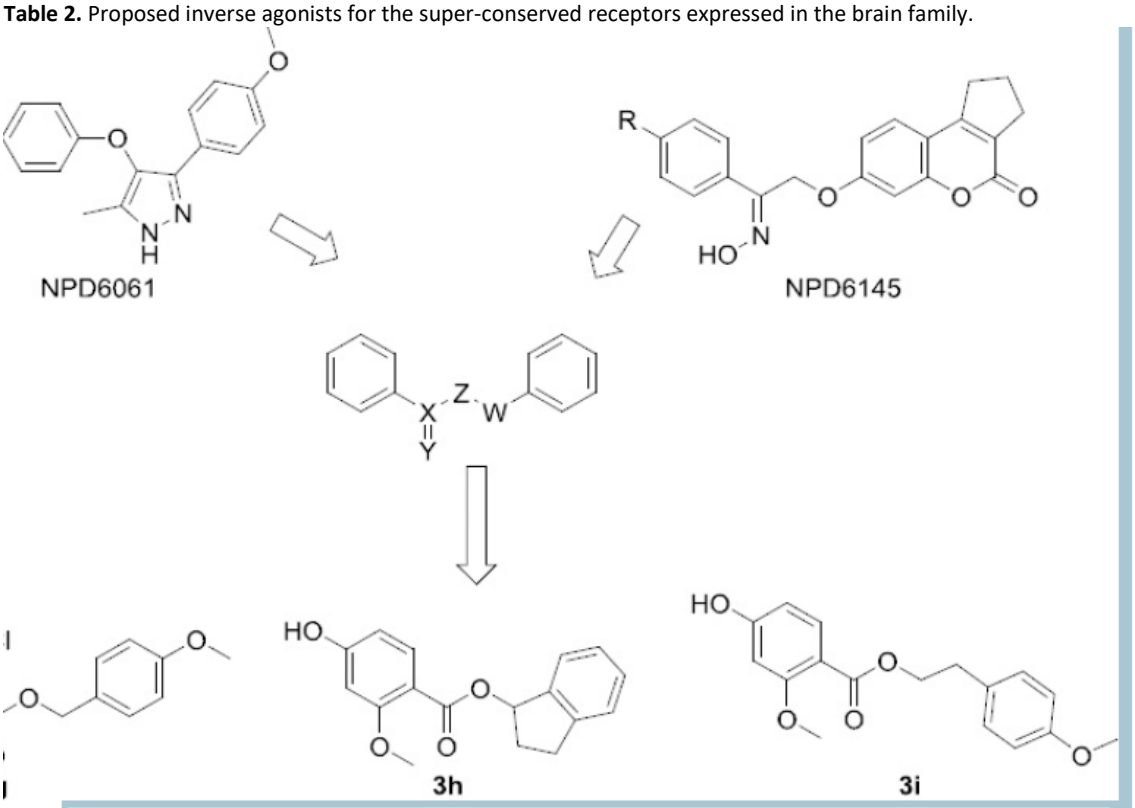
cerebral cortex, suggesting that this gene plays a role in cerebral differentiation. GPR85 was also detected in neural crest derivatives and developing teeth.

Table 2. Proposed inverse agonists for the super-conserved receptors expressed in the brain family.



Compound	GPR27–Gsα IC_{50} (μM)	GPR85–Gsα IC_{50} (μM)	GPR 173–Gsα IC_{50} (μM)
2g	2.3 ± 0.5	1.7 ± 0.2	2.6 ± 0.9
3h	1.5 ± 0.6	1.3 ± 0.4	1.1 ± 0.7
3i	0.62 ± 0.3	0.52 ± 0.3	2.6 ± 0.9

Data taken from [31].

GPR85 & AUTISM SPECTRUM DISORDER

Recently, GPR85 has also been linked to autism [39,40], attention deficit hyperactivity disorder [41], Tourette's syndrome and intellectual disability [42]. Based on this, it has been postulated that GPR85 (SREB2: rs56080411 and rs56039557) genetic variants, like other genetic risk factors connected to schizophrenia risk, may play a role in several neurodevelopmental psychiatric diseases.

The genetic etiology of autism spectrum disorder (ASD) is complicated. Schizophrenia and ASD share certain symptoms and mutant genes, such as NLGN, NRXN and SHANK3. During research on the shared molecular foundation of mental illnesses such as schizophrenia and ASD, Jelen´ *et al.* discovered that the C-terminal sequence of GPR85 was related to NLGN and PDZ proteins in the brain, including PSD-95 [43].

One possible molecular cause is an imbalance of excitatory and inhibitory receptors linked to the NLGN–PSD95–SHANK complex by the binding of PDZ. Fujita et al. focused on GPR85 as a possible gene for ASD, since its C-terminal amino acid sequence (Thr-Cys-Val-Ile [YCVI]) is classified as a type II PDZ-binding motif and GPR85 is linked to schizophrenia [44]. They discovered that GPR85 is connected to

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PSD-95, which is linked to NLGN in the brain, while looking for molecules that bind with GPR85. In the study, they employed a pull-down experiment and immunoblot analysis to seek proteins that connect to GPR85's C-terminal region, as well as a GPR85 gene mutation in ASD patients. The intracellular location of mutant GPR85 and its impact on cell and neuron morphology was investigated using immunofluorescence. As a result, the C-terminal sequences of GPR85 and NLGN interacted with PSD-95 at PDZ1 and PDZ3, respectively. Both T1033C (M152T) and G1239T mutations in GPR85 were found in two male ASD patients from separate Japanese families (V221L). These mutations were found in a region involved in G protein interaction and signaling. Unlike wild-type GPR85, mutant GPR85 was more selectively accumulated and seen in ASD patients' disrupted dendritic development, suggesting that the related NLGN–PSD-95 receptor complex may be a possibility for the molecular etiology of ASD.

Radulescu *et al.* employed functional MRI (fMRI) paradigms to test the notion that these changes have an impact on brain function in healthy adults (hippocampal formation and amygdaloid complex) [45]. Due to the well-known effects of different sexes on the amygdala [46] and hippocampus activation [47] during face assessment and emotional recall [48], they investigated the impact of biological sexes through SREB2 genotype interactions. Furthermore, sex (in terms of biological gender) influences the onset and occurrence of mental illnesses, including depression and schizophrenia [49].

The study found that risk-associated polymorphisms in SREB2 are connected to phenotypes in the dorsolateral prefrontal cortex and amygdala of men that are similar to those reported in schizophrenia patients, but the tendency is reversed in women. As a result, the SREB2 effect on cortical circuitry linked to schizophrenia risk might be more sophisticated or gender-specific.

GPR85 & SCHIZOPHRENIA

A statistical relationship between SREB2 gene variants and an increased prevalence of psychosis [45,50] has been found, linking SREB2/GPR85 to a variety of neurodevelopmental psychiatric diseases, including schizophrenia.

GPR85's role in regulating brain size is one of the most important results from GPR85 investigations in genetically engineered mice. Matsumoto *et al.* created an SREB2-overexpressing transgenic (Tg) mouse, which revealed that SREB2 plays a role in regulating brain size, influencing a variety of behaviors and perhaps predicting schizophrenia vulnerability [51]. In one experiment, GPR85-overexpressing transgenic mice had reduced brain mass and features comparable to prior experimental models of schizophrenia, such as decreased social interaction, abnormal sensorimotor gating and poor memory. GPR85 KO mice showed greater brain mass and performed better in recalling training stimuli compared with wild-type littermates.

Researchers revealed that the Akt/PKB pathway is involved in pathogenic alterations caused by SREB protein expression variations. This signaling system is required for neuronal development and is linked to schizophrenia [51]. They discovered that the activity of the Akt/PKB pathway was lower in SREB2 Tg mice's brains and greater in SREB2 KO mice's brains. Chen *et al.* discovered that SREB2 regulates adult neurogenesis in the hippocampal nucleus, which affects cognitive function [50]. In SREB2 Tg (overexpression) and KO (null-mutant) mice, bromodeoxyuridine incorporation and immunohistochemistry were utilized to measure adult neurogenesis and neonatal neuron dendritic

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morphology. The cognitive responses of SREB2 mutant mice were evaluated in relation to changes in adult neurogenesis. They discovered that SREB2 Tg mice had lower progenitor cell proliferation and newborn neuron numbers, but SREB2 KO mice had better newborn neuron survival with no difference in proliferation [50].

Doublecortin labeling revealed dendritic morphological defects in freshly generated neurons in SREB2 Tg mice. In a spatial pattern separation test, SREB2 Tg mice exhibited a decreased ability to distinguish spatial connections, whereas SREB2 KO mice had a greater ability. SREB2 Tg and KO mice displayed reciprocal abnormalities in a Ymaze working memory task. According to these findings, SREB2 could be a negative regulator of adult neurogenesis and, as a result, cognition. Inhibition of SREB2 activity may be a novel method of promoting adult neurogenesis and cognitive abilities in psychiatric patients' core symptoms.

GPR173 (SREB3)

Like other subfamily members of SREB, GPR173 is most prominently expressed in the brain, especially in astrocytes [52,53]. However, its expression has been reported in a variety of tissues [12,54]. These include tissue with rich nerve networks, such as the dental pulp, as well as tissue in the human ovaries and small intestine [52,55,56]. GPR173 has been proven to be linked to mediating hypothalamus activity and to be the putative receptor for the pleiotropic peptide phoenixin (PNX) [52,57].

PNX AS A PUTATIVE LIGAND FOR GPR173

PNX is part of the C4orf52 C-terminus family and it appears in two different active forms: a 20-amino-acid-long form called PNX-20 and the shorter, 14-amino-acid-long PNX-14 [58–60]. Studies have shown that it hides a preeminent potential for the treatment of a wide range of diseases, and it has already been proven to be linked to beneficial effects such as reduced anxiety and improved memory [52,61,62]. Regarding its function in the brain, the depletion of GPR173 RNA (with a siRNA-based approach) has been shown to abolish PNX-20 effects on gonadotropin-releasing hormone (GnRH) expression, suggesting that GPR173 might be a PNX putative cellsurface receptor [54,63]. PNX was found to be a regulator of pituitary gonadotropin secretion by modulation of the expression of the GnRH receptor [59]. GnRH is an essential peptide for various processes – in particular, for sexual development. Studies on zebrafish have shown that GPR173 and PNX might be candidates for the functioning of these physiological processes [64]. PNX is produced by cleavage of SMIM20. SMIM20 regulates energy homeostasis and reproduction, which suggests a role for PNX (and GPR173) in diseases associated with dysfunction of reproduction, such as polycystic ovary syndrome (PCOS) [65]. It was found that levels of PNX-14 and GPR173 in the ovary of PCOS rats were increased, but in periovarian adipose tissue, GPR173 protein levels decreased [65]. The involvement of GPR173 in the reproductive system was also studied with human non-luteinized granulosa cell lines. PNX treatment stimulated the proliferation of granulosa cells, increased estradiol production and induced the expression of genes related to follicle development [56]. Recently, PNX, follicle-stimulating hormone (FSH), luteinizing hormone (LH) and 17β-estradiol concentrations were measured in women with

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endometriosis by Kulinska *et al.* PNX levels were reduced and LH/FSH ratios were increased, as were 17β-estradiol concentrations [66]. Interestingly, *SMIM20* expression remained unchanged [66]. Measurement of PNX and LH/FSH ratio could provide new possibilities in the diagnosis of endometriosis in women [66].

Since GPR173 and PNX are expressed in the hypothalamus, their involvement in vasopressin and oxytocin secretion has been studied as well. Intracerebroventricular PNX treatment of rats led to a vasopressin increase but no change in oxytocin levels [67]. Notably, the neural and hormonal effects of PNX and GPR173 were found to be important for the influence of thirst behavior. Treatment of PNX-20 significantly increased water drinking in rats. Therefore, it was suggested that PNX caused these effects by interacting with neural centers controlling thirst. Previously described stimulation of AVP release has also been linked to these effects [68]. GPR173's role in the gut–brain axis also remains to be explored further [52,61,62]. It has been suggested that besides its neuronal functions, GPR173 signaling is connected to osteoblastic differentiation in C3T3-E1 cells [69].

The suggested GnRH signaling was shown by Treen etal. in 2016. PNX-20 proved to modulate GnRH expression via the PKA/cAMP pathway mediated by CREB [54,62]. Since CREB activates PGC-1α, Yang et al. studied its role in GPR173/PNX-20 function [54]. It was shown that PNX-20, in fact, induces PGC-1α. Associated with this, the induction of NRF-1 and TFAM could be demonstrated. According to these results, Yang et al. found that PNX-20 promoted neuronal mitochondrial biogenesis and an increased number of cellular mitochondrial copies [54]. Therefore, PNX-20 and its putative receptor GPR173 could be a target of research into viable drugs for the treatment of neurodegenerative diseases [54].

Recently, the validity of GPR173 pairing with PNX was questioned [70] and is therefore still a subject of discussion. According to established criteria for new receptor deorphanization, a new pairing should be confirmed independently by at least two different labs and on different assay systems (PubMed ID: 23686350 and 29454621). Thus, additional evidence from other labs is needed to validate the direct interaction between PNX and GPR173.

DISEASES ASSOCIATED WITH PNX

In keeping with the hypothesis that PNX-20 is a GPR173 ligand, Wei *et al.* have studied PNX-20's potential as an anti-atherosclerotic treatment [52]. Research has revealed that the oxidation of native LDL to ox-LDL induces atherosclerosis, the leading cause of vascular disease worldwide [71]. ox-LDL is assumed to activate the inflammatory pathway through NF-κB and lead to cell transformation [52,72]. In an in vitro model, Wei *et al.* used ox-LDL to trigger atherogenesis in human aortic endothelial cells (HAECs) [52]. The influence of PNX-20 treatment on the attachment of monocytes to HAECs and the ox-LDL-induced endothelial dysfunction was studied thoroughly [52]. It was found that the introduction of three doses of ox-LDL to HAECs significantly reduced the expression of GPR173 mRNA to 32% and GPR173 protein concentration to 36% [52].

Equally important for the treatment of atherosclerosis is the reduction of ox-LDL-induced monocyte attachment to endothelial cells by PNX-20. Treatment of cells with PNX-20 and ox-LDL reduced the attachment of THP-1 monocytes significantly in comparison with cells without PNX-20 treatment.

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Measurements of real-time PCR analysis led to the assumption that the two major cellular adhesion molecules, ICAM-1 and VCAM-1, are involved in these effects [52].

Notably, PNX-20 effects on reactive oxygen species (ROS) have been studied as well. PNX-20 has been proven to reduce ROS in ox-LDL-treated HAECs, showing the importance of GPR173 function as a possible treatment for atherosclerosis [52]. This reduction of ROS is suggested to be linked to the reduction of NOX-4 expression, which is known to modulate the production of mitochondrial ROS [52]. PNX's role in ROS was also researched in the treatment of neuroinflammatory diseases. In 2020, Sun et al. showed that inflammation in dental pulp cells caused by lipopolysaccharides could be reduced with treatment with PNX-20 [55]. They found that PNX-20 reduced the expression of pro-inflammatory cytokines, cell adhesion molecules, MMP-2 and MMP-9 [52,55]. Additionally, the release of pro-inflammatory cytokines and inflammatory mediators was found to be reduced after the treatment [55]. Wang *et al.* further studied these processes. They treated primary astrocytes from mice with lipopolysaccharides to induce an endoplasmic reticulum stress response. This response and the NLRP3 inflammasome activation were attenuated by treatment with PNX-14. Therefore, it was suggested that these effects could be linked to the suppression of ROS by PNX [53]. A final siRNA knockdown experiment targeted at GPR173 suggested that the receptor was involved in PNX effects [53].

In search of a possible explanation for these effects, Wei *et al.* suggested the involvement of GPR173 in NF-κB activity. NF-κB is responsible for different detrimental effects by, among other things, inducing the -overproduction of pro-inflammatory cytokines [52]. In fact, a reduction in NF-κB activity after PNX-20 addition to ox-LDL-treated HAECs was proven. Its nuclear levels were reduced by up to twofold and its luciferase activity from 87-fold to 37-fold in comparison with HAECs treated only with ox-LDL [52]. In conclusion, pleiotropic PNX-20 is therefore an important actor in protecting endothelial cells from cellular stress and, hence, in the future treatment of atherosclerosis [52]. Its role in neurodegenerative diseases and neuronal and hormonal functions is equally significant and should be researched further. Levels of PNX were found to be elevated in the blood and aqueous humor of patients with Type 2 diabetes and cataracts with and without diabetic retinopathy [73]. Celik and Aydin suggest that according to their findings, PNX may also be a part of the pathophysiology of Type 2 diabetes with cataracts [73].

Conclusion & future perspective

GPR27 (SREB1), GPR85 (SREB2) and GPR173 (SREB3) are a group of orphan G protein-coupled receptors that constitute the SREB subfamily. Recently, they have gained significant attraction due to their expression in the CNS and the high degree of sequence conservation among vertebrates. GPR27 has been linked to lipid metabolism and pancreatic cell insulin secretion and regulation; GPR85 has been linked to schizophrenia, autism and neuronal plasticity; and GPR173 has been linked to atherosclerosis, neurodegenerative diseases and important hormonal and neuronal functions (see Table 3). The physiological activities of SREBs are currently unclear due to the absence of specific ligands for each of them or validated endogenous agonists. Specific ligands are beneficial in functional assessments. Hanson's group recently described a novel class of sulfonamides as selective and specific GPR27 agonists, including 2,4-dichloro-*N*-(4-[*N*-phenylsulfamoyl]phenyl)benzamide (**I**; pEC_{50}: 6.44;

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E_{max}: 100%) and 4-chloro-2,5-difluoro-*N*-(4-[*N*-phenylsulfamoyl]phenyl)benzamide(**7y**;pEC_{50}: 6.85;E_{max}:37%)as full and partial agonists, respectively. The solubility and cellular toxicity of this compound's class have been assessed in parallel and proved to have limited influence at pharmacologically relevant concentrations. Thus, compounds I and 7y could be potent tool compounds to further characterize the (patho)physiological roles of GPR27. Additionally, homology models of these receptors might be used to conduct screening campaigns and discover new ligands.

Table 3. Receptors of the super-conserved receptors expressed in the brain family, the tissues where they are most prominently expressed and possible therapeutic targets.

Receptor	Expression	Possible therapeutic targets	Ref.
GPR27	Parathyroid gland, thyroid gland, brain, granulocytes, fallopian tube	Lipid metabolism, pancreatic β-cell insulin secretion and regulation, lactate homeostasis	[20] [7,20] [30]
GPR85	Skeletal muscle, brain, placenta	Autism, schizophrenia, neuronal plasticity	[39,40] [45,50,51] [18]
GPR173	Brain (cerebral cortex, cerebellum), pituitary gland, ovary	Atherosclerosis, neurodegenerative diseases, type 2 diabetes with cataracts, polycystic ovary syndrome, endometriosis	[52] [54] [73] [65] [66]

Gene expression data accessed from https://www.proteinatlas.org/, November 2021.

A couple of results regarding the non-selective ligands for GPR27, GPR85 and GPR173 have been published. However, these ligands have yet to be confirmed, and future research should be cautiously verified.

The validity of the GPR173–PNX combination has recently been questioned; therefore, it is still a topic of debate [70]. To prove PNX's function in GPR173 activity, more evidence is needed. Still, given the SREB family's links to a variety of common disorders, we believe the SREB family will establish its importance in the near future, particularly in terms of pharmacological activities and medicinal chemistry approaches.

Executive summary

- The G protein-coupled receptor subfamily called super-conserved receptors expressed in the brain consists of three members: GPR27 (SREB1), GPR85 (SREB2) and GPR173 (SREB3).
- They are almost exclusively expressed in the CNS.
- GPR27 has been found to influence glucose homeostasis and lipid metabolism; agonists have been discovered by medicinal chemists.
- It has been suggested that GPR85 plays vital roles in neuronal plasticity, schizophrenia and autism.
- Recently, inverse agonists for GPR85 have been proposed.
- GPR173 is a potential receptor for the pleiotropic peptide phoenixin and has been connected to modulating hypothalamic function.
- Phoenixin studies suggested GPR173's participation in atherosclerosis and neurodegenerative diseases.

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References

Papers of special note have been highlighted as: • of interest

1. Huang Y, Todd N, Thathiah A. The role of GPCRs in neurodegenerative diseases: avenues for therapeutic intervention. *Curr. Opin. Pharmacol.* 32, 96–110 (2017).
2. Harmar AJ, Hills RA, Rosser EM *et al.* IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels. *Nucleic Acids Res.* 37(Suppl. 1), S680–S685 (2009).
3. Azam S, Haque M, Jakaria M, Jo SH, Kim IS, Choi DK. G-protein-coupled receptors in CNS: a potential therapeutic target for intervention in neurodegenerative disorders and associated cognitive deficits. *Cells* 9(2), 506 (2020).
4. Breton TS, Sampson WGB, Clifford B *et al.* Characterization of the G protein-coupled receptor family SREB across fish evolution. *Sci. Rep.* 11(1), 12066 (2021).
5. Fredriksson R, Schiöth HB. The repertoire of G-protein-coupled receptors in fully sequenced genomes. *Mol. Pharmacol.* 67(5), 1414–1425 (2005).
6. Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. *Nat. Rev. Drug Discov.* 16(12), 829–842 (2017).
7. Chopra DG, Yiv N, Hennings TG, Zhang Y, Ku GM. Deletion of Gpr27 *in vivo* reduces insulin mRNA but does not result in diabetes. *Sci. Rep.* 10(1), 5629 (2020).

• **This publication investigated the role of GPR27 in glucose homeostasis.**

8. Pillaiyar T, Rosato F, Wozniak M *et al.* Structure-activity relationships of agonists for the orphan G protein-coupled receptor GPR27. *Eur. J. Med. Chem.* 225, 113777 (2021).

- **The powerful agonists of the first identified class of GPR27 agonists were found to be soluble and free of cellular toxicity, according to the structure–activity relationships.**

9. Yang D, Zhou Q, Labroska V *et al.* G protein-coupled receptors: structure- and function-based drug discovery. *Signal Transduct. Target. Ther.* 6(1), 7 (2021).
10. Chung S, Funakoshi T, Civelli O. Orphan GPCR research. *Br. J. Pharmacol.* 153(Suppl. 1), S339–S346 (2008).
11. Laschet C, Dupuis N, Hanson J. The G protein-coupled receptors deorphanization landscape. *Biochem. Pharmacol.* 153, 62–74 (2018).
12. Matsumoto M, Saito T, Takasaki J *et al.* An evolutionarily conserved G-protein coupled receptor family, SREB, expressed in the central nervous system. *Biochem. Biophys. Res. Commun.* 272(2), 576–582 (2000).
13. Regard JB, Sato IT, Coughlin SR. Anatomical profiling of G protein-coupled receptor expression. *Cell* 135(3), 561–571 (2008).
14. Hellebrand S, Wittenberger T, Schaller HC, Hermans-Borgmeyer I. Gpr85, a novel member of the G-protein coupled receptor family, prominently expressed in the developing mouse cerebral cortex. *Brain Res. Gene Expr. Patterns* 1(1), 13–16 (2001).
15. Breton TS, Berlinsky DL. Characterizing ovarian gene expression during oocyte growth in Atlantic cod (*Gadus morhua*). *Comp. Biochem. Physiol. D* 9, 1–10 (2014).
16. Ku GM, Kim H, Vaughn IW *et al.* Research resource: RNA-seq reveals unique features of the pancreatic β-cell transcriptome. *Mol. Endocrinol.* 26(10), 1783–1792 (2012).
17. Ku GM, Pappalardo Z, Luo CC, German MS, McManus MT. An siRNA screen in pancreatic beta cells reveals a role for Gpr27 in insulin production. *PLoS Genet.* 8(1), e1002449 (2012).
18. Matsumoto M, Beltaifa S, Weickert CS *et al.* A conserved mRNA expression profile of SREB2 (GPR85) in adult human, monkey, and rat forebrain. *Brain Res. Mol. Brain Res.* 138(1), 58–69 (2005).
19. Regard JB, Kataoka H, Cano DA *et al.* Probing cell type-specific functions of Gi *in vivo* identifies GPCR regulators of insulin secretion. *J. Clin. Invest.* 117(12), 4034–4043 (2007).
20. Nath AK, Ma J, Chen ZZ *et al.* Genetic deletion of gpr27 alters acylcarnitine metabolism, insulin sensitivity, and glucose homeostasis in zebrafish. *FASEB J.* 34(1), 1546–1557 (2020).
21. Martin AL, Steurer MA, Aronstam RS. Constitutive activity among orphan class-A G protein coupled receptors. *PLoS One* 10(9), e0138463 (2015).
22. Yanai T, Kurosawa A, Nikaido Y *et al.* Identification and molecular docking studies for novel inverse agonists of SREB, super conserved receptor expressed in brain. *Genes Cells* 21(7), 717–727 (2016).

- **The discovery of new non-selective inverse agonists for the super-conserved receptors expressed in the brain family.**

23. Dupuis N, Laschet C, Franssen D *et al.* Activation of the orphan G protein–coupled receptor GPR27 by surrogate ligands promotes β-arrestin 2 recruitment. *Mol. Pharmacol.* 91(6), 595–608 (2017).

- **The first GPR27 agonists were discovered in this study, and they are selective versus the closely related receptors GPR85 and GPR173, respectively.**

24. Lu S, Jang W, Inoue A, Lambert NA. Constitutive G protein coupling profiles of understudied orphan GPCRs. *PLoS One* 16(4), e0247743 (2021).
25. Spaeth JM, Gupte M, Perelis M *et al.* Defining a novel role for the Pdx1 transcription factor in islet β-cell maturation and proliferation during weaning. *Diabetes* 66(11), 2830–2839 (2017).
26. Jain S, Ruiz De Azua I, Lu H, White MF, Guettier JM, Wess J. Chronic activation of a designer G(q)-coupled receptor improves β cell function. *J. Clin. Invest.* 123(4), 1750–1762 (2013).
27. Dickinson ME, Flenniken AM, Ji X *et al.* High-throughput discovery of novel developmental phenotypes. *Nature* 537(7621), 508–514 (2016).
28. Persaud SJ. Islet G-protein coupled receptors: therapeutic potential for diabetes. *Curr. Opin. Pharmacol.* 37, 24–28 (2017).
29. Hossain S, Mineno K, Katafuchi T. Neuronal orphan G-protein coupled receptor proteins mediate plasmalogens-induced activation of ERK and Akt signaling. *PLoS One* 11(3), e0150846 (2016).
30. Dolanc D, Zorec TM, Smole Z *et al.* The activation of GPR27 increases cytosolic L-lactate in 3T3 embryonic cells and astrocytes. *Cells* 11(6), 1009 (2022).
31. Sakai A, Yasui T, Watanave M *et al.* Development of novel potent ligands for GPR85, an orphan G protein-coupled receptor expressed in the brain. *Genes Cells* doi:10.1111/gtc.12931 (2022).

- **The GPR85 inverse agonist was investigated for its role in potassium channel opening.**

32. Hellebrand S, Schaller HC, Wittenberger T. The brain-specific G-protein coupled receptor GPR85 with identical protein sequence in man and mouse maps to human chromosome 7q31. *Biochim. Biophys. Acta* 1493(1–2), 269–272 (2000).
33. Jeon J, Kim C, Sun W, Chung H, Park SH, Kim H. Cloning and localization of rgpr85 encoding rat G-protein-coupled receptor. *Biochem. Biophys. Res. Commun.* 298(4), 613–618 (2002).
34. Abrous DN, Koehl M, Le Moal M. Adult neurogenesis: from precursors to network and physiology. *Physiol. Rev.* 85(2), 523–569 (2005).
35. Aimone JB, Wiles J, Gage FH. Computational influence of adult neurogenesis on memory encoding. *Neuron* 61(2), 187–202 (2009).
36. Ehninger D, Kempermann G. Neurogenesis in the adult hippocampus. *Cell Tissue Res.* 331(1), 243–250 (2008).
37. Geuze E, Vermetten E, Bremner JD. MR-based *in vivo* hippocampal volumetrics: 2. Findings in neuropsychiatric disorders. *Mol. Psychiatry* 10(2), 160–184 (2005).
38. Lucassen PJ, Heine VM, Muller MB *et al.* Stress, depression and hippocampal apoptosis. *CNS Neurol. Disord. Drug Targets* 5(5), 531–546 (2006).
39. Trikalinos TA, Karvouni A, Zintzaras E *et al.* A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol. Psychiatry* 11(1), 29–36 (2006).

Published in: Future Medicinal Chemistry (2022), vol. 14, n°12, pp. 899-913
DOI: 10.4155/fmc-2022-0006
Status: Postprint (Author's version)

40. Voineagu I, Wang X, Johnston P *et al.* Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474(7351), 380–384 (2011).
41. Anney RJ, Lasky-Su J, O'dúshláine C *et al.* Conduct disorder and ADHD: evaluation of conduct problems as a categorical and quantitative trait in the international multicentre ADHD genetics study. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 147b(8), 1369–1378 (2008).
42. Patel C, Cooper-Charles L, McMullan DJ, Walker JM, Davison V, Morton J. Translocation breakpoint at 7q31 associated with tics: further evidence for IMMP2L as a candidate gene for Tourette syndrome. *Eur. J. Hum. Genet.* 19(6), 634–639 (2011).
43. Jeleń F, Oleksy A, Smietana K, Otlewski J. PDZ domains – common players in the cell signaling. *Acta Biochim. Pol.* 50(4), 985–1017 (2003).
44. Fujita-Jimbo E, Tanabe Y, Yu Z *et al.* The association of GPR85 with PSD-95–neuroligin complex and autism spectrum disorder: a molecular analysis. *Mol. Autism* 6, 17 (2015).
45. Radulescu E, Sambataro F, Mattay VS *et al.* Effect of schizophrenia risk-associated alleles in SREB2 (GPR85) on functional MRI phenotypes in healthy volunteers. *Neuropsychopharmacology* 38(2), 341–349 (2013).
46. Hamann S. Sex differences in the responses of the human amygdala. *Neuroscientist* 11(4), 288–293 (2005).
47. Mackiewicz KL, Sarinopoulos I, Cleven KL, Nitschke JB. The effect of anticipation and the specificity of sex differences for amygdala and hippocampus function in emotional memory. *Proc. Natl Acad. Sci. USA* 103(38), 14200–14205 (2006).
48. Andreano JM, Cahill L. Sex influences on the neurobiology of learning and memory. *Learn. Mem.* 16(4), 248–266 (2009).
49. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. *Int. Rev. Psychiatry* 22(5), 417–428 (2010).
50. Chen Q, Kogan JH, Gross AK *et al.* SREB2/GPR85, a schizophrenia risk factor, negatively regulates hippocampal adult neurogenesis and neurogenesis-dependent learning and memory. *Eur. J. Neurosci.* 36(5), 2597–2608 (2012).
51. Matsumoto M, Straub RE, Marenco S *et al.* The evolutionarily conserved G protein-coupled receptor SREB2/GPR85 influences brain size, behavior, and vulnerability to schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* 105(16), 6133–6138 (2008).

- **This study revealed that SREB2 has a role in regulating brain size, influencing a variety of behaviors and perhaps predicting schizophrenia vulnerability.**

52. Wei X, Lin H, Zhang B *et al.* Phoenixin-20 prevents ox-LDL-induced attachment of monocytes to human aortic endothelial cells (HAECs): a protective implication in atherosclerosis. *ACS Chem. Neurosci.* 12(6), 990–997 (2021).

Published in: Future Medicinal Chemistry (2022), vol. 14, n°12, pp. 899-913
DOI: 10.4155/fmc-2022-0006
Status: Postprint (Author's version)

53. Wang J, Zheng B, Yang S, Tang X, Wang J, Wei D. The protective effects of phoenixin-14 against lipopolysaccharide-induced inflammation and inflammasome activation in astrocytes. *Inflamm. Res.* 69(8), 779–787 (2020).

54. Yang Y, Lv Y, Liu J, Zhang S, Li Y, Shi Y. Phoenixin 20 promotes neuronal mitochondrial biogenesis via CREB–PGC-1α pathway. *J. Mol. Histol.* 51(2), 173–181 (2020).

55. Sun G, Ren Q, Bai L, Zhang L. Phoenixin-20 suppresses lipopolysaccharide-induced inflammation in dental pulp cells. *Chem. Biol. Interact.* 318, 108971 (2020).

56. Nguyen XP, Nakamura T, Osuka S *et al.* Effect of the neuropeptide phoenixin and its receptor GPR173 during folliculogenesis. *Reproduction* 158(1), 25–34 (2019).

57. Schalla MA, Stengel A. Phoenixin– a pleiotropic gut-brain peptide. *Int. J. Mol. Sci.* 19(6), 1726 (2018).

- **This study has proven GPR173 to be linked to mediating hypothalamus activity and being the putative receptor for the pleiotropic peptide phoenixin.**

58. Rajeswari JJ, Unniappan S. Phoenixin-20 stimulates mRNAs encoding hypothalamo-pituitary-gonadal hormones, is pro-vitellogenic, and promotes oocyte maturation in zebrafish. *Sci. Rep.* 10(1), 6264 (2020).

59. Yosten GL, Lyu RM, Hsueh AJ *et al.* A novel reproductive peptide, phoenixin. *J. Neuroendocrinol.* 25(2), 206–215 (2013).

60. Lyu RM, Huang XF, Zhang Y *et al.* Phoenixin: a novel peptide in rodent sensory ganglia. *Neuroscience* 250, 622–631 (2013).

61. Dinan TG, Cryan JF. Brain–gut–microbiota axis – mood, metabolism and behaviour. *Nat. Rev. Gastroenterol. Hepatol.* 14(2), 69–70 (2017).

62. Treen AK, Luo V, Belsham DD. Phoenixin activates immortalized GnRH and kisspeptin neurons through the novel receptor GPR173. *Mol. Endocrinol.* 30(8), 872–888 (2016).

63. Stein LM, Tullock CW, Mathews SK *et al.* Hypothalamic action of phoenixin to control reproductive hormone secretion in females: importance of the orphan G protein-coupled receptor Gpr173. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 311(3), R489–R496 (2016).

64. Ceriani R, Calfún C, Whitlock KE. Phoenixin(smim20), a gene coding for a novel reproductive ligand, is expressed in the brain of adult zebrafish. *Gene Expr. Patterns* 39, 119164 (2021).

65. Kalamon N, Blaszczyk K, Szlaga A *et al.* Levels of the neuropeptide phoenixin-14 and its receptor GRP173 in the hypothalamus, ovary and periovarian adipose tissue in rat model of polycystic ovary syndrome. *Biochem. Biophys. Res. Commun.* 528(4), 628–635 (2020).

66. Kulinska KI, Andrusiewicz M, Dera-Szymanowska A *et al.* Phoenixin as a new target in the development of strategies for endometriosis diagnosis and treatment. *Biomedicines* 9(10), 1427 (2021).

67. Gasparini S, Stein LM, Loewen SP *et al.* Novel regulator of vasopressin secretion: phoenixin. *Am J Physiol Regul Integr Comp Physiol* 314(4), R623–R628 (2018).

68. Haddock CJ, Almeida-Pereira G, Stein LM, Yosten GLC, Samson WK. A novel regulator of thirst behavior: phoenixin. *Am J Physiol Regul Integr Comp Physiol* 318(6), R1027–R1035 (2020).
69. Gu Z, Xie D, Ding R, Huang C, Qiu Y. GPR173 agonist phoenixin 20 promotes osteoblastic differentiation of MC3T3-E1 cells. *Aging (Albany NY)* 13(4), 4976–4985 (2020).
70. Yañez-Guerra LA, Thiel D, Jékely G. Pre-metazoan origin of neuropeptide signalling. *Mol. Biol. Evol.* doi:10.1093/molbev/msac051 (2022).
71. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of atherosclerosis and the potential to reduce the global burden of atherothrombotic disease. *Circ. Res.* 118(4), 535–546 (2016).
72. Lu J, Mitra S, Wang X, Khaidakov M, Mehta JL. Oxidative stress and lectin-like ox-LDL-receptor LOX-1 in atherogenesis and tumorigenesis. *Antioxid. Redox Signal* 15(8), 2301–2333 (2011).
73. Celik F, Aydin S. Blood and aqueous humor phoenixin, endocan and spexin in patients with diabetes mellitus and cataract with and without diabetic retinopathy. *Peptides* 150, 170728 (2022).